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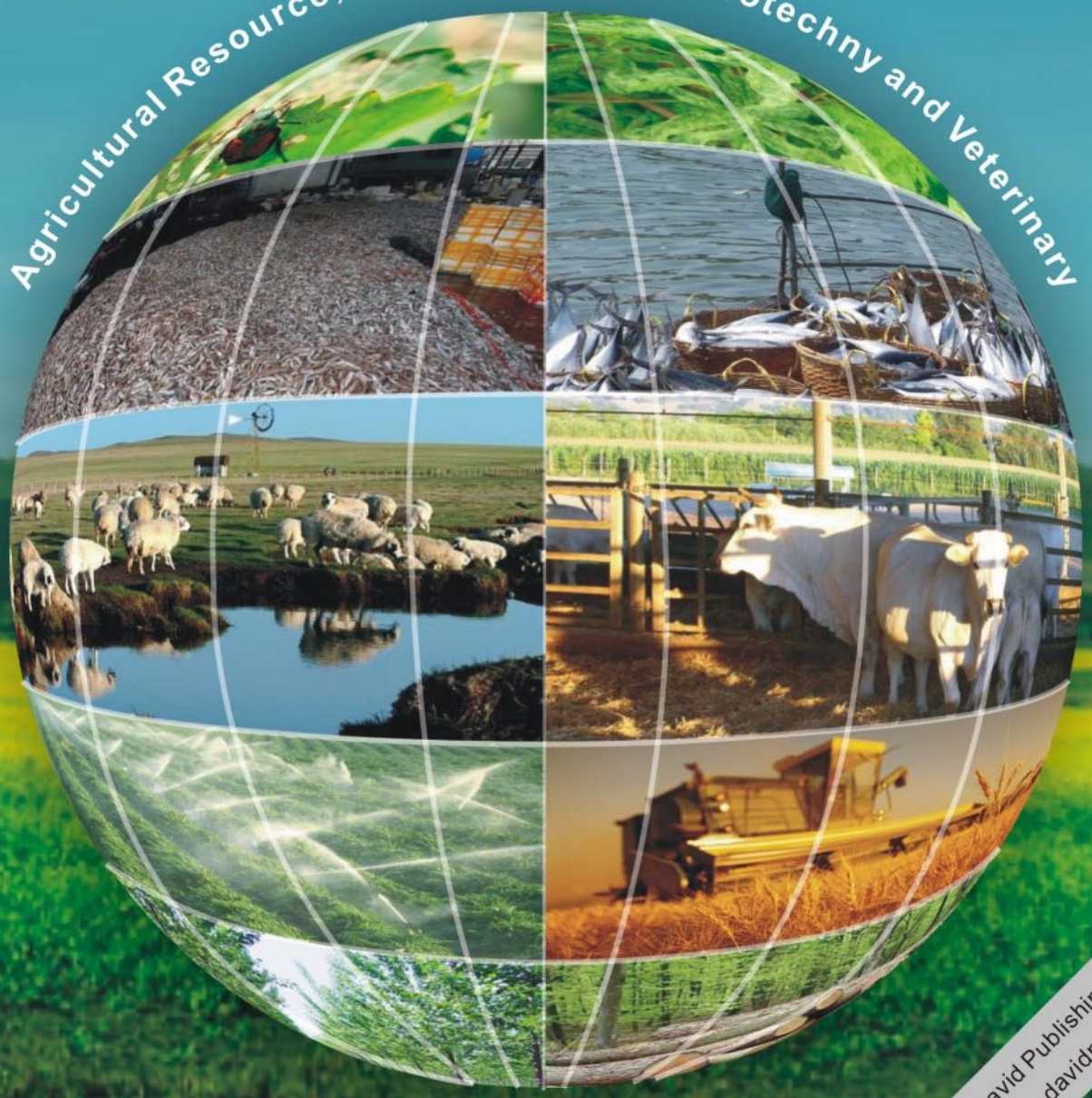
ISSN 2161-6256

Journal of Agricultural Science and Technology A

Volume 1, Number 2, June 2011

Agricultural Resource; Plant Protection; Zootechny and Veterinary



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Journal of Agricultural Science and Technology A

Volume 1, Number 2, June 2011 (Serial Number 2)

David Publishing Company
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Publication Information:
Journal of Agricultural Science and Technology A (Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250) is published monthly in hard copy (ISSN 2161-6256) by David Publishing Company located at 1840 Industrial Drive, Suite 160, Libertyville, Illinois 60048, USA.

Aims and Scope:
Journal of Agricultural Science and Technology A, a monthly professional academic journal, particularly emphasizes new research results in realm of agriculture, forestry, animal husbandry and veterinary, fisheries, agricultural engineering, food science, agricultural economy and so on. Articles interpreting practical application of up-to-date technology are also welcome.

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Abstracted/Indexed in:
Database of EBSCO, Massachusetts, USA
Chinese Database of CEPS, American Federal Computer Library Center (OCLC), USA
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Ulrich's Periodicals Directory
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Subscription Information:
Price (per year)
Print $520, Online $360
Print and Online $680

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Journal of Agricultural Science and Technology A

Volume 1, Number 2, June 2011 (Serial Number 2)

Contents

Research Papers

Journal of Agricultural Science and Technology A 1 (2011) 149-155
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Determination of Hesperidin and Limonin Levels as Part of the Defense Mechanism of Some Lemon Varieties [*Citrus limon* (L.) Burm f.] against Mal Secco Disease [*Phoma tracheiphila* (Petri) Kanc.et Ghik.]

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Received: May 25, 2010 / Published: June 20, 2011.

Abstract: In this paper, it was aimed to identified and quantified hesperidin and limonin compounds using HPLC (High Performance Liquid Chromatography) techniques against to developing of mal secco disease caused by *Phoma tracheiphila. S*ix *citrus lemon* varieties (Meyer, Kütdiken, Enterdonato, Yediveren, Sweet lemon and Euroka) were infected by *P. tracheiphila* and artificial inoculation were applied *in vivo* conditions. Before and after inoculation, leaf, branch and stem samples were taken from each lemon varieties. The results show that the amount of hesperidin and limonin concentration was increased after the inoculations at various levels based on the lemon cultivars. Various concentrations (1, 5, 10, 25, 50, 75, 100 ppm) of hesperidin and limonin compounds were also tested under *in vitro* conditions to compare response of *P. tracheiphila* development. According to the results, hesperidin and limonin compounds play an important role against to *P. tracheiphila* development and Sweet Lemon variety was found to be the most resistance both observation and HPLC results.

Key words: HPLC, *Phoma tracheiphila*, *Citrus limon*, hesperidin, limonin.

1. Introduction

The lemon [*Citrus limon* (L.) Burm. f.] has an important place at all citrus species which are produced in the worldwide. Each plant species is affected by many different kinds of fungi, bacteria, and etc. [1]. Like the other plants, lemons are infected by some pathogens at producing areas. But *Phoma tracheiphila* is known to be the most common problem and causes commercial losses and as a result it's limiting the lemon production areas at all growing production area in the world [2]. Recent studies showed that the chemical application of this pathogen, don't exposed successful result due to the it is known tracheomy-cotiv fungi [2-4]. According to the previous studies, results show that the application of systemic fungicides or other chemicals weren't found a definite solution [2-5].

As other all plants, citrus are defend themselves against pathogens by combinations of weapons from two arsenals; one of them is structural characteristics and the other one is biochemical reactions that take place in cell and tissues of the plant [1]. These biochemicals are playing a role as phytoalexins and there are produced by plant as defense mechanisms in response to microbial infection [6, 7]. Some studies have looked at the possible role that phenolic compounds might play as phytoalexins in some *Citrus* species [8-13]. Phenolic compounds are secondary metabolites which are produced after the plants are infected by disease factors [14-18]. After infection with

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pathogen so many phenolic compounds can be synthesized and can play an important role against to disease development [19]. Most citrus species accumulate substantial quantities of flavonoids during the development of their different organs [20-23]. All the flavonoids described in citrus spp. can be classified into three groups: Flavanones, flavones and flavonols [24]. Also hesperidin is the principal flavanone in lemon and citrus species [25-28]. There are many reports on antifungal activity of hesperidin [9-11, 27-29].

The other important phenolic compound group is limonoid. Limonin is the most prevalent of the citrus limonoids. Limonoids are highly oxygenated triterpenoid compounds that are found in all citrus tissues as limonoid. A-ring lactones, glucosides or aglycones. Limonoid A-ring lactones are water-soluble and tasteless metabolic precursors to limonoid aglycones and glucosides and exist mainly in juice sacs and leaves. Limonoid glucosides, also water-soluble and tasteless, are found in especially high concentrations in seed and fruit tissues. In contrast, limonoid aglycones are fairly water-insoluble and some, including limonin, the most common aglycone in citrus, have a bitter taste. Aglycones are predominately distributed in leaves and seeds and are sometimes present in juices, but generally only at low concentrations [30]. In addition, some authors reported that limonin was shown as anti-viral, antifungal, anti-bacterial, antineoplast and antimalarial activities [31, 32].

In this paper, we aimed to detect relationship between hesperidin and limonin levels with response of mal secco disease in six lemon varieties using HPLC (High Performance Liquid Chromatography). As far as we know, there are no such investigations previously reported. For this purpose, mal secco disease caused by *P. tracheiphila* was infected to the six lemon varieties under greenhouse conditions. Artificial inoculations were also applied with including various levels of hesperidine and limonin *in vivo* conditions.

2. Materials and Methods

2.1 Plant Materials

This study was done during 2008 and 2009 growing periods at Subtropical Fruits Research and Experimental Center, University of Çukurova. The one and half years old seedlings of Meyer, Kütdiken, Enterdonato, Yediveren, Sweet lemon and Euroka lemon varieties were used as a plant material. For each variety ten seedlings were used.

2.2 Isolation of Pathogen and Identification

The one year old shoots infected by *P. tracheiphila* which were taken from the Kütdiken lemon variety isolated and purified with PDA media and these were examined under the flouresan microscope [33]. Disks were taken from the purified culture and artificial inoculation were applied to the experimental lemon varieties.

2.3 Artificial Inoculation of P. tracheiphila

Isolated *P. tracheiphila* culture was used for infection of six lemon varieties as artificially. An artificial inoculation of *P. tracheiphila* disks infected into the stems of six lemon varieties and wrapped with grafting band. For each variety twenty plants were used for each lemon varieties in greenhouse condition.

2.4 Preparation of Plant Materials for HPLC Analysis

When the *P. tracheiphila* symptoms were observed, plant samples were taken from the three different part (middle-ages leaves, branches (4.5-5.5 mm diameters) and stems (8-9 mm diameters) of six lemon varieties both infected and noninfected plants. The plant materials were cut in to the small piece about 0.5-1 mm for phenolic extraction HPLC analysis. The plant materials were stored at -20 °C until HPLC analysis.

2.5 Identification and Quantification of Hesperidin and Limonin Compounds

From each plant materials 0.5 mg sample weighed and then extracted for two hours with 2 ml of dimethyl

sulfoxide (DMSO). Then the extract was filtered through a 0.45 μm nylon filter before analyzed with High Pressure Liquid Chromatography (HPLC). The Ultra pure water: methanol: acetonitrile: acetic acid (15:2:2:1) was used as a mobile phase. HPLC column SB C-18 (150 mm, 4.6 mm × 50 mm) was used for the identification and quantification of both compounds. As for the hesperidin analysis; the detection was done using a UW detector and set at 280 nm and column oven was heated at 40 °C. Limonin analysis were also done using same column but UW detector was set at 210 nm and column oven was heated at 30 °C.

2.6 In vitro Conditions

The hesperidin and limonin standards were dissolved in acetonitrile (1mg/1ml) and then cold sterilization were done using sterile filter (0.22 μm). Those standards were applied into the PDA medium at various levels (0 (Control), 1, 5, 10, 25, 50, 75, 100 ppm) and *P. tracheiphila* disks were planted into the place and incubated at +17 °C.

3. Results and Discussion

3.1 Artificial Inoculation of P. tracheiphila

After the artificial inoculation of seedlings of six lemon varieties in the greenhouse disease symptoms were observed. The disease symptoms are mostly observed on sensitive varieties (Kütdiken and Yediveren) firstly later on it was started the other varieties.

3.2 Incidence of P. tracheiphila

P. tracheiphila was infected to the Yediveren, Euroka, Interdonato, Sweet Lemon, Kütdiken, Meyer lemon varieties artificially. First symptoms were observed 78 days after inoculation. The primer symptoms were observed in Kütdiken variety. One month after inoculation color changes were observed on cottons at the infection region. After the inoculation, fungi started too grown and xylem tissues started to blocked then leaves become yellowish. At the end leaves were broken off without petiole and further stage shoots started to dried and die back started. When the infected branches were cut into the horizontal shape orange color was seen (Fig. 1).

Results show that symptoms and disease levels were differed in experimental lemon varieties. The earliest symptoms were observed in Kütdiken lemon variety, Yediveren and Euroka lemon varieties followed by. Whilem the symptoms were observed in Interdanato and Meyer lemon varieties later and lower scale than Kütdiken variety. Furthermore, very little symptoms were observed in Sweet Lemon variety. Incidence of disease symptoms were given in Fig. 2.

In previous studies, Cutuli et al. [2] reported that Meyer lemon variety was found to be resistant whereas Interdonato variety was found to be highly resistant against to mal secco disease in Israel and France. According to the our results, Sweet Lemon variety showed highest resistance when compared to the Meyer and Enterdonato lemon varieties, respectively. Similar results with us were reported by Tuzcu et al. [34].

3.3 Identified and Quantified of Hesperidin and Limonin Levels by HPLC

Hesperidin and limonin levels of leaf, branch and stem samples of six lemon varieties were analyzed by

Fig. 1 A view of after the artificial inoculation of *P tracheiphila* infection stages.

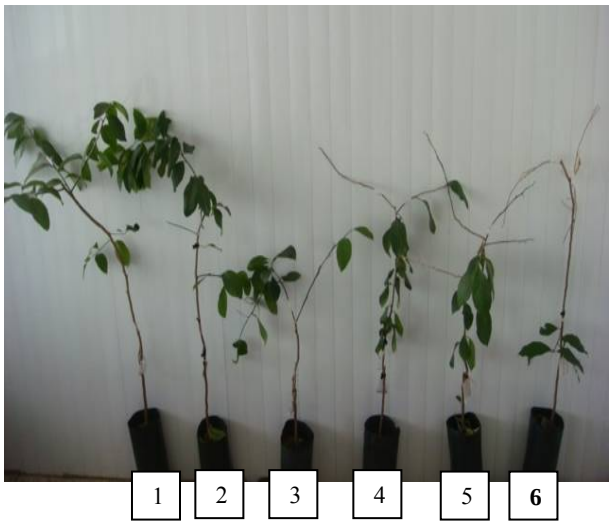

Fig. 2 A view of after the artificial inoculation of *P. traacheiphila:* 1. Sweet Lemon; 2. Meyer; 3. Interdonato; 4. Euroka; 5. Yediveren; 6. Kütdiken.

HPLC after artificial inoculation of P. *tracheiphila* and obtained results were shown in Table 1. As seen in Table 1 hesperidin level was found to be the highest in branch samples while lowest in leaves.

After the inoculation, hesperidin levels of leaf stem and branches were increased and these increasement levels were differed among the genotypes. As seen in Fig. 1 the highest increasement of hesperidin level was detected in Sweet Lemon, Interdonato and Meyer varieties, respectively. Euroka, Yediveren and Kütdiken varieties followed by. Similar results with us were published by Ben-Aziz [9]. The same author reported that hesperidin showed antifungal effect against to *P. tracheiphila.* In another study, Del Ri´o et al. [11] reported that hesperidin inhibited *Phytophthora citrophthora* development.

As seen in Table 2 the highest increasement percentage of branches was detected in Sweet Lemon (152.2%) and followed by Interdonato (130.3%), Meyer (112.3%), Yediveren (94.9%), Euroka (86.0%) and Kütdiken (39.1%) varieties, respectively. Similar results were obtained from the stem. The highest increasement percentage were obtained from in Sweet Lemon (429.1%), and followed by Meyer (288.4%), Interdonata (120.3%) Euroka (92.1%), Yediveren (88.9%) and Kütdiken (86.2%), respectively.

Chutia et al., [31] reported that limonin plays an

Table 1 Hesperidin levels of before and after artificial inoculation of *P. tracheiphila* (mg/g).

Varieties	Leaf				Branch				Stem			
	Before		After		Before		After		Before		After	
Yediveren	0.85	b*	2.00	a	5.02	a	7.25	b	0.48	b	0.64	c
Euroka	1.00	a	1.50	b	3.13	b	6.62	c	1.77	a	2.40	a
Interdonato	0.19	e	0.69	c	1.26	d	4.66	d	0.19	d	1.01	c
Sweet lemon	0.29	de	1.48	b	1.13	e	7.67	a	0.38	c	2.37	a
Meyer	0.35	cd	0.95	c	2.57	c	7.40	ab	0.34	c	1.76	b
Kütdiken	0.47	c	0.70	c	1.17	e	2.37	e	0.51	b	0.56	c

Mean values in the column followed by the same letter(s) are not significantly different at $P = 0.05$.

Table 2 Limonin levels of before and after artificial inoculation of *P. tracheiphila* (mg/g).

Varieties	Leaf				Branch				Stem			
	Noninoculated		Inoculated		Noninoculated		Inoculated		Noninoculated		Inoculated	
Yediveren	0.29	e*	0.59	e	15.70	a	30.80	b	0.72	e	1.36	e
Euroka	0.77	b	1.50	c	2.86	e	5.32	e	1.14	d	2.19	d
Interdonato	0.59	c	2.17	b	15.60	a	36.00	a	3.01	a	6.63	b
Sweet lemon	0.50	d	2.15	b	7.83	b	19.80	c	1.89	b	10.00	a
Meyer	0.28	e	1.19	d	3.83	C	8.13	d	1.29	c	5.01	c
Kütdiken	1.42	a	3.01	a	3.25	d	4.52	f	1.16	d	2.16	d

Mean values in the column followed by the same letter(s) are not significantly different at $P = 0.05$.

important role against to fungal disease development.

According to the results, increasement of hesperidin percentages were found to be higher than limonin in Sweet Lemon variety (Fig. 3). However, limonin contents of leaf, branch and stem were found to be higher than hesperidin contents.

3.4 Various Concentrations of Hesperidin and Limoninin against to P. tracheiphila Development

At this stage various concentration of hesperidin and limonin (1, 5, 10, 25, 50, 75 and 100 ppm) compounds were added PDA media and development of *P. tracheiphila* disks were evaluated. The developments of *P. tracheiphila* were measured when the fungi covered all the petri dish (control) and results were given in Table 3. As seen in Table 3 increaesement of hesperidin and limonin level effect positively fungi development.

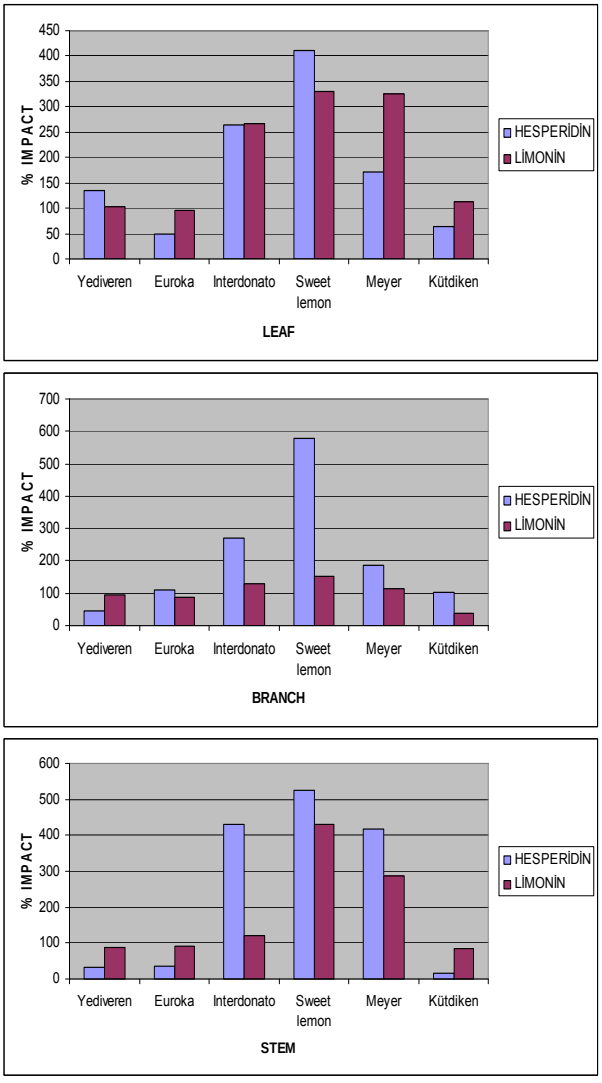

Fig. 3 Increasement of limonin and hesperidin percentages of leaf, branch and stem samples of six lemon varieties (%).

Table 3 Effects of various hesperidin and limoninin concentrations against to *P. tracheiphila* development.

Concentrations (ppm)	Hesperidin		Limonin	
	Colony diameter (mm)	Impact (%)	Colony diameter (mm)	Impact (%)
0	22.10	---	22.10	---
1	21.40	3.16	21.83	1.22
5	21.30	3.61	18.67	15.52
10	20.50	7.24	15.67	29.09
25	18.00	18.55	15.58	29.50
50	13.95	36.87	9.92	55.11
75	11.25	49.09	6.08	72.48
100	9.13	58.68	3.25	85.29

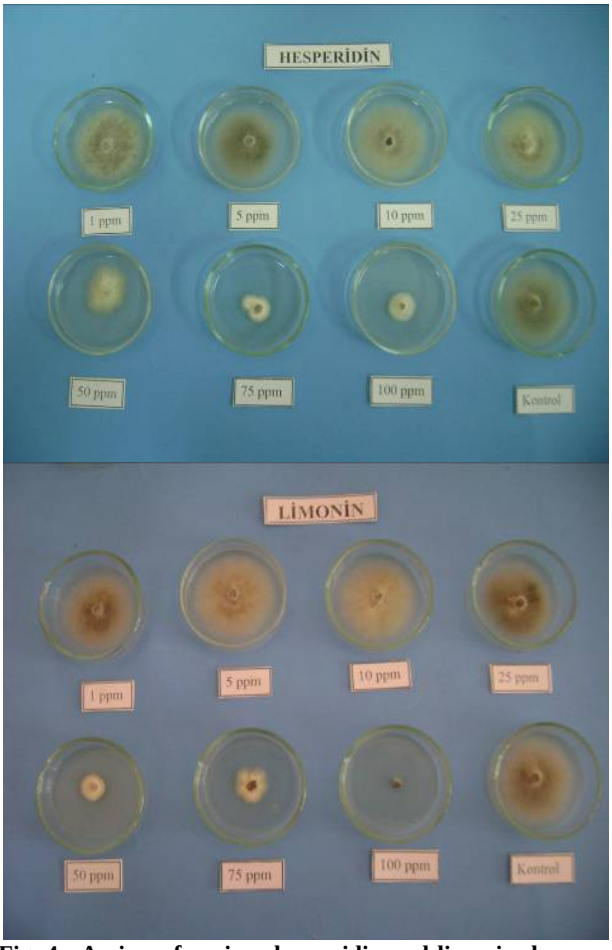

Fig. 4 A view of various hesperidin and limonin doses on growth of *P. tracheiphila* development.

Opposite to the artificial inoculation results, limonin was found to be more effective than hesperidin in *in vitro* experiment (Fig. 4).

As seen in Fig. 4, the highest limonin and hesperidin concentrations was obtained from 100 ppm of their dosages. The results agreed with the previous study Fourie [35] on *Phytophthora nicotianae.* The same author reported that hesperidine played an important role for inhibit the fungal disease development.

The results of various hesperidin and limoninin concentrations against to *P. tracheiphila* development were given in Table 3. As seen in Table 3 colony diameter was decreased while impact percentages increased according to the increasement of hesperidin and limonin concentrations. 100 ppm concentration was found to be the most effective one for inhibition of *P. tracheiphila* development.

4. Conclusion

According to the *in vivo* results, Sweet Lemon variety showed highly resistance compared to the Meyer and Interdonato lemon varieties, respectively. After the artificial inoculation, hesperidin and limonin levels of leaf, stem and branches were increased and these increasement levels were differed among the genotypes. In addition, increasement of hesperedin percentages were found to be higher than limonin in Sweet Lemon variety (Fig. 3). However, limonin contents of leaf, branch and stem were found to be higher than hesperidin contents. Furthermore, hesperidin and limonin levels were found to be the highest in branch samples while lowest in leaves.

As for the in vitro results, colony diameter was decreased while impact percentages increased according to the increasement of hesperidin and limonin concentrations. 100 ppm concentration was found to be the most effective one for inhibition of *P. tracheiphila* development. As a conclusion, hesperidin and limonin showed antifungal effect against to *P. tracheiphila.*

Acknowledgment

This project was supported by Academic Research Projects Unit of University of Çukurova, Project No:Z.F. 2009. YL.8.

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Journal of Agricultural Science and Technology A 1 (2011) 156-163
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Effects of Cultivar, Frying Temperature and Slice Thickness on Oil Uptake and Sensory Quality of Potato Crisps Processed from Four Kenyan Potato Cultivars

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Received: December 17, 2010 / Published: June 20, 2011.

Abstract: The effects of potato cultivar, frying temperature and slice thickness on oil uptake and sensory quality of potato crisps were investigated in four Kenyan cultivars. Potato tubers were peeled, washed and cut into slices of thickness 1.0 mm, 1.5 mm and 2.0 mm. Each size was fried at a constant temperature of 170 °C for 3-5 minutes. For frying temperature evaluation, the potatoes for all cultivars were cut into a uniform thickness of 1.5 mm and fried at temperatures of 160, 170 and 180 °C for 2-5 minutes. Crisps made from the four cultivars differed significantly ($P \leq 0.05$) in oil absorbed which ranged from 35.12% in Dutch Robyjn to 36.52% in clone 391,691.96. Tuber dry matter differed significantly ($P \leq 0.05$) among the cultivars ranging from 20.99% in clone 391691.96 to 25.29% in variety Dutch Robyjn. Tuber dry matter content was found to be negatively correlated to oil content of crisps; oil content increased with decrease in dry matter content. For each cultivar, the oil content of crisps differed significantly ($P \leq 0.05$) with temperatures and was higher at frying temperatures of 160 °C and lowest at 180 °C, respectively. The oil content was significantly ($P \leq 0.05$) higher in slices of 1.0 mm thick than in slices of 1.5 mm and 2.0 mm; the amount of oil absorbed decreased with increase in slice thickness. There was significant correlation ($P \leq 0.05$, r = -0.834) between oil content as determined in the laboratory and sensory scores. Results showed that high dry matter, slice thickness and temperature of frying resulted in reduced oil absorption by crisps during processing

Key words: Slice thickness, frying temperatures, oil content, potato crisps, cultivar.

1. Introduction

Potato crisps are popular fried snacks in the world. They are increasingly becoming important snack foods to many Kenyans and especially those living in the main urban centres. Potato crisps are among the most important products in the Kenyan potato processing industry. The demand for crisps increases rapidly, especially during festive seasons. The amount of oil absorbed during deep frying of crisps is becoming important. The amount of oil absorbed influences flavor of crisps and number of calories supplied by the food. Fried foods may contain amounts of oil that in some cases is more than 40% of the weight of the total product [1, 2]. French fries made from Kenyan cultivars, for example, contain about 12% oil [3] and crisps can contain oil contents of up to 45% depending on potato cultivar, and processing parameters [2, 4, 5]. Consumption of crisps is therefore of concern to nutritionists who advocate a decreased or increased oil intake in diets depending on the part of the World they are based. Crisps with low oil content and desirable sensory attributes are therefore expected to be accepted by the increasing numbers of health conscious consumers.

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Many factors have been reported to affect the oil content of French fries and crisps including oil quality, frying temperature and duration, slice thickness, product shape and composition (moisture content, solids, fat, gel-strength), pre-frying techniques (blanching, drying and frying) and any added coating such as methylcellulose or colloids [6-9]. Gamble et al. [1] found the loss of moisture and the oil uptake in French fries during frying to be interrelated; a reduction of the initial moisture content by drying was recommended to reduce the oil uptake into potatoes. Deep-fat frying of potato slices for crisps production involves an initial, very short period of heating at high moisture level resulting in gelatinization of starch followed by a rapid dehydration period to a final moisture content of about 2%. There exists an intimate contact between the frying oil and the surface of the potato slices that ensures high heat and mass transfer rates. In addition, the frying oil is taken up by the potato slices to a final oil content of approximately 35% for most industrial manufactured crisps [10].

The frying process as a function of time has been well described [11]. It has been noted that the residence time of frying determines the amount of oil absorbed by the potato slices; the longer the time, the greater the amount of oil absorbed. Literature information on the relation of cultivar, dry matter content, frying temperature and slice thickness to final oil content vary and to some extent is contradictory. In earlier investigation by Ufheil and Escher [10], an increase of fat content with decreasing initial dry matter, decreasing slice thickness, decreasing oil temperature and increasing frying time was found, the relative influence of the four factors being quite different.

Selection of appropriate variety and proper control of process parameters are very important in determining the oil uptake by crisps [12]. The present study was therefore designed to investigate the influence of potato cultivar, frying temperature and slice thickness on oil content and sensory quality of crisps processed from four Kenyan potato cultivars.

2. Materials and Methods

2.1 Potatoes for Processing

Two potato clones coded as 391,691.96 and 393,385.39 from the International Potato Center (CIP) and two varieties (Tigoni and Dutch Robyjn) were grown at the National Potato Research Center Tigoni (2,100 m above sea level) in the year 2009/10. These were grown under the standard cultural conditions [13]. After maturity, the crop was dehaulmed two weeks before harvesting. Then, the tubers were harvested and allowed to cure in a common dark store under ambient air conditions (17-22 °C /84-92% rh) for two weeks at the National Potato Research Center in Tigoni. They were then processed into crisps in Tigoni and the products analyzed for oil content in our laboratories in the Department of Food Science, Nutrition and Technology, University of Nairobi.

2.2 Potato Crisps Processing

Potato tubers were peeled, and sliced using an automatic electric slicer (Hitech Systems, Saudi Arabia) to uniform thicknesses of 1.0 mm, 1.5 mm and 2.0 mm. The slices were washed in cold water to remove surface starch, dried with a cloth towel and fried at a constant temperature of 170 °C for 3-5 min in an institution size, batch type, deep oil fryer (E 6 ARO S.A., La Neuveville, Switzerland) containing about 7 litres of "Cheff" corn oil (Premier Oil Mills Ltd., Nairobi, Kenya). For frying temperature evaluations, the potatoes for all cultivars were cut into a uniform thickness of 1.5 mm and triplicate samples of 100 g slices were fried in oil heated at temperatures of 160 °C, 170 °C and 180 °C for 2-5 min. The fried slices were removed and excess oil drained off for 1 min, placed on plates, cooled ready for evaluation.

2.3 Determination of Moisture Content

The moisture contents of the fresh tubers and processed products were determined by standard analytical method [14]. Triplicate samples of approximately 5 g were accurately weighed in aluminum

dishes and dried in an air-oven at 105 °C to constant weight. The dried samples were cooled in a dessicator to room temperature and weighed. Loss of weight due to drying was converted to percent moisture content.

2.4 Oil Content Determination

After drying, potato crisps were finely ground in a blender and triplicate 5 g samples were accurately weighed and placed into thimbles. They were extracted in 16-hr Soxhlet apparatus using analytical grade petroleum ether (boiling point 40-60 °C) as described by Lulai and Orr [15]. The petroleum ether was evaporated away in a rotary vacuum evaporator and the residual oil dried in an air-oven at 80 °C for 2 hrs. The weight of the residue was calculated as percent oil content.

2.5 Sensory Evaluation

For sensory evaluation, coded samples were presented to 20 panelists, all familiar with potato crisps. Panel members scored for flavor, oiliness and overall acceptability on a 7-point hedonic rating scale varying from 1 (dislike very much) to 7 (like very much). A score of 4 was the lower limit of acceptability [16].

2.6 Data Analysis

All these experiments were replicated three times, and the average values are reported. Analysis of variance (ANOVA) and least significant difference test for the variables were conducted using the Statistical Analysis System (SAS version 9). Correlation analysis was performed to determine linear relationship between tuber dry matter content and oil content, and also between oil content determined in laboratory and sensory scores. Differences at $P \leq 0.05$ were considered significant.

3. Results and Discussion

3.1 Influence of Tuber Dry Matter on Oil Content Correlation of Dry Matter

Influence of tuber dry matter content on oil content of crisps is illustrated in Fig. 1. Tuber dry matter differed significantly ($P \leq 0.05$) among the cultivars. Dry matter content ranged from 20.99% in clone 391,691.96 to 25.29% in variety Dutch Robyjn. Crisps made from the potato tubers differed significantly ($P \leq 0.05$) in oil content which ranged from 35.12% Dutch Robyjn to 36.52% in clone 391,691.96. Tuber dry matter content was found to be negatively correlated to oil content of crisps; oil uptake increased with decrease in tuber dry matter. The range of dry matter content, 20.99% to 25.29%, in potato tubers falls within recommended levels for crisps processing in Kenya and East Africa [12]. On the basis of dry matter content as a selection criterion, therefore, all the cultivars were suitable for crisps processing. Potato tuber dry matter content is a very important characteristic in determining suitability of cultivars for processing into crisps.

Significantly ($P \leq 0.05$) more oil was absorbed in crisps processed from tubers of clone 391691.96 compared to Dutch Robyjin due to variation in dry matter contents. There was a significant ($P \leq 0.05$) correlation between the oil content of crisps and dry matter content of potato tubers. The regression line $Y = -3.123X + 135.16$ was developed to estimate oil content of crisps (Y) on the basis of dry matter content (X) in a potato tuber. In earlier studies of oil uptake by potato crisps, Gravoueille [17] noted that dry matter was a major factor for the potato processing industry, and that it was required to be between 23% and 25% to minimize the oil uptake and improve yields.

Ufheil and Escher [10] found a close relationship between dry matter and oil content of potato crisps; oil content decreasing with increase in dry matter. Potatoes with high dry matter (> 20%) have been shown to produce high yield of French fries with lower oil content than those of lower dry matter [3]. Hagenimana et al. [18] working on sweet potatoes, found a linear relationship between dry matter content and oil uptake in thin sliced crisps, with oil content decreasing with increase in dry matter. Tubers with high dry matter generally give higher yields of crisps,

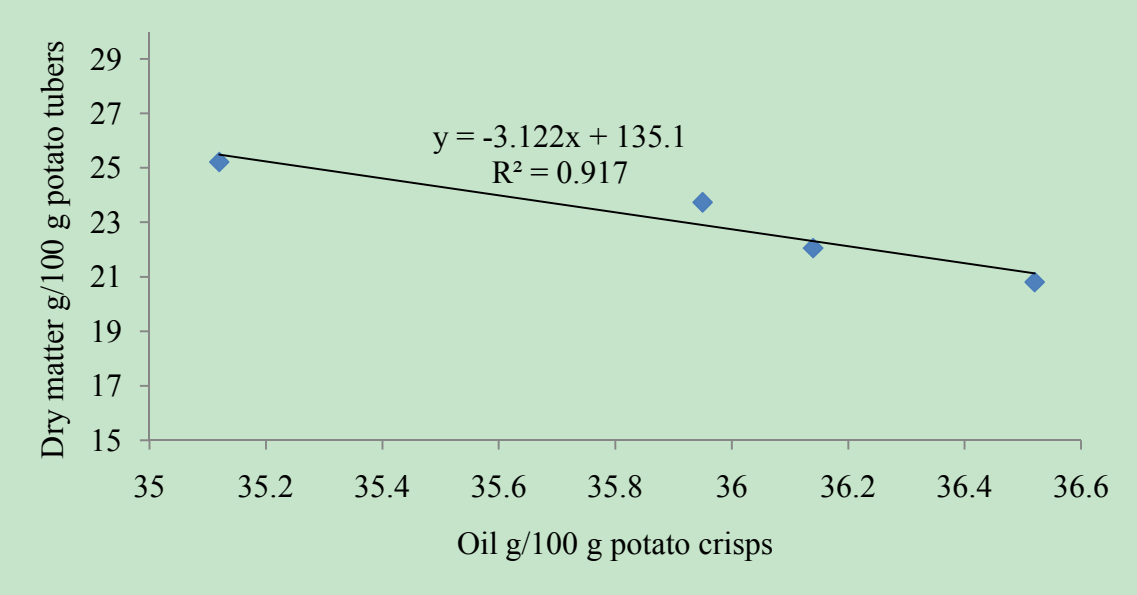

Fig. 1 Correlation between dry matter in raw potato cultivars and oil content of crisps.

have lower oil absorption and therefore are more economical to process [19].

3.2 Effects of Frying Temperature and Slice Thickness on Oil Content

Table 1 shows variation of oil content of crisps processed from the four potato cultivars, depending on the frying temperature.

For each cultivar, the oil content of crisps fried at different temperatures differed significantly ($P \leq 0.05$). There was also significant ($P \leq 0.05$) interaction in oil uptake between cultivar and frying temperature. The oil content was significantly ($P \leq 0.05$) higher at 160 °C than at 170 °C and 180 °C, respectively. The oil content decreased with increase in frying temperature. On average, the oil content decreased by 2.2% between 160 and 170 °C, and also between 170 and 180 °C, respectively. It therefore indicates that there was a decrease of about 4% of oil absorption by the potato cultivars when temperature was increased from 160 °C to 180 °C. These results slightly compare to the findings of Kita et al. [2] who observed that with every 20 °C increase in frying temperature, oil absorption was reduced by 3%, on average, irrespective of the type of oil used in frying crisps.

Table 1 Oil content of crisps, depending on frying temperature.

Cultivar	Frying temperature		
	160 ℃	170 ℃	180 ℃
391,691.96	38.42 ± 0.00 a	36.51 ± 0.01 a	34.32 ± 0.02 a
393,385.39	38.14 ± 0.08 a	36.13 ± 0.07 a	34.03 ± 0.09 a
Dutch Robyjn	37.12 ± 0.06 b	35.12 ± 0.05 b	33.08 ± 0.06 b
Tigoni	38.15 ± 0.04 a	35.95 ± 0.04 ab	33.45 ± 0.03 b

Values with the same letters in the same column are not significantly different at $P \leq 0.05$;
Values are means of triplicate samples ± standard deviation.

Many factors have been reported to affect oil uptake by crisps including oil quality, frying temperature and duration, product shape, moisture content, solids content, coating agents and slice thickness [7, 8, 20, 21]. Ziaiifar et al. [9] agree that oil uptake is a complex phenomenon resulting from interactions between oil and products that undergo numerous physical, chemical, and structural transformations during frying. Frying temperature has been reported to be one of the most important factors affecting the quantity of fat absorbed by potato crisps [2]. In the present study the oil uptake by crisps was significantly ($P \leq 0.05$) correlated with the frying temperatures among the cultivars; the oil content was significantly higher at 160 °C than at 170 °C and 180 °C,

respectively. This behavior agrees with results of similar study by Moyano and Pedreschi [22], who worked on a potato variety Panda under different treatments and found that higher frying temperatures lead to lower oil absorption by crisps. Similar results were also reported by Pedreschi and Moyano [23]. Kita et al. [2] compared oil content of crisps fried at different temperatures in different vegetable oils and found oil uptake to reduce with increase in temperature irrespective of the type of oil. Crust formation on crisps occurs on the surface of the potato slice during frying and as it becomes thicker, resistance to evaporation water from the inner part of the crisp increases. The rate of evaporation is reduced after crust formation thereby reducing oil uptake. As frying temperatures increase, the rate of crust formation increases due to a higher rate of evaporation which in turn decreases the amount of oil absorbed by crisps [24].

Variation in amount of oil absorbed by crisps with slice thickness is summarized in Table 2. For each cultivar, the oil content of crisps differed significantly ($P \leq 0.05$) with slice thickness. There was significant ($P \leq 0.05$) interaction in oil uptake between cultivar and slice thickness. The oil content was significantly ($P \leq 0.05$) higher in 1.0 mm thick slices than in 1.5 mm and 2.0 mm thick slices; the amount of oil absorbed decreased with increase in slice thickness. Clone 391,691.96 and Dutch Robyjn were most affected by slice thickness variation. Increasing slice thickness from 1.0 mm to 1.5 mm decreased the amount of oil absorbed by 10% on average, while from 1.5 mm to 2.0 mm it decreased by about 4 %. There was therefore a large decrease (14%) in oil absorbed by the potato cultivars when slice thickness was increased from 1.0 mm to 2.0 mm.

Potato crisps vary in slice thickness depending on the processors and countries. In United Kingdom, for example, crisps are very thin pieces (1.27-1.78 mm thick) of sliced raw potatoes that are fried to a final oil content of 33-38 g/100 g (wet basis) [22]. In Kenya,

Table 2 Oil content of crisps, depending on slice thickness.

Cultivar	Slice thickness		
	1.0 mm	1.5 mm	2.0 mm
391,691.96	48.54 ± 0.04 a	36.52 ± 0.00 a	33.52 ± 0.17 a
393,385.39	44.64 ± 0.15 c	36.14 ± 0.08 a	33.27 ± 0.26 a
Dutch Robyjn	46.49 ± 0.01 b	35.12 ± 0.06 b	29.80 ± 2.36 c
Tigoni	43.01 ± 0.55 d	35.95 ± 0.04 ab	32.23 ± 0.25 b

Values with the same letters in the same column are not significantly different at $P \leq 0.05$;
Values are means of triplicate samples ± standard deviation.

slice thickness is regulated to range from 1.0 mm to 1.5 mm [25]. It therefore means that processing any of the four cultivars into crisps with slices of 1.0 mm thickness will produce crisps with higher oil content than the required maximum 40% as stipulated by Kenya Bureau of Standards [25]. Oil content and surface area of crisps have been well related; the larger the surface area, the higher the oil content of the slices [26]. Thinner slices have been shown to absorb significantly more frying oil compared to large slices due to their larger surface area to volume ratio [2]. Baumann and Escher [16] also noted that slice thickness is inversely related to oil uptake since most oil in crisps is deposited on the surface; the surface area to volume ratio and specific surface for oil absorption decreases with increase in slice thickness.

3.3 Sensory Quality Characteristics of Crisps

Effect of frying temperature on flavor, oiliness and overall acceptability for crisps processed from 4 cultivars is presented in Table 3.

Crisps from all the 4 cultivars were acceptable to the panelists notwithstanding the frying temperature. Significant ($P \leq 0.05$) differences were noted in flavor and overall acceptability with variations in temperature among the cultivars. Scores on flavor did not show an apparent pattern as compared to overall acceptability in which case there were higher scores in crisps fried at 180 °C. Analysis of variance for the sensory attributes for each separate frying temperature indicated that there were significant ($P \leq 0.05$) differences in scores for all the evaluated attributes of

crisps made from different cultivars. In oiliness, there was a significant ($P \leq 0.05$) higher score for crisps processed at 180 °C than at 170 and 160 °C, respectively. The highest score for oiliness was observed in Dutch Robyjn (4.9), while clone 391,691.96 had the lowest score (4.2). Significant correlation ($P \leq 0.05$, r = -0.834) between oil content as determined in the laboratory and sensory scores on oiliness were observed in crisps indicating that the amount of oil absorbed by a cultivar would be detected by the consumer and hence influence the score; higher score for crisps of low oil content.

Effect of slice thickness on sensory quality characteristics of flavor, oiliness and overall acceptability for crisps processed from 4 potato cultivars are presented in Table 4.

Crisps from all the 4 cultivars were acceptable to the panelists. Significant ($P \leq 0.05$) differences were noted in flavor and overall acceptability with variations in slice thickness among crisps made from the cultivars. Except in cv. 393,385.39, scores on overall acceptability were higher in slice thickness of 1.0 mm

Table 3 Sensory quality characteristics of crisps, depending on frying temperature.

Cultivar	Frying temperature/℃	Flavor	Oiliness	Overall acceptability
Dutch Robyjn	160	5.5 ± 1.44 a	4.5 ± 0.67 cb	5.3 ± 0.96 a
	170	5.2 ± 0.70 ab	4.8 ± 0.62 b	5.2 ± 0.72 a
	180	5.3 ± 0.86 ab	5.5 ± 0.90 a	5.6 ± 0.90 a
393,385.39	160	5.4 ± 0.79 a	4.4 ± 1.24 bc	4.9 ± 1.24 b
	170	4.5 ± 0.52 b	4.9 ± 1.24 b	5.2 ± 0.83 a
	180	5.4 ± 1.38 a	5.4 ± 1.34 a	5.3 ± 1.22 a
391,691.96	160	4.2 ± 0.72 c	3.8 ± 1.03 d	4.3 ± 1.15 c
	170	4.4 ± 1.00 cb	4.4 ± 1.24 bc	4.9 ± 0.90 b
	180	4.2 ± 1.11 c	4.4 ± 1.31 bc	4.0 ± 0.85 c
Tigoni	160	4.3 ± 0.65 c	3.6 ± 0.49 d	4.8 ± 1.05 b
	170	4.9 ± 1.08 b	4.1 ± 0.51 c	4.5 ± 1.31 c
	180	5.1 ± 1.08 ab	5.5 ± 0.79 a	5.6 ± 1.24 a

Evaluation was done on 7-point hedonic scale. A score of 4 was the acceptable lower limit. All figures are mean ± standard deviation.
[3]Values with the same letters in the same column are not significantly different at $P \leq 0.05$.

Table 4 Sensory quality characteristics of crisps, depending on slice thickness.

Cultivar	Slice thickness	Flavor	Oiliness	Overall acceptability
Dutch Robyjn	2.0 mm	5.4 ± 1.08 a	5.6 ± 1.24 a	5.3 ± 0.98 ab
	1.5 mm	5.0 ± 1.35 ab	5.3 ± 1.30 ab	5.5 ± 1.17 a
	1.0 mm	5.1 ± 1.16 ab	5.3 ± 1.05 ab	5.9 ± 0.67 a
393,385.39	2.0 mm	6.0 ± 0.60 a	6.1 ± 0.66 a	6.2 ± 0.39 a
	1.5 mm	5.2 ± 1.11 a	5.6 ± 0.90 a	5.8 ± 0.62 a
	1.0 mm	4.8 ± 1.11 b	5.0 ± 1.21 b	5.3 ± 0.96 ab
391,691.96	2.0 mm	5.1 ± 0.90 ab	5.6 ± 0.79 a	5.6 ± 0.90 a
	1.5 mm	5.8 ± 0.97 a	5.6 ± 1.08 a	5.8 ± 0.93 a
	1.0 mm	4.8 ± 0.75 b	5.6 ± 1.24 a	5.8 ± 0.75 a
Tigoni	2.0 mm	5.0 ± 1.44 ab	5.7 ± 0.98 a	5.2 ± 1.29 ab
	1.5 mm	5.5 ± 1.17 a	5.2 ± 1.27 b	5.5 ± 1.31 a
	1.0 mm	5.3 ± 1.07 a	5.8 ± 1.28 a	5.6 ± 1.00 a

Evaluation was done on 7-point hedonic scale. A score of 4 was the acceptable lower limit; All figures are mean ± standard deviation.
[3]Values with the same letters in the same column are not significantly different at $P \leq 0.05$.

than 1.5 and 2.0 mm. this may have been due to the brittle and crispy nature of small sliced crisps. Sensory scores on oiliness were significantly ($P \leq 0.05$) higher for crisps of slice thickness 2.0 mm and 1.5 mm in comparison to slices of 1.0 mm. This is an indicating that consumers are able to differentiate crisps, depending on the levels of absorbed oil as influenced by slice thickness.

4. Conclusion

Variety, frying temperature and slice thickness are important factors influencing oil uptake by crisps processed from all the four Kenyan potato cultivars studied. There is a need for processors to wisely choose the potato cultivar in order to produce crisps with desired oil content. All the four cultivars produced acceptable crisps, especially when processed at moderately higher temperature (170-180 °C) and slice thickness (1.5-2.0 mm) that would ensure moderate retention of oil and hence much lower oil contents of crisps.

Acknowledgments

The authors wish to acknowledge the financial and technical support from the National Potato Research Centre (KARI) and DAAD. The roles played by potato breeders; Dr. J. Landeo (CIP), Mr. S. Nderitu, Mr. J. Karinga and Mr. J. Onditi (NPRC) who ensured that the potato materials were available are acknowledged. Appreciation goes to the Food Science Department team at KARI-Tigoni led by Mrs. N. Ngone and Mr. K. Bethuel who offered overwhelming support during the sample preparation and evaluation. Laboratory analysis was accomplished with assistance from Mrs. A. Kimende, Mr. A. Sikuku, Mr. J. M'Thika and Ms. R. Kamau, Laboratory Technicians, University of Nairobi.

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Journal of Agricultural Science and Technology A 1 (2011) 164-169
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Single Nucleotide Polymorphism Genotyping of Calpastatin Gene Using the ARMS Compared with the RFLP

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Received: August 12, 2010 / Published: June 20, 2011.

Abstracts: Calpastatin is an endogenous inhibitor of calpain which is responsible for the breakdown of myofibrillar proteins. The association of Single Nucleotide Polymorphism (SNP) in the calpastatin gene with meat tenderness is an important topic in meat production. Therefore efficient procedure to investigate the SNP is necessary. The objectives of this study were to detect the SNP of calpastatin gene at domain L marker (G/C transversion) of the Kamphaengsaen beef breed (KPS cattle; n = 26) by the Amplification Refractory Mutation System (ARMS) compared with the Restriction Fragment Length Polymorphism (RFLP) methods and to determine the genotypes of the KPS cattle at that marker. Genomic DNA of calpastatin gene extracted from blood of the KPS cattle was detected with ARMS and RFLP methods. The ARMS system has utilized two primer pairs to amplify the two different alleles of a polymorphism in single PCR reaction to detected single base mutation. In this method, the alleles-specific primers had a mismatch at 3′ terminal base and a second deliberate mismatch at position -2 from 3′ terminus. While the RFLP method detected a polymorphism using PCR-base technique follow by *Rsa*I restriction enzyme. Amplification of the ARMS method revealed that the results were not different from the conventional method of RFLP. Analysis of genotypes revealed that the KPS cattle inherited the CC, CG and GG genotypes at domain L marker. These were reliable when verified by nucleotide sequence analysis of PCR products. The animals were genotyped and determined for tenderness phenotype with this marker that predicted variation of an intronic polymorphism at domain L of the calpastatin gene. Therefore, the ARMS method was simple, efficient technique, and suitable for detecting SNP at domain L marker of the calpastatin gene.

Key words: Single Nucleotide Polymorphism (SNP), Amplification Refractory Mutation System (ARMS), Restriction Fragment Length Polymorphism (RFLP), calpastatin gene, meat tenderness.

1. Introduction

Beef tenderness is the major component of palatability because it has a major impact on consumer satisfaction. Difficulty in availability of phenotypic data until slaughter makes genetic polymorphism study suitable for increasing the accuracy of selection and improves rates of genetic progress for meat tenderness [1, 2].

Calpastatin is a specific inhibitor of μ-calpain and m-calpain proteases (EC.3.4.22.17) and regulates postmortem proteolysis. Increasing of its activity

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postmortem is highly related with reduction of meat tenderness [3]. The calpastatin molecule contains a unique N-terminal region (domain L) and four homologous C-terminal regions (domain 1-4). Each of the four repeating units is responsible for the inhibition of calpain and the function of domain L remains not clear [4, 5]. However, the region of domain L containing amino acid residues 54-64 (EGKPKEHTEPK) regulates the Ca^{2+} channel-activating function [6].

Recently, Schenkel et al. [7] reported that a single nucleotide polymorphism (SNP) was identified in the calpastatin gene at domain L of beef. The calpastatin SNP was associated with shear force measures across days of postmortem ageing and related to increase the tenderness of Longissimus dorsi muscle. Observed genotypes were segregated in G/C substitution. Genotype CC had more tender than GG and CG had an intermediate tenderness. This polymorphism has been genotyped using the restriction fragment length polymorphism (PCR-RFLP) technique. However, a point mutation may create or destroy a restriction enzyme recognition site, PCR products may (or may not) be cleaved when conducted with the restriction enzyme [8].

The amplification refractory mutation system (ARMS) procedure is simple, reliable and rapid. The system is a PCR-base method of detecting single base mutations. ARMS utilizes two primer pairs to amplify the two different alleles of any point mutation polymorphism in a single PCR reaction [9]. The primer is synthesized in two forms, the "normal" form is refractory to PCR on "mutant" template DNA and the "mutant" form is refractory to PCR on "normal" DNA. The oligonucleotides with a mismatched 3′ residue will not function as primers in the PCR under appropriate conditions [8]. Both of ARMS and PCR-RFLP techniques were equally reliable to detect the mutation. However, the ARMS technique was found to be more rapid and economical than the PCR-RFLP technique [10]. Therefore, ARMS technique can be successfully identified any mutations in livestock populations such as the ryanodine receptor mutation (RYR1); influences the rate of pH fall by favoring the calcium release in muscle cells [11], melanocortin-4 receptor (MC4R); an important mediation of leptin's effects on food intake and body weight [12] and diacylglycerol acyltransferase (DGAT); plays a major role in triacylglycerol synthesis [13].

The objectives of this study were to detect the SNP of calpastatin gene at domain L marker of the Kamphaengsaen beef breed (KPS cattle) by the tetra-primer ARMS-PCR approach compared with PCR-RFLP methods and to determine the genotypes of the KPS cattle at that marker.

2. Materials and Methods

2.1 DNA Isolation and Primer Design

The animal used in this study was the crossbred cattle in Thailand, that is the Kamphaengsaen beef breed or KPS cattle. The KPS cattle was developed using the Charolais breed (*Bos Taurus*) top crossing to the crossbred (*Bos indicus*) which is a Brahman cross with a Thai native cattle. It was established by the Department of Animal Science, Kasetsart University, Thailand. The blood samples of 26 KPS cattle from the Kasetsart University Kamphaengsaen Campus Beef Farmers Cooperative and Prucsasri Farm in Nakhon Pathom province were investigated. The genomic DNA was extracted from blood sample according to a DNA extraction method from Nelson and Krawetz [14]. All samples were genotyped for the calpastatin SNP using ARMS and RFLP methods.

The primers were designed using sequence base on the reported sequence (GenBank accession number AY008267: exons 5, 6 and partial coding sequence), corresponding to domain L of calpastatin nucleotide sequence. The ARMS approach has utilized two primer pairs (P2-P3-P4-P5 primers; Fig. 1 and Table 1) to amplify the two different alleles of a polymorphism in single PCR reaction to detected single base mutation. In this method, we are introducing the alleles-specific

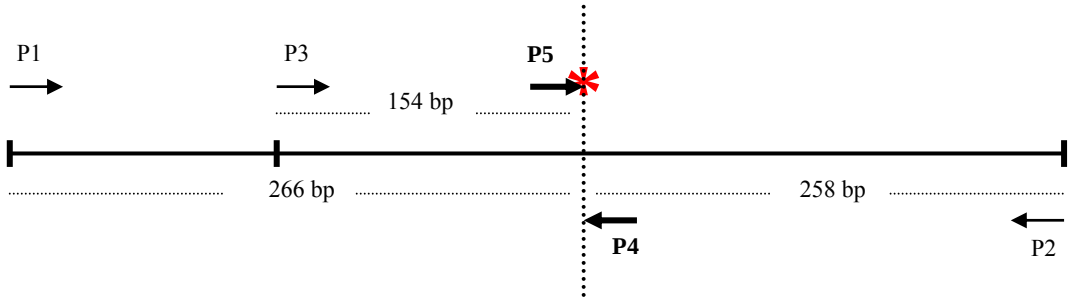

Fig. 1 Diagram of marker-specific primer of genomic calpastatin gene. The position of the SNP indicated by a star.

Table 1 Marker-specific primer sequences of genomic calpastatin gene*.

Method	Primer	Primer sequences (5′-3′)	Amplicon size
		forward primer: (adapted from Schenkel et al. [7])	
RFLP	P1	AGA AGT AAA GCC AAA GGA ACA CAC AGA	524 bp
		reverse primer: (Schenkel et al. [7])	
	P2	ATT TCT CTG ATG GTG GCT GCT CAC T	
		forward inner primer:	
ARMS	P5	GAA GGA ATT GCA TTG TTT CAA ATT **G**TC	258 bp (C allele)
		reverse inner primer:	
	P4	ATG TGA CAA ATT TCA CTT TGG G**C**A **C**	154 bp (G allele)
		forward outer primer:	
	P3	GAA CTC TCA TCT TTC AAC ACT TAA G	412 bp
		reverse outer primer:	(from 2 outer- primers)
	P2	ATT TCT CTG ATG GTG GCT GCT CAC T	

* The mismatched bases are shown underline.

primers which had a mismatch at 3′ terminal base of primer P4 and a second deliberate mismatch at position-2 from 3′ terminus of P4 and P5 primers to improve in some incident of a single 3′ mismatched base thus allow amplification to proceed.

2.2 Amplification Refractory Mutation System (ARMS) Method

The amplification reaction was performed in a final volume of 100 μL containing 65.5 μL of distilled water, 10 μL of 10 × Taq buffer with $(NH_4)_2SO_4$, 8 μL of 25 m*M* $MgCl_2$, 2 μL of 10 m*M* dNTP, 1 μL of each P2, P3, P4 and P5 primer, 0.5 μL of *Taq* DNA polymerase (Fermentus) and 10 μL of DNA template. The PCR was carried out in a PTC-200 Peltier Thermal Cycler machine (MJ Research) using the following conditions which were 95 °C for (5 min) for the first cycle and 95 °C (30 s) for denaturation, 56.5 °C (30 s) for annealing, and 72 °C (30 s) for extension, with total of 40 cycles and 72 °C for (7 min) for final extension. Ethidium bromide stained DNA fragments were visualized on 1.8% agarose gels.

2.3 Restriction Fragment Length Polymorphism (RFLP) Method

The RFLP method detected a polymorphism by PCR-base technique. The PCR master mix was performed in a final volume of 100 μL containing 72.5 μL of distilled water, 10 μL of 10 × PCR buffer, 3 μL of 50 m*M* $MgCl_2$, 2 μL of 10 m*M* dNTP, 1 μL of each P1 and P2 primer, 0.5 μL of *Taq* DNA polymerase (Invitrogen) and 10 μL of DNA template. The PCR cycling conditions were 95 ℃ (5 min) for the first cycle and 95 ℃ (30 s) for denaturation, 62 ℃ (30 s)

for annealing, and 72 ℃ (30 s) for extension, with total of 35 cycles and 72 ℃ for (7 min) for final extension. The PCR reactions were performed on the PTC-200 Peltier Thermal Cycler.

Five microliter aliquots of the amplification products were digested with 1 μL of *Rsa*I (Fermentus), 1 μL of 10 × Buffer Tango^{TM} and 8 μL of distilled water at 37 ℃ (3 h). DNA fragments were separated on 1.8% agarose gel. The gel was stained with ethidium bromide and exposured to ultraviolet light.

2.4 Sequencing of Amplified DNA Fragments

The PCR products were sequenced by auto sequencing on the ABI PRISM model 3100 Genetic Analyzer by the Genome Institute. The nucleotide sequences were aligned with the sequences from GenBank accession number AY008267 and AY834770 using ClustalW Multiple alignment (1994) and BioEdit version 7.0.5.2 (1999).

3. Results and Discussion

Results for the calpastatin SNP at domain L of the KPS cattle from the one-tube method of ARMS analysis were separated into 3 genotypes (CC, CG and GG).

Two allele-specific amplicons were generated using two pairs of primers. One pair, P2-P5 was deliberated a mismatch at position -2 from 3′ terminus (Table 1), proceed an amplicon representing the CC genotype (Fig. 2A) with the expected 258 bp product. While another expected 154 bp product was generated an amplicon representing the GG genotype (Fig. 2A) when the primer pair, P3-P4, was introduced into a mismatch at 3′ terminal base and a deliberate mismatch at position -2 from 3′ terminus (Table 1). Besides, the ARMS detected both of 258 bp and 154 bp products representing the heterozygous CG genotype. However, the interactions between two outer primers, P2-P3, affected unwanted 412 bp product.

The use of the classical PCR-RFLP technique for the genotyping of the calpastatin SNP resulted in similar genotype findings as that from ARMS method. Amplified products of 524 bp were obtained by using P1-P2 primers. After genomic DNA was digested with recognizing sequence GT/AC of *Rsa*I at position 266 bp of the SNP (Fig. 1), revealed the GG genotype characterized by the presence of 266 and 258 bp fragments, GC heterozygous genotype characterized by 524, 266 and 258 bp fragments and CC genotype characterized by 524 bp fragments. The 266 and 258 fragments presented as overlaid under electrophoresis conditions conducted. Restriction fragment patterns of the KPS cattle samples were shown by Fig. 2.

Screening of the 26 samples revealed 14 CC genotypes, 9 CG genotypes and 3 GG genotypes. The accuracy of the calpastatin SNP at domain L of the KPS cattle genotyping by the ARMS and RFLP were confirmed through the direct sequencing of PCR

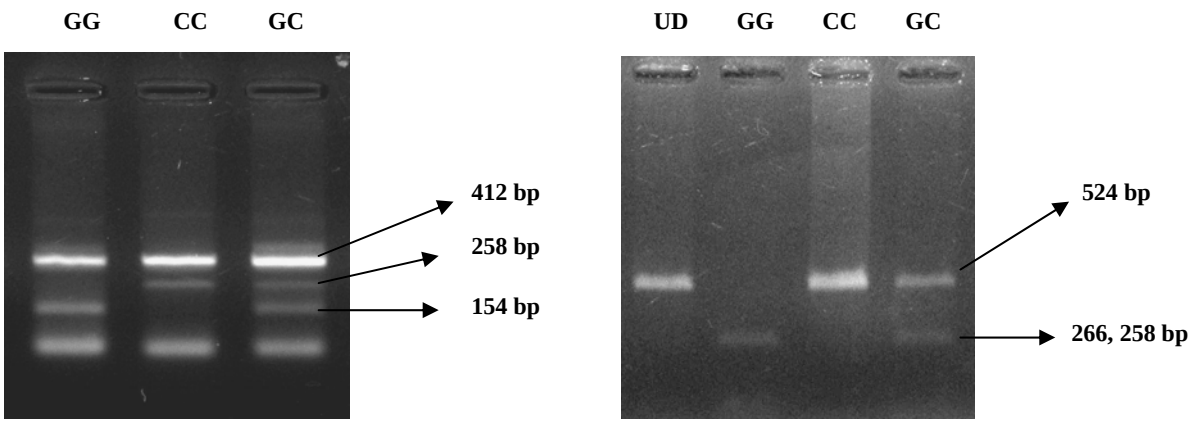

Fig. 2 Agarose gel showing calpastatin gene of the KPS cattle by ARMS (A) and RFLP digested with *Rsa*I (B). Lane UD = un-digested product.

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AY008267  AGCCTTTTTT TTTTT--CCC TTATTTTTGT GAAGGATAAA ATTTTGAACT CTCATCTTTC  58
AY834770  .......... .....TT... .......... .......... .......... ..........  60
KPS_1     .......... .....--... .......... .......... .......... ..........  58
KPS_2     .......... .....--... .......... .......... .......... ..........  58
KPS_3     .......... .....--... .......... .......... .......... ..........  58

AY008267  AACACTTAAG TCCTACCTAG AATGGCAGTT ATTTGTTTTT CTGTTAAAAC GGCACCTCTG  118
AY834770  .......... .......... .......... .......... .......... ..........  120
KPS_1     .......... .......... .......... .......... .......... ..........  118
KPS_2     .......... .......... .......... .......... .......... ..........  118
KPS_3     .......... .......... .......... .......... .......... ..........  118

AY008267  TGTGGCATCA GCAGGTATTG CAATTTGCTT GTGTGATTCT TGCTGAATTT GGAAGGAAGG  178
AY834770  .......... .......... .......... .......... .......... ...G......  180
KPS_1     .......... .......... .......... .......... .......... ...G......  178
KPS_2     .......... .......... .......... .......... .......... ...G......  178
KPS_3     .......... .......... .......... .......... .......... ...G......  178

                                          *
AY008267  AATTGCATTG TTTCAAATTT TCTACCCAAA GTGAAATTTG TCACATGTAA ATCATACTAA  238
AY834770  .......... .......... .......... .......... .......... ..........  240
KPS_1     .......... .......... .......... .......... .......... ..........  238
KPS_2     .......... .......... .G........ .......... .......... ..........  238
KPS_3     .......... .......... .S........ .......... .......... ..........  238
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Fig. 3 Nucleotide sequence alignment of the calpastatin gene from PCR amplification of the KPS cattle compared with the sequences from GenBank accession No. AY008267 and AY834770.
Denote conservation by plotting identities to a standard as a dot (.), deletion as a desh (-), and S = C or G. Position of polymorphisms indicated by a star.

products and compared with the sequences from the GenBank. The sequences of calpastatin gene from the KPS cattle corresponded to the GenBank accession number AY008267 and AY834770 that contained exons 5, 6 and partial coding sequence (Fig. 3).

These results indicated that the genotypes determined by the ARMS method were consistent with those determined by the classical restriction endonuclease digestion method and direct sequencing of PCR products.

Therefore, the ARMS method was an efficient technique and suitable for use to identify genotype at the domain L marker of the calpastatin SNP. Moreover, the one-tube technique suited for the routine analysis in order to predict meat tenderness since this developed manner was rapid, simple, inexpensive [8, 9] and avoided any inhibitor in this reaction.

4. Conclusion

Amplification of the ARMS method revealed that the results were not different from the conventional method of RFLP. Analysis of genotypes revealed that the KPS cattle inherited the CC, CG and GG genotypes at domain L marker. They were reliable when verified by nucleotide sequence analysis of PCR products. The animals were genotyped and determined tenderness phenotype for this marker that predicted variation of an intronic polymorphism at domain L of the calpastatin gene.

Acknowledgments

The authors thank Kasetsart University Kamphaengsaen Campus Beef Farmers Cooperative and Prucsasri Farm, Kamphaengsaen, Nakhon Pathom.

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Journal of Agricultural Science and Technology A 1 (2011) 170-181
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Soil Aggregates, Organic Matter, and Labile C and N Fractions after 37 Years of N, P and K Applications to an Irrigated Subtropical Soil under Maize-Wheat Rotation

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Received: August 23, 2010 / Published: June 20, 2011.

Abstract: Physical, chemical and biological soil properties in surface (0-5 cm) and subsurface soil (5-15 cm) were determined in a field experiment conducted with seven treatments consisted of different combinations of fertilizer N (0, 100 and 200 kg N ha^{-1}), P (0, 22 and 44 kg P_2O_5 ha^{-1}) and K (0, 41 and 82 kg K_2O ha^{-1}) applied both to summer-grown maize (*Zea mays* L.) and winter-grown wheat (*Triticum aestivum* L.) crops continuously for 37 years under irrigated subtropical conditions. Application of N, P and K significantly increased water stable aggregates and had profound effects in increasing the mean weight diameter as well as the formation of macro-aggregates, which were highest in both surface (81%) and subsurface (74%) soil layers with application of 100 kg N + 22 kg P_2O_5 + 41 kg K_2O ha^{-1} ($N_{100}P_{22}K_{41}$). The $N_{100}P_{22}K_{41}$ treatment also enhanced total organic C (TOC) from 4.4 g kg^{-1} in no-NPK control to 4.8 g kg^{-1} in surface layer and from 3.3 to 4.1 g kg^{-1} in subsurface layer leading to the 20% higher TOC stocks in 0-15 cm soil. The labile C and N fractions such as water soluble C, particulate and light fraction organic matter, potentially mineralizable N and microbial biomass were also highest under the optimized balanced application of $N_{100}P_{22}K_{41}$. Relatively higher increase in all labile fractions of C and N as proportion of TOC and total N, respectively suggested that these are potential indicators to reflect changes in management practices long before changes in TOC and TN are detectable. These results demonstrated that optimized balanced application of N, P and K is crucial for improving soil health ensuring long-term sustainability of farming systems in semiarid subtropical soils.

Key words: Total organic C, water stable aggregate, water soluble C, particulate and light fraction organic matter, potentially mineralizable N, microbial biomass C and N, soil health.

1. Introduction

Soil health, the capacity of the soil to promote growth of plants, protect watersheds by regulating the infiltration, partitioning of precipitation, and to prevent water and air pollution by buffering potential pollutants is largely determined by water stable aggregate (WSA) and soil organic matter (SOM). Soil aggregates, the basic units of soil structure composed of primary soil particles and binding agents, are formed and stabilized by SOM, organic materials and microorganisms [1, 2]. In turn, microbes and SOM are heterogeneously distributed across different sizes of soil aggregates. Therefore, WSA plays an important role in nutrient cycling and in supplying substrate for microbial processes that lead to structural stability. SOM, precisely estimated as total organic carbon (TOC), also acts as storehouse for the supply of nutrients to the growing crops.

However, labile organic matter fractions are readily accessible sources to microorganisms, turnover rapidly (weeks or months), and have direct impact on plant nutrient supply. Labile organic matter fractions generally include water soluble C (WSC), particulate organic matter (POM), light-fraction organic matter (LFOM), soil microbial biomass (SMB), and potentially mineralizable N (PMN). Microbial biomass

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C (MBC) and microbial biomass N (MBN) generally have higher mineralization rates than SOM. These labile fractions serve as both source and sink for mineral nutrients and organic substrates in a short-term, and as a catalyst for conversion of plant nutrients from stable organic form over a longer period, thereby influencing crop productivity and nutrient cycling. As these are more sensitive to management practices than TOC, they are potential indicators of soil health [3]. Since turnover rate of N in MBN is very rapid, it may reflect changes in management practices long before changes in TN are detectable [4].

In contrast to temperate climate where decomposition is slow, harsh hot and dry climate in semiarid, arid, subtropical and tropical environments causes fast decomposition. While the soils in these regions are inherently poor in SOM, the use of inadequate and imbalanced chemical fertilizers over a long period adversely affects soil health and productivity [5]. However, comparative effects of fertilizer N, P and K applied continuously to crops in semiarid subtropical soils have seldom been investigated. Such information is needed to identify crop nutrient management practices for sustaining or improving soil heath leading to the environmental safety, conservation of resources and the success of sustaining farming systems.

The objectives of the present study were to determine the effects of long-term and continued application of N, P and K fertilizers on several physical, chemical and biological properties of soil and their variations in surface and subsurface soil layers. The study was, therefore, undertaken to investigate the effect of different rates of N, P and K application for 37 years on soil aggregation, TOC and labile fractions of C and N in an irrigated subtropical soil under maize-wheat rotation.

2. Materials and Methods

2.1 Experimental Site and Treatments Details

A field experiment was initiated in 1970 to study the effect of various combinations of N, P and K under annual maize-wheat rotation on Fatehpur sandy loam soil (*Typic Haplustept*) in semiarid subtropical region at the Punjab Agricultural University Research Farm, Ludhiana, India, which is situated at 30°56′ N, 75°48′ E and 247 m above mean sea level. The subtropical region has summer and winter crop-growing seasons. While summer is characterized by high temperature and rainfall (monsoons), the winter is often dry with low temperature and is suitable for growing field crops such as wheat under irrigated conditions. The annual rainfall ranges from 600 to 1,200 mm of which 75-85% occurs during the July-September period.

Fatehpur soil is sandy loam in texture throughout the soil profile up to 150 cm with little decrease in sand and corresponding increase in silt as described earlier by Garg and Aulakh [6]. The soil (0-15 cm) characteristics at the beginning of study were: pH 8.2; electrical conductivity (1:2 soil water ratio), 0.11 dS m^{-1}; SOC, 3.6 g kg^{-1} and Olsen-P, 12 kg P ha^{-1}. The deep Fatehpur sandy loam soil is suitable for most of field crops including maize and wheat under irrigated conditions, and could produce high yields with proper management of fertilizers and irrigation.

The details of treatments of the field experiment have been reported earlier by Garg and Aulakh [6] and Brar et al. [7]. Briefly, seven treatments selected for the present study consisted of different combinations of fertilizer N (0, 100 and 200 kg N ha^{-1}), P (0, 22 and 44 kg P_2O_5 ha^{-1} equivalent to 0, 10 and 20 kg P ha^{-1}) and K (0, 41 and 82 kg K_2O ha^{-1} equivalent to 0, 34 and 68 kg K ha^{-1}) applied both to summer-grown maize (June-October) and winter-grown wheat (November-April) crops each year (Table 1). N, P and K were applied through urea, diammonium phosphate and muriate of potash, respectively. After pre-sowing irrigation, the individual plots were conventionally-tilled where the soil was tilled to a depth of 10-12 cm by one pass of disking followed by two passes with a tine cultivator and planking to create a well pulverized seed bed before seeding of each crop. The crops were

Table 1 Description of fertilizers treatments in maize-wheat field experiment.

Treatment No.	Treatments to maize	Treatments to wheat
T_1	$N_0P_0K_0$ †	$N_0P_0K_0$
T_2	$N_{100}P_0K_0$	$N_{100}P_0K_0$
T_3	$N_{200}P_0K_0$	$N_{200}P_0K_0$
T_4	$N_{100}P_{22}K_0$	$N_{100}P_{22}K_0$
T_5	$N_{200}P_{44}K_0$	$N_{200}P_{44}K_0$
T_6	$N_{100}P_{22}K_{41}$	$N_{100}P_{22}K_{41}$
T_7	$N_{200}P_{44}K_{82}$	$N_{200}P_{44}K_{82}$

†N = fertilizer N (kg N ha^{-1}); P = fertilizer P (kg P_2O_5 ha^{-1}); K = fertilizer K (kg K_2O ha^{-1}).

irrigated (7.5 cm) as and when required by taking into consideration the moisture received through rainfall. At harvesting of each crop at maturity, above-surface crop residues except stubbles were removed.

2.2 Soil Sampling and Analyses

In April 2007, after the completion of 37 cycles of maize-wheat rotation, representative soil samples were taken from 21 plots (7 treatments × 3 replications) by combining four soil cores taken with auger separately for 0-5 and 5-15 cm soil layers of each individual plot (8 m × 5 m size). In one portion of each sample, soil clods were hand-broken to 5-8 mm for estimation of WSA. Aggregate size distribution was determined by wet sieving method [8]. The soil clods were spread uniformly on the top most sieve of a nest of sieves having pore diameter 2, 1, 0.5, 0.25 and 0.11 mm. The nest of sieves was oscillated up and down by a pulley arrangement for 30 min at a frequency of 30 cycles per min in salt free water. The WSA of different sizes were collected and weighed from the respective sieves separately after oven drying the sieves at 50 ℃. Second portion of each soil sample was ground and passed through 2 mm sieve to use for determining TOC, POM-C, POM-N, LFOM-C and LFOM-N, MBC and MBN. The TOC content was determined by using Walkley and Black's rapid titration method [9] and computed using Eq. (1):

$$\text{TOC stock (Mg C ha}^{-1}) = \text{TOC content (g C kg}^{-1}) \times \text{Db (Mg m}^{-3}) \times \text{Soil layer (m)} \times 10 \quad (1)$$

Where, Db is bulk density of the particular soil layer (Db values for 0-5 cm and 5-15 cm soil layer were 1.52 and 1.53 Mg m^{-3}, respectively).

The WSC was determined by the method described by McGill et al. [10]. For the estimation of POM by the method of Cambardella and Elliott [11], 20 g air-dry soil was dispersed in 60 mL of sodium hexametaphosphate solution (5 g L^{-1}) by shaking on a reciprocal shaker for 18 h. The soil suspension was poured over a 53-μm sieve, and after rinsing several times with distilled water, the sand and organic material retained on the sieve was dried over night at 60 °C, weighed and ground to determine C [9] and N content by digestion in concentrated sulphuric acid followed by Kjeldahl's steam distillation method. For the estimation of LFOM by the method of Compton and Boone [12], 15 g air-dry soil was placed in a centrifuge tube with 30 mL $ZnBr_2$ having a density of 1.70 g cm^{-3}. The tubes were shaken by hand for 30 sec and then centrifuged at 1000 rpm for 15 min. The floating LFOM was siphoned off and placed on filter paper. The centrifugation-siphon process was repeated at least four times until all floating material was recovered. The floating material on the filter paper was washed with distilled water, dried for 48 h at 60 °C, weighed and ground to determine C and N content as described above.

PMN in soil was determined by the method described by Keeney [13], where 10 g air-dry soil was taken in a test tube with distilled water (1:2) and incubated for 7 days under waterlogged conditions at 40 °C. The mineralized NH_4^+-N was determined by the Kjeldahl's distillation method. The amount of PMN (mg NH_4^+-N kg^{-1} 7 d^{-1}) was determined by subtracting the concentration of NH_4^+-N at the beginning of incubation.

For the estimation of soil microbial biomass C and N by the chloroform fumigation and incubation method [14], soil moisture was adjusted to 55% field water capacity, pre-incubated at 25 °C for 7 days in the dark, and each soil sample was subdivided into two subsamples for fumigated and non-fumigated

treatments. For MBC, soil samples, equivalent to 30 g dry weight, were fumigated with $CHCl_3$ for 24 h at 25 °C. After removing the $CHCl_3$, each soil sample was incubated at 25 °C for a period of 10 days in closed tight Mason jar along with vials containing 1.0 mL 2 M NaOH. The flush of CO_2-C released upon fumigation was determined from titration with HCl. The MBC was computed using Eq. (2):

$$\text{MBC (mg kg}^{-1}) = (\text{Fc-UFc})/\text{Kc} \quad (2)$$

Where, Fc is CO_2 evolved from the fumigated soil, UFc is CO_2 evolved from the unfumigated soil, and Kc is a factor with value of 0.41 [15].

For MBN, fumigated and non-fumigated soil samples after 10-day incubation were extracted with 2 M KCl (5:1 ratio of extractant: soil) for 1 h and inorganic N was determined by the Kjeldahl distillation as described by Keeney and Nelson [16]. The MBN was computed using Eq. (3):

$$\text{MBN (mg kg}^{-1}) = (\text{Fn-UFn})/\text{Kn} \quad (3)$$

Where, Fn is mineral N from fumigated soil, UFn is mineral N from unfumigated soil, and Kn is a factor with value of 0.57 [17].

2.3 Statistical Analysis

Data on TOC, WSC, WSA, POM, PMN, LFOM, MBC and MBN for applied N, P and K treatments were analyzed statistically using randomized block design (RBD).

3. Results

3.1 Water Stable Aggregates

Total WSA in 0-5 cm and 5-15 cm soil layers of the 37-year maize-wheat experiment was 66% and 63%, respectively in no-fertilizer control ($N_0P_0K_0$), which increased in different NPK treatments (Fig. 1). Application of 100 and 200 kg N ha^{-1} alone in $N_{100}P_0K_0$ and $N_{200}P_0K_0$ treatments showed significant increase in total WSA content in 0-5 cm soil layer. Fertilizer P application @ 22 and 44 kg P_2O_5 ha^{-1} in $N_{100}P_{22}K_0$ and $N_{200}P_{44}K_0$ treatments further significantly increased the total WSA content over N treatments. However,

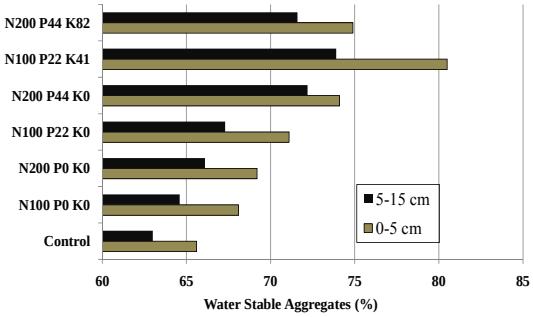

Fig. 1 Effect of different fertilizer treatments on water stable aggregates in 0-5 and 5-15 cm soil layers after 37-year maize-wheat rotation.
LSD ($P \geq 0.05$) for water stable aggregates (%) of 0-5 and 5-15 cm soil layers is 1.3 and 1.4, respectively.

application of K along with N and P showed an increase of only 9% and 1% in total WSA when applied @ 41 and 82 kg K_2O ha^{-1}. As compared to surface layer, total WSA decreased in 5-15 cm subsurface layer. The effects of NPK were similar to those in 0-5 cm layer but had relatively lower magnitude.

Table 2 shows distribution of aggregates in different size classes (> 2, 1-2, 0.5-1.0, 0.25-0.5 and 0.11-0.25 mm) at two soil depths. In all treatments, the proportion of macro-aggregates (0.25 to > 2 mm) was 1.8 to 2.6 fold higher than micro-aggregates (0.11- < 0.25 mm). Among the macro-aggregates, 0.25-0.50 mm fraction constituted the greatest proportion (21-25%) followed by 0.5-1 mm (13-16%), 1-2 mm (5-10%) and > 2 mm fraction (1-5%) in both soil layers. Balanced application of $N_{100}P_{22}K_{41}$ significantly improved total WSA and the macro-aggregate in both 0-5 cm and 5-15 cm soil layers. However, micro-aggregates were neither influenced by fertilizer treatments nor by soil depth. Mean weight diameter (MWD) ranged from 0.36 to 0.49 mm in the 0-5 cm soil depth and 0.35 to 0.43 mm in the 5-15 cm soil depth (Table 2). Again, the MWD was significantly higher in $N_{100}P_{22}K_{41}$ treatment plots in both the soil layers and decreased with soil depth.

3.2 Total Organic C, N and C/N Ratio

TOC content in 0-5 cm and 5-15 cm soil layers of different treatments was 4.4 g kg^{-1} and 3.3 g kg^{-1}, respectively in control and was further increased in

Table 2 Effect of different fertilizer treatments on soil aggregate size distribution, and mean weight diameter (MWD) in surface (0-5 cm) and subsurface (5-15 cm) soil layers in 37-year maize-wheat rotation.

Treatments	Macro-aggregates (%)				Total	Micro-aggregate (%)	MWD mm
	> 2 mm	1-2 mm	0.5-1.0 mm	0.25-0.5 mm		0.11- < 0.25 mm	
0-5 cm soil layer							
$N_0P_0K_0$†	1.4	5.4	15.0	21.4	43.2	22.5	0.36
$N_{100}P_0K_0$	2.0	5.6	16.0	22.8	46.3	21.8	0.38
$N_{200}P_0K_0$	2.7	7.5	15.4	22.7	48.3	20.8	0.41
$N_{100}P_{22}K_0$	3.8	9.0	15.8	22.3	50.9	20.3	0.45
$N_{200}P_{44}K_0$	4.4	10.2	16.4	22.8	53.8	20.4	0.47
$N_{100}P_{22}K_{41}$	5.4	10.4	16.4	24.8	57.1	23.5	0.49
$N_{200}P_{44}K_{82}$	5.4	10.0	15.5	22.2	53.1	21.8	0.48
Mean	3.6	8.3	15.8	22.7	50.4	21.6	0.43
LSD (0.05)	*0.5*	*0.9*	*ns**	*0.8*	*1.5*	*2.1*	*0.01*
5-15 cm soil layer							
$N_0P_0K_0$†	1.2	4.7	14.2	20.5	40.7	22.3	0.35
$N_{100}P_0K_0$	1.3	4.9	14.6	21.7	42.5	22.1	0.35
$N_{200}P_0K_0$	1.8	6.5	13.4	23.7	45.4	20.8	0.38
$N_{100}P_{22}K_0$	2.7	7.9	13.4	23.1	47.1	20.2	0.42
$N_{200}P_{44}K_0$	3.2	8.5	14.2	21.9	47.8	24.4	0.42
$N_{100}P_{22}K_{41}$	3.6	9.2	14.7	23.2	50.6	23.2	0.43
$N_{200}P_{44}K_{82}$	3.2	8.9	14.1	22.3	48.5	23.2	0.43
Mean	2.4	7.2	14.1	22.3	46.1	22.3	0.40
LSD (0.05)	*0.2*	*0.8*	*0.9*	*ns*	*1.9*	*1.8*	*0.01*

†N = fertilizer N (kg N ha^{-1}); P = fertilizer P (kg P_2O_5 ha^{-1}); K = fertilizer K (kg K_2O ha^{-1}).

**ns* = non-significant

different NPK treatments (Fig. 2). Application of 100 and 200 kg N ha^{-1} alone in $N_{100}P_0K_0$ and $N_{200}P_0K_0$ treatments increased TOC content by 0.1 and 0.3 g kg^{-1}, respectively in 0-5 cm soil layer (Fig. 2). Fertilizer P application @ 22 and 44 kg P_2O_5 ha^{-1} in $N_{100}P_{22}K_0$ and $N_{200}P_{44}K_0$ treatments increased the TOC content by 0.2 and 0.1 g kg^{-1} over $N_{100}P_0K_0$ and $N_{200}P_0K_0$ treatments, respectively. Similarly, application of K along with N and P showed an increase of 0.2 and 0.04 g kg^{-1} TOC when applied @ 41 and 82 kg K_2O ha^{-1}. TOC content was comparatively lower in subsurface layer (5-15 cm) than surface layer (0-5 cm) suggesting decrease in TOC content with soil depth. But only application of 200 kg N ha^{-1} significantly increased TOC in top soil, whereas in the subsoil layer, both 100 and 200 kg N ha^{-1} increased TOC significantly (Fig. 2).

TOC stock in 0-5 cm and 5-15 cm soil layers of $N_0P_0K_0$ plot was 3.3 Mg C ha^{-1} and 5.0 Mg C ha^{-1}, respectively and further increased in different NPK

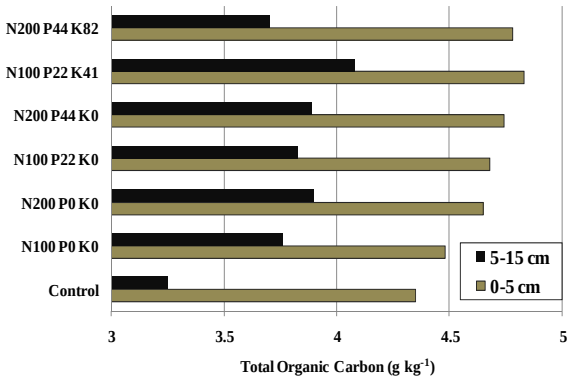

Fig. 2 Effect of different fertilizer treatments on total organic carbon content in 0-5 and 5-15 cm soil layers after 37-year maize-wheat rotation.

LSD ($P \geq 0.05$) for total organic C (g kg^{-1}) of 0-5 and 5-15 cm soil layers is 0.17 and 0.13, respectively.

treatments (Table 3). Application of 100 and 200 kg N ha^{-1} alone in $N_{100}P_0K_0$ and $N_{200}P_0K_0$ treatments increased TOC stock by 0.1 and 0.2 Mg C ha^{-1}, respectively in 0-5 cm soil layer. Whereas, fertilizer P application @ 22 and 44 kg P_2O_5 ha^{-1} in $N_{100}P_{22}K_0$ and $N_{200}P_{44}K_0$ treatments increased the TOC stock by 0.2

Table 3 Effect of different fertilizer treatments on TOC stocks, C/N ratio, water soluble carbon (WSC), and proportion of WSC in TOC content (%) in 0-5 cm and 5-15 cm soil layers after 37-year maize-wheat rotation.

Treatments	TOC stock (Mg C ha^{-1})			C/N ratio[#]		WSC (mg kg^{-1})		WSC (% of TOC)	
	0-5 cm	5-15 cm	Total	0-5 cm	5-15 cm	0-5 cm	5-15 cm	0-5 cm	5-15 cm
$N_0P_0K_0$[†]	3.31	4.97	8.28	6.5	6.3	23.0	17.3	0.53	0.53
$N_{100}P_0K_0$	3.40	5.75	9.15	6.4	6.2	29.1	21.0	0.65	0.56
$N_{200}P_0K_0$	3.54	5.96	9.50	6.3	6.1	31.1	22.6	0.67	0.58
$N_{100}P_{22}K_0$	3.56	5.86	9.42	6.2	5.7	31.9	24.1	0.68	0.63
$N_{200}P_{44}K_0$	3.60	5.95	9.55	6.1	5.3	35.0	23.7	0.74	0.61
$N_{100}P_{22}K_{41}$	3.67	6.24	9.91	6.0	5.6	32.4	23.6	0.67	0.58
$N_{200}P_{44}K_{82}$	3.64	5.66	9.30	5.9	4.7	35.8	24.5	0.75	0.66
Mean	3.53	5.77	9.30	6.2	5.6	31.2	23.5	0.67	0.59
LSD (0.05)	*0.13*	*0.20*	*0.25*	-	-	*0.67*	*0.68*	-	-

[†]N = fertilizer N (kg N ha^{-1}); P = fertilizer P (kg P_2O_5 ha^{-1}); K = fertilizer K (kg K_2O ha^{-1}).

[#] Ratio of TOC content to total N content.

and 0.1 Mg C ha^{-1}, over $N_{100}P_0K_0$ and $N_{200}P_0K_0$ treatments, respectively. Similarly, application of K along with N and P showed an increased TOC stock by 0.1 and 0.04 Mg C ha^{-1} when applied @ 41 and 82 kg K_2O ha^{-1}. TOC stock in 5-15 cm subsurface layer was higher than 0-5 cm layer because of higher soil mass (soil thickness was 10 cm in 5-15 cm layer as compared to 5 cm in 0-5 cm layer). Overall TOC stock in 0-15 cm soil layer ranged from 8.3 to 9.9 Mg C ha^{-1} and out of it, 37 to 40% was present in surface 5 cm layer and 60 to 63% in next 10 cm layer.

The TN content ranged from 665 to 817 mg kg^{-1} in 0-5 cm and 513 to 782 mg kg^{-1} in 5-15 cm soil layer, indicating increase due to different NPK fertilizer treatments (Fig. 3). In 0-5 cm layer, the C/N ratio was 6.5 in control, which became lower with the application of fertilizers and was 5.9 with $N_{200}P_{44}K_{82}$ treatment (Table 3). Similarly in 5-15 cm layer, the C/N ratio was lowered from 6.3 in control to 4.7 with $N_{200}P_{44}K_{82}$ treatment.

3.3 Water Soluble C

WSC content in 0-5 cm and 5-15 cm soil layers after 37-year of maize-wheat experiment was 23.0 mg kg^{-1} and 17.3 mg kg^{-1}, respectively in control and was further increased in different NPK treatments (Table 3). Application of 100 and 200 kg N ha^{-1} alone in $N_{100}P_0K_0$ and $N_{200}P_0K_0$ treatments significantly increased WSC content in 0-5 cm soil layer. Fertilizer P application @

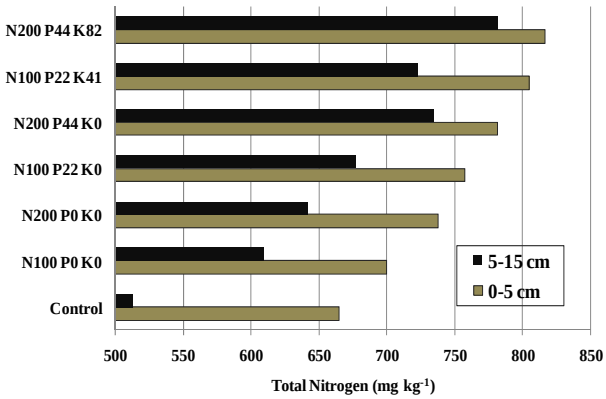

Fig. 3 Effect of different fertilizer treatments on total nitrogen content in 0-5 and 5-15 cm soil layers after 37-year maize-wheat rotation.

LSD ($P \geq 0.05$) for total N (mg kg^{-1}) of 0-5 and 5-15 cm soil layers is 54 and 47 mg kg^{-1}, respectively.

22 and 44 kg P_2O_5 ha^{-1} in $N_{100}P_{22}K_0$ and $N_{200}P_{44}K_0$ treatments further significantly increased the WSC content over $N_{100}P_0K_0$ and $N_{200}P_0K_0$ treatments, respectively. Similarly, application of K along with N and P showed relatively a small increase of 0.5 and 0.8 mg kg^{-1} WSC content when applied @ 41 and 82 kg K_2O ha^{-1}. In 5-15 cm subsurface layer, the effects of NPK on WSC were similar to those in 0-5 cm layer but had relatively lower magnitude (Table 3). These results also revealed that WSC content decreased with soil depth, and thin surface layer (0-5 cm) contained much higher WSC content than 5-15 cm soil layer. WSC accounted for 0.5 to 0.8% and 0.5 to 0.7% of TOC in 0-5 cm and 5-15 cm soil layer, respectively (Table 3).

The enhanced proportion of WSC indicated that the increase in WSC with the application of NPK fertilizers was relatively more than TOC, especially in 0-5 cm surface layer.

3.4 Particulate Organic Matter C and N

POM-C content in 0-5 cm and 5-15 cm soil layers of 37-year maize-wheat experiment was 0.8 and 0.6 g kg^{-1}, respectively in control, which further increased in different NPK treatments (Table 4). Application of 100 and 200 kg N ha^{-1} alone in $N_{100}P_0K_0$ and $N_{200}P_0K_0$ treatments significantly increased POM-C content in 0-5 cm soil layer. Fertilizer P application @ 22 and 44 kg P_2O_5 ha^{-1} in $N_{100}P_{22}K_0$ and $N_{200}P_{44}K_0$ treatments further significantly increased the POM-C content over $N_{100}P_0K_0$ and $N_{200}P_0K_0$ treatments, respectively. In 5-15 cm subsurface layer, the effects of NPK on POM-C were similar to those in 0-5 cm layer but had relatively lower magnitude (Table 4). As was noted for TOC and WSC, POM-C content also decreased with soil depth, and thin surface layer (0-5 cm) contained higher POM-C content than 5-15 cm layer. For different NPK fertilizer treatments in 0-5 cm and 5-15 cm soil layer, POM-C accounted for 17 to 27% and 17 to 26% of TOC content, respectively (Table 4). The enhanced proportion of POM-C indicated that increase in POM-C with the application of NPK fertilizers was relatively more than TOC.

POM-N content in 0-5 cm and 5-15 cm soil layers after 37 cycles of maize-wheat rotation was 22 and 18 mg kg^{-1}, respectively in control, which further increased in different NPK treatments (Table 4). Application of 100 and 200 kg N ha^{-1} alone in $N_{100}P_0K_0$ and $N_{200}P_0K_0$ treatments significantly increased POM-N content in 0-5 cm soil layer. Fertilizer P application @ 22 and 44 kg P_2O_5 ha^{-1} in $N_{100}P_{22}K_0$ and $N_{200}P_{44}K_0$ treatments further significantly increased the POM-N content over $N_{100}P_0K_0$ and $N_{200}P_0K_0$ treatments, respectively. Application of K along with N and P showed an increase of 10 and 23 mg kg^{-1} POM-N content when applied @ 41 and 82 kg K_2O ha^{-1}. In 5-15 subsurface layers, the effects of NPK on POM-N were similar to those in 0-5 cm layer but had relatively lower magnitude (Table 4). These results also revealed that POM-N content decreased with depth. For different fertilizer treatments in 0-5 cm and 5-15 cm soil layer, POM-N accounted for 3 to 17% and 3 to 13% of TN content, respectively, indicating the increase in the proportion of POM-N with the application of NPK fertilizers.

3.5 Light Fraction Organic Matter C and N

LFOM-C content in 0-5 cm and 5-15 cm soil layers was 52 and 38 mg kg^{-1}, respectively in control and was further increased due to different NPK treatments (Table 5). Application of 100 and 200 kg N ha^{-1} alone in $N_{100}P_0K_0$ and $N_{200}P_0K_0$ treatments significantly increased LFOM-C content in 0-5 cm soil layer. Fertilizer P application @ 22 and 44 kg P_2O_5 ha^{-1} in $N_{100}P_{22}K_0$ and $N_{200}P_{44}K_0$ treatments increased the LFOM-C content by 9 and 12 mg kg^{-1}, over $N_{100}P_0K_0$ and $N_{200}P_0K_0$ treatments, respectively. In 5-15 cm subsurface layer, the effects of NPK on LFOM-C were similar to those in 0-5 cm layer but had relatively lower magnitude (Table 5). These results also revealed that LFOM-C content decreased with depth and thin surface layer (0-5 cm) contained higher LFOM-C content than 5-15 cm soil layer. For different fertilizer treatments in 0-5 cm and 5-15 cm soil layers, LFOM-C accounted for 1 to 4% of TOC (Table 5) indicating the increase in the proportion of LFOM-C in TOC with the application of NPK fertilizers.

LFOM-N content in 0-5 cm and 5-15 cm soil layers was 6 and 5 mg kg^{-1}, respectively, which further increased in different NPK treatments (Table 5). Application of 100 and 200 kg N ha^{-1} alone in $N_{100}P_0K_0$ and $N_{200}P_0K_0$ treatments significantly increased LFOM-N content in 0-5 cm soil layer. In subsurface layer, the effects of NPK on LFOM-N were similar to those in 0-5 cm layer but had relatively lower magnitude (Table 5). These results also revealed that LFOM-N content decreased with soil depth, and 0-5

Table 4 Effect of different fertilizer treatments on particulate organic matter C (POM-C) and N (POM-N) in 0-5 cm and 5-15 cm soil layers and their proportion in total organic C (TOC) and total N (TN) after 37-year maize-wheat rotation.

Treatments	POM-C (g kg^{-1})		POM-C (% of TOC)		POM-N (mg kg^{-1})		POM-N (% of TN)	
	0-5 cm	5-15 cm	0-5 cm	5-15 cm	0-5 cm	5-15 cm	0-5 cm	5-15 cm
$N_0P_0K_0$†	0.75	0.55	17.3	16.8	22.3	17.7	3.4`	3.4
$N_{100}P_0K_0$	0.86	0.71	19.3	19.0	75.2	56.5	10.7	9.3
$N_{200}P_0K_0$	0.93	0.76	19.9	19.5	99.8	77.2	13.5	12.0
$N_{100}P_{22}K_0$	0.97	0.78	20.7	20.2	94.7	72.2	12.5	10.7
$N_{200}P_{44}K_0$	1.05	0.85	22.0	21.9	112.7	84.7	14.4	11.5
$N_{100}P_{22}K_{41}$	1.31	1.06	27.2	26.0	104.4	77.4	13.0	10.7
$N_{200}P_{44}K_{82}$	1.04	0.76	21.7	20.6	135.8	104.5	16.6	13.4
Mean	0.99	0.78	21.2	20.6	92.1	70.0	12.2	10.5
LSD (0.05)	0.06	0.07	-	-	2.4	2.6		

†N = fertilizer N (kg N ha^{-1}); P = fertilizer P (kg P_2O_5 ha^{-1}); K = fertilizer K (kg K_2O ha^{-1}).

Table 5 Effect of different fertilizer treatments on light fraction organic matter C (LFOM-C) and N (LFOM-N) in 0-5 cm and 5-15 cm soil layers and their proportion in total organic C (TOC) and LFOM-N after 37-year maize-wheat rotation.

Treatments	LFOM-C (mg kg^{-1})		LFOM-C (% of TOC)		LFOM-N (mg kg^{-1})		LFOM-C/LFOM-N Ratio	
	0-5 cm	5-15 cm	0-5 cm	5-15 cm	0-5 cm	5-15 cm	0-5 cm	5-15 cm
$N_0P_0K_0$†	52.2	37.5	1.2	1.2	6.0	4.6	8.7	8.1
$N_{100}P_0K_0$	77.9	56.7	1.7	1.5	7.4	5.9	10.6	9.6
$N_{200}P_0K_0$	93.8	76.4	2.0	2.0	8.5	7.2	11.1	10.5
$N_{100}P_{22}K_0$	86.9	83.0	1.9	2.2	6.6	7.6	13.2	10.9
$N_{200}P_{44}K_0$	105.3	95.8	2.2	2.5	9.3	8.7	11.4	11.0
$N_{100}P_{22}K_{41}$	173.8	147.2	3.6	3.6	12.8	11.5	13.6	12.8
$N_{200}P_{44}K_{82}$	145.8	125.1	3.0	3.4	11.4	10.9	12.8	11.4
Mean	105.1	88.8	2.2	2.3	8.8	8.1	11.6	10.6
LSD (0.05)	10.3	9.4	-	-	0.6	0.6	-	-

†N = fertilizer N (kg N ha^{-1}); P = fertilizer P (kg P_2O_5 ha^{-1}); K = fertilizer K (kg K_2O ha^{-1}).

cm surface layer contained higher LFOM-N content than 5-15 cm soil layer. LFOM-C/LFOM-N ratio ranged from 9 to 14 and 8 to 13, respectively, revealing higher increase in the proportion of LFOM-C than LFOM-N with the application of NPK fertilizers.

3.6 Potentially Mineralizable N

PMN content both in 0-5 cm and 5-15 cm soil layers of was 5 mg kg^{-1} 7d^{-1} in control and was further increased in different NPK treatments (Table 6). Application of 100 and 200 kg N ha^{-1} alone in $N_{100}P_0K_0$ and $N_{200}P_0K_0$ treatments significantly increased PMN content in 0-5 cm soil layer. Fertilizer P application @ 22 and 44 kg P_2O_5 ha^{-1} in $N_{100}P_{22}K_0$ and $N_{200}P_{44}K_0$ treatments increased the PMN content by 2 and 1 mg kg^{-1} 7d^{-1}, over $N_{100}P_0K_0$ and $N_{200}P_0K_0$ treatments, respectively. Likewise, the application of K along with N and P showed increase of 3 and 1 mg kg^{-1} 7d^{-1} PMN when applied @ 41 and 82 kg K_2O ha^{-1}. In subsurface layer, the effects of different combinations of applied NPK on PMN were similar to those in 0-5 cm layer but had relatively lower magnitude (Table 6). These results also revealed that PMN content decreased with soil depth.

3.7 Microbial Biomass C and N

MBC content, in 0-5 cm soil layer was 123 mg kg^{-1} in control; this increased further due to different NPK treatments (Table 6). Application of 100 and 200 kg N ha^{-1} alone in $N_{100}P_0K_0$ and $N_{200}P_0K_0$ treatments significantly increased MBC content in 0-5 cm soil layer. Fertilizer P application @ 22 and 44 kg P_2O_5 ha^{-1} in $N_{100}P_{22}K_0$ and $N_{200}P_{44}K_0$ treatments further

Table 6 Effect of different fertilizer treatments on potentially mineralizable N (PMN), microbial biomass C (MBC) and N (MBN) in 0-5 cm soil layer, and their proportion in total organic C (TOC) and total N (TN) after 37-year maize-wheat rotation.

Treatments	PMN (mg kg^{-1} 7 d^{-1})		MBC (mg kg^{-1})	MBC (% of TOC)	MBN (mg kg^{-1})	MBN (% of TN)
	0-5 cm	5-15 cm	0-5 cm	0-5 cm	0-5 cm	0-5 cm
$N_0P_0K_0$†	5.1	5.2	123.3	2.8	8.6	1.3
$N_{100}P_0K_0$	11.2	7.4	146.8	3.3	11.2	1.6
$N_{200}P_0K_0$	11.9	9.0	161.3	3.5	13.3	1.8
$N_{100}P_{22}K_0$	12.9	10.0	176.2	3.8	15.9	2.1
$N_{200}P_{44}K_0$	13.2	10.7	185.9	3.9	21.1	2.7
$N_{100}P_{22}K_{41}$	15.4	12.9	202.8	4.2	25.0	3.1
$N_{200}P_{44}K_{82}$	14.6	11.5	199.2	4.2	22.1	2.7
Mean	12.1	9.5	170.8	3.7	16.7	2.2
LSD (0.05)	1.9	2.2	7.5	-	1.2	-

†N = fertilizer N (kg N ha^{-1}); P = fertilizer P (kg P_2O_5 ha^{-1}); K = fertilizer K (kg K_2O ha^{-1}).

significantly increased the MBC content over $N_{100}P_0K_0$ and $N_{200}P_0K_0$ treatments, respectively. Correspondingly, application of K along with N and P showed further significant increase of 27 and 13 mg kg^{-1} MBC when applied @ 41 and 82 kg K_2O ha^{-1}. Maximum MBC content of 203 mg kg^{-1} in 0-5 cm layer was observed in $N_{100}P_{22}K_{41}$ treatment. The proportion of MBC ranged from 3 to 4% of TOC content, indicating increase due to different NPK fertilizer treatments.

MBN content in 0-5 cm soil layer was 9 mg kg^{-1} in control and increased further due to different NPK treatments (Table 6). Application of 100 and 200 kg N ha^{-1} alone in $N_{100}P_0K_0$ and $N_{200}P_0K_0$ treatments significantly increased MBN content in 0-5 cm soil layer. Fertilizer P application @ 22 and 44 kg P_2O_5 ha^{-1} in $N_{100}P_{22}K_0$ and $N_{200}P_{44}K_0$ treatments further significantly increased the MBN content over $N_{100}P_0K_0$ and $N_{200}P_0K_0$ treatments, respectively. Similarly, application of K along with N and P showed further significant increase of 9 and 1 mg kg^{-1} MBN when applied @ 41 and 82 kg K_2O ha^{-1}. Again, maximum MBN content of 25 mg kg^{-1} in 0-5 cm layer was observed in $N_{100}P_{22}K_{41}$ treatment. The proportion of MBN ranged from 1 to 3% of TN content (Table 6), again illustrating increase due to different NPK fertilizer treatments.

4. Discussion

The balanced application of NPK fertilizers for 37 years to maize-wheat rotation significantly improved total WSA (Fig. 1) as well as the proportion of macro-aggregates of 0.25 to > 2 mm size, which constituted the major proportion of WSA (Table 2). Maximum total WSA of 81% in 0-5 cm and 74% in 5-15 cm layer was developed under $N_{100}P_{22}K_{41}$ treatment, which produced greatest amount of crop residue, root biomass and rhizo-deposition, which help enhanced binding of soil particles and aggregate formation [18]. Also, certain polysaccharides formed during decomposition of organic materials as well as the cementing action of the bacteria and fungi lead to the formation of stable aggregates [1]. The beneficial effect of applied P was presumably due to the role of phosphate ions in binding of soil particles that protects the soil aggregation and improves the soil structure [19].

The effects of optimum and balanced application of NPK fertilizers on the TOC content (Fig. 2) and TOC stocks (Table 3) were more pronounced in 0-5 cm thin surface layer than 5-15 cm layer. Similarly, the C/N ratio became lower with the application of fertilizers (Table 3). Surface layer retained more plant materials and residues than subsurface, which led to enhance the TOC content. In earlier studies with cereals and legumes at the same site, N and P applications to 4-year rice-wheat rotation did not affect TOC content [20] but increased TOC from 3.2 to 3.6 g kg^{-1} in a 4-year groundnut-sunflower [21], and from 2.7 to 4.3 g kg^{-1} in a 30-year groundnut-based cropping systems [22]. The

present study further demonstrated that optimum application of $N_{100}P_{22}K_{41}$ in maize-wheat rotation for 37 years exhibited greatest benefit of increased TOC content both in 0-5 cm soil layer (11%) and 5-15 cm soil layer (13%), and correspondingly enhanced TOC stocks in 0-15 cm soil by 20% (from 8.3 to 9.9 Mg C ha^{-1}) due to higher crop biomass produces with adequate nutrition and therefore higher amounts of crop stubbles, root biomass and fallen leaves returned to soil after each harvest.

WSC accounted for 0.5 to 0.8% and 0.5 to 0.7% of TOC both in 0-5 cm and 5-15 cm soil layer, respectively, indicating relatively more increase in WSC than TOC with the application of NPK fertilizers, especially in 0-5 cm surface layer (Table 3). WSC, an active pool of organic C, serves as both source and sink for mineral nutrients and organic substrates in a short-term, and as a catalyst for conversion of plant nutrients from stable organic form over a longer period thereby influencing crop productivity and nutrient cycling.

While POM-C accounted for 17 to 27% of TOC content, POM-N accounted for 3 to 17% of TN content (Table 4). The significant increases in POM-C, POM-N, LFOM-C and LFOM-N were evident with the application of NPK fertilizers (Tables 4 and 5). POM, dominated by undecomposed plant residues that retain recognizable cell structures including fungal hyphae, seeds, spores, and fungal skeletons, is an active fraction of SOM, which supplies nutrients to the growing plants [23]. POM-C and POM-N provide estimates of the intermediate pool of SOM between the active and passive pools [11] and provide substrate for microorganisms and influence soil aggregation [24, 25]. LFOM, composed primarily of plant derived remains, and microbial and micro-faunal debris and other incompletely decomposed organic residues [23], is more sensitive to management practices than POM [26]. PMN, a measure of the soil capacity to supply mineral N, constitutes an important measure of the soil health due to its strong relationship with the capability of soil to supply N for crop growth. These labile C and N fractions are the potential indicators of soil health because change in the N pool will be detectable before the changes in total soil N or organic matter [3].

MBC is an active component of SOM and constitutes an important soil health parameter as carbon contained within microbial biomass is a stored energy for microbial process. Thus MBC and MBN, the measure of potential microbial activity, are strongly related to soil aggregate stability. Since turnover rate of N in MBN is very rapid, it reflects changes in management practices long before changes in TN are detectable [4].

Maximum contents of WSC, POM-C, LFOM-C, POM-N and LFOM-N in 0-5 cm and 5-15 cm layer were observed with the application of $N_{100}P_{22}K_{41}$or $N_{200}P_{44}K_{82}$ treatment. Maximum PMN content of 15 mg kg^{-1} $7d^{-1}$ in 0-5 cm and 13 mg kg^{-1} $7d^{-1}$ in 5-15 cm layer observed in $N_{100}P_{22}K_{41}$ treatment. Similarly, the content and stock of TOC and contents of MBC and MBN were highest with the continuous application of $N_{100}P_{22}K_{41}$ treatment, which continued to produce high crop yields during the long-experiment [7, 27]. Application of $N_{200}P_{44}K_{82}$ treatment did not further increase crop yields perhaps due to excessive supply of N that produces excessive vegetative growth resulting in lodging of the crops [28]. Thus, application of optimized levels of N, P and K fertilizers provides a better option in significantly improving the aggregate stability, MWD, storage of nutrients in labile pools, and C sequestration in semiarid subtropical soils that are inherently low in organic matter and nutrients.

5. Conclusions

Results of this 37-year field study with maize-wheat cropping rotation in a semiarid subtropical soil indicate that the content of TOC, POM-C, POM-N, LFOM-C, LFOM-N and PMN decreased with soil depth, and thin surface layer (0-5 cm) contained much higher concentration of these labile pools than 5-15 cm subsurface layer. The surface soil layer had substantially

higher levels of all soil health parameters than subsurface layer, presumably due to higher retention of crop stubbles, fallen leaves and root biomass. The enhanced proportions of WSC, POM-C, LFOM-C, MBC in TOC and that of POM-N, LFOM-N, MBN in TN with the supply of optimum and balanced N, P and K indicate that the improvement in labile forms of both C and N was relatively rapid than TOC and TN suggesting that active C and N pools reflect changes due to balanced NPK fertilization long before changes occur in TOC and TN. Further, 8 to 14-fold higher increase in the proportion of LFOM-C than LFOM-N with the application of NPK fertilizers indicated higher sequestration of plant-derived C than N.

The optimum and balanced use of 100 kg N, 22 kg P_2O_5 and 41 kg K_2O ha^{-1} to both maize and wheat crops increased water stable aggregate from 66% in control to 81% in surface layer and 63 to 74% in subsurface soil layer. This NPK treatment enhanced the proportion of macroaggregtes by 14 and 10%, respectively in surface and subsurface soil layer. Similarly, the content and stock of TOC and contents of POM-C, LFOC-C, PMN, MBC and MBN were highest with the continuous application of 100 kg N, 22 kg P_2O_5 and 41 kg K_2O ha^{-1}, which produced highest crop yields. In conclusion, optimized supply of N, P and K fertilizers to crops play a significant role in building up/restring soil health and productivity with co-benefits of improved C sequestration in semiarid subtropical soils inherently low in organic matter and nutrients. These findings will hopefully encourage the use of optimum and balanced fertilizer nutrients as the healthy and productive soils are indispensable for successful agriculture and prosperity.

Acknowledgments

The financial support to first author (Shrvan Kumar) from Indian Council of Agricultural Research, New Delhi in the form of Junior Research Fellowship is acknowledged. Authors are thankful to Dr. N. S. Dhillon, Department of Soils, PAU, Ludhiana for facilitating the soil sampling from the long-term maize-wheat experimental field.

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Journal of Agricultural Science and Technology A 1 (2011) 182-186
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Estimate the Emergence of *Pectinophora gossypiella* Saunders. (Lepidoptera: Gelechiidae) with Degree Days in the Region of Thessaly, Greece

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Received: August 23, 2010 / Published: June 20, 2011.

Abstract: The pink bollworm (*Pectinophora gossypiella*), is one of the most damaging pests of cotton growing in the region of Thessaly in Greece. The time of exit of the adults in spring is an important factor that affects the infestation index in the crop during the summer. Mathematical models by Sevacherian & El-Zik, and Huber, which were implemented in California, were used in this study to determine the beginning, the peak of the adults output and the end of them during the summer. A data comparison between California and region of Thessaly were applied since California and Thessaly are on the same latitude with similar meteorological conditions. The results showed that the emergence occurs when the insect completes 259 DD according to the method described by Sevacherian & El-Zik, while according to the method described by Huber 430-454 DD are needed. It was observed that either according to the method described by Sevacherian and El-Zik or according to the method described by Huber, the values (DD) showed that the appearance of adults varies between -262 DD to 59 DD and -872 DD to 115 DD respectively.

Key words: Pink bollworm, *Pectinophora gossypiella*, degree days, insect emergence.

1. Introduction

Pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) was reported by many authors as a serious pest of cotton causing significant yield loss [1-5]. Pink bollworm larvae damage mainly the bolls of cotton. The female moth lays eggs on a cotton boll, and when the larvae emerge from the eggs burrow into bolls, and damage seeds through feeding [6]. Once a larva has been inside the boll, chemical treatments are ineffective [7].

The pink bollworm, *P. gossypiella* is one of the most common and damaging pests of cotton plants growing in Greece. It is native in several regions of Thessaly where cotton plants are cultivated as a single-crop. The insect appears 3-5 generations per year depending on weather conditions and crop availability, affecting the cotton bolls by 15% in July, 15-45% in August, 45-75% in September, 75-90% in October, reaching 100% in November [8].

In the region of Thessaly the main control tools are observance of pink bollworm peaks with pheromones traps and appliance of insecticides to control the pest. Our observation showed that treating the plants with pink bollworm insecticides, early in the season will cause an extensive damage to the crop by other species, as the cotton aphid (*Aphis gossypiii* Glover) or mites later on. Moreover, because of the danger of secondary outbreaks farmers apply insecticides for a long period after recording the second generation pest peak in most cases unnecessarily, increasing rapidly the chemical cost.

So, the rate of development in pink bollworm populations is directly affected by the temperature [9-11]. The time of appearance of the pest adults a) late in the spring is an important factor in the

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development of infestation in the crop and b) early in the summer is also an important factor for effective applications of insecticides. The precise knowledge of these periods of pest behavior is an important factor for wise applications of insecticides (an objective of this study).

High temperatures during the spring months accelerate larval diapauses and increases adults output [12]. Degree day model based on insect development rates could be an excellent estimation tool for pest management based on region conditions (another objective of this study).

Generally in Greece, the appearance of adults begins in May and continues until late of September (personal observations, studies with pheromone traps). Because of the danger of the summer pest peak (summer peak emergence) for cotton pink bollworm an accurate prediction model is needed. A useful tool for monitoring and treatment decisions is provided by UC Pest management Guidelines web pages, "how to manage pests, calculating degree days" (http://www.ipm.ucdavis.edu/WEATHER/ddretrieve.html). Moreover based on bibliography the output of adults occurs when 675 degree days (DD) are completed, start counting on first January followed by a second maximum output (subsequent peak emergence) when 965 DD are completed [13].

Further, we believe that prediction model based on the degree days calculation of the insect population will be a key for insect pre-calling population determined for an effective insect control strategy to overcome losses by pink bollworm especially due to early pest damage (the main objective of this study).

2. Materials and Methods

During the first 10 days of October 2003 through 2008, from a cotton farm in central Thessaly, approximately 7 ha in size, mature bolls of Acala J2 variety were randomly sampled. In each sample, each year collected 400 bolls randomly. From the 400 bolls 100 of them were opened randomly to examine the infestation rate, while the remaining 300 bolls were placed in entomological cages (50 bolls/cage) and exposed to the environmental conditions of the sample area. Further, following the method described by Sevacherian [14], observations were taken from 1st January as described by Matthews and Tunstall [15].

Observations were taken every 10 days from 1st January to mid April while from mid-April to mid-August observations were daily. The adults found or captured in all observations were recorded and then removed from the cage. Meteorological data of maximum, minimum and average temperature over 24 hours were obtained from the Department of Larissa Cotton Board (Central Greece). Degree days were calculated according to the methods described by Sevacherian & El-Zik and Huber (in http://www.ipm.ucdavis.edu/PHENOLOGY/ma-pink_bollworm.html and in Ref. [12]).

The lower threshold for pink bollworm development according to Sevacherian & El-Zik model was 15.6 °C, whereas the lower and the upper thresholds for pink bollworm development according to Huber's method were 12.8 °C and 30 °C.

Further, accumulated percentages of emerged pink bollworm moths were made based on heat units accumulated beginning from 1st January to determine a) start of spring emergence, b) peak of spring emergence, c) end of spring emergence and d) summer generation time (adult to adult), estimated for each tested model as presented in http://www.ipm.ucdavis.edu/PHENOLOGY/ma-pink_bollworm.html. All data analyses were made in Microsoft Excel Packages 2002.

3. Results and Discussion

The bolls infestation had a variation from 42-74%. The lowest infestation occurred in 2004 compared with the highest infestation observed during the years 2006 and 2007. Probably diapause of pest is related to the crop maturity [11]. The first exit of the insect for the year 2004-2005 was observed on May 30 while

the same observation for the year 2006-2007 and 2007-2008 was recorded on 25 of May. The results showed that the stages of *Pectinophora gossypiella* emergence for Spring-Summer period, (means from all studied years), are listed in Tables 1 and 2, according to Sevacherian & El-Zik (1983) and Huber's methods.

The results showed that pink's bollworm emergence (start of spring emergence), in the region of Thessaly occurred when the pest completed 134-154 DD °C, (with a mean of 144 DD °C) according to the method described by Sevacherian & El-Zik (Table 1), while according to the method described by Huber 218.9-259.3 DD °C, with a mean of 239.1 DD °C are needed (Table 2). In California the start of spring emergence values are 111.1 DD °C and 277.8 DD °C, respectively.

The peak of spring emergence, in the region of Thessaly occurred when the insect completed 372.9-458.3 DD °C (with a mean of 415.6 DD) according to the method described by Sevacherian & El-Zik (Table 1), while according to the method described by Huber 557.3-635.1 DD °C, with a mean of 596.2 DD are needed (Table 2). In California the start of spring emergence values are 375.0 DD °C and DD 655.6 °C, respectively.

The end of spring emergence, in the region of Thessaly occurred when the insect completed 528.6 DD °C according to Sevacherian & El-Zik method (Table 1) or needed 738 DD °C according to Hube's methods (Table 2). In California the start of spring emergence values are 611.1 DD °C and 738.1 DD °C, respectively.

Finally the peak of summer emergence, in the region of Thessaly occurred when the insect completed 391.5 DD °C according to Sevacherian & El-Zik method (Table 1) or needed 508.1 DD °C according to Hube's methods (Table 2). In California the start of spring emergence values are 537.2 DD °C and 444.4 DD °C, respectively.

It is observed that according to the method described by Sevacherian and El-Zik, the values varies from -262 DD to 59 DD, and according to the method described by Huber's, the observed values varies between -872 DD to 115 DD. Adjustment to the method of Sevacherian and El-Zik, is observed during the start of spring emergence, with the spread of the values varies to +59 DD, and during the peak of spring

Table 1 Appearance of *Pectinophora gossypiella* emergence in the region of Thessaly during the Spring-Summer period in all studied years, compared with the reported values in California. The values are calculated in DD according to Sevacherian & El-Zik method.

	California		Thessaly-Greece	
	DD (°C)	DD (°F)	DD (°C)	DD (°F)
Start of spring emergence	111.1	200.0	144.0	259,2
Peak of spring emergence	375.0	675.0	415.6	748.0
End of spring emergence	611.1	1,100.0	528.6	951.5
Peak of summer emergence (adult to adult)	537.2	967.0	391.5	704.7

Table 2 Appearance of *Pectinophora gossypiella* emergence in the region of Thessaly during the Spring-Summer means values in all studied years, with the reported values in California. The values are calculated in DD according to Huber's method.

	California		Thessaly-Greece	
	DD (°C)	DD (°F)	DD (°C)	DD (°F)
Start of spring emergence	277.8	500.0	239.1	430.4
Peak of spring emergence	656.6	1,180.0	596.2	1,073.1
End of spring emergence	1,222.2	2,200.0	738.1	1,328.5
Peak of summer emergence (adult to adult)	444.4	800.0	508.1	914.7

emergence, with the spread of values varies to +73 DD. Furthermore, adjustments to the method of Huber may occur in start of spring emergence, with a spread of the values to -50 DD.

Moreover for the years 2003-2008 in the region of Thessaly, the adults of *P. gossypiella* start the spring emergence during the period of 25-30 May. The peak of spring emergence occurred on 30 June and the end of the emergence occurred on mid-June.

The present data shows that both models are potentially useful for monitoring: a) the start of spring emergence, b) the peak of spring emergence and c) the peak of summer emergence with the appropriate modification as presented in Tables 1 and 2.

Phenology models predicting timing of events in an organism's development based on simple linear regression of development rate and temperature are widely used. Similarly in this research our field tested data are very close to Humber's method of DD calculation, as the linear relationship occurs; between pink bollworm emergence time in the region of California and in the region of Thessaly (Fig. 1). The Sevacherian & El-Zik method occurred only for the start of spring emergence or the peak of spring emergence (Fig. 2). The model lacks the linearity and the ability to predict in close value the end of the spring emergence as Fig. 2 shows, probably due to model threshold temperature.

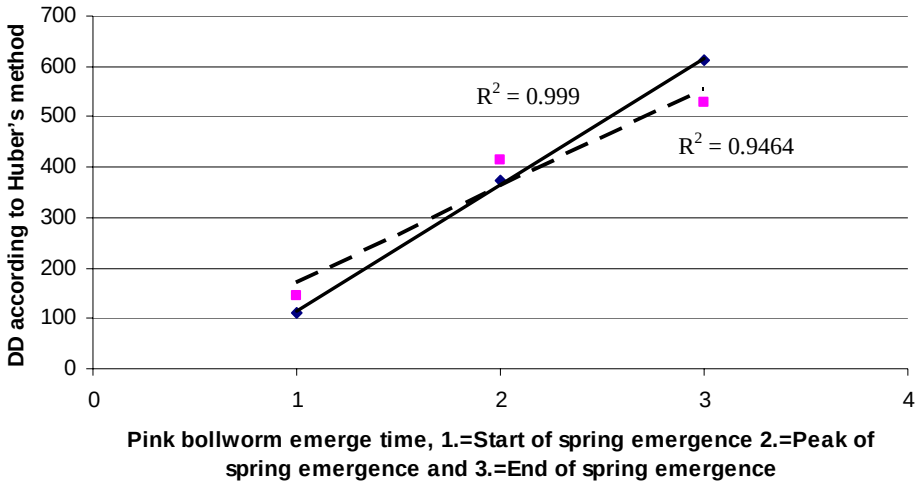

Fig. 1 Regression analysis for pink bollworm emergence time in California (direct line) and in the region of Thessaly (dot line) according to Huber's method.

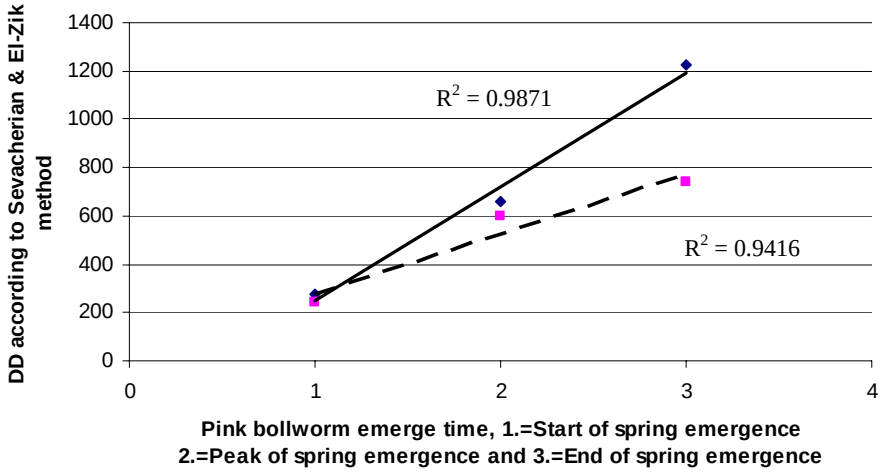

Fig. 2 Regression analysis for pink bollworm emergence time in California (direct line) and in the region of Thessaly (dot line) according to Sevacherian & El-Zik method.

We concluded that geographical area and cultural practices have to be adjusted for the normal variation in phenology tested models for pink bollworm between California and Thessaly. Under this situation the precise knowledge of the pest behaviour is very important as reported by Sevacherian et al. [15] and Matthews and Tunstall [13].

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Journal of Agricultural Science and Technology A 1 (2011) 187-193
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Heating and Cooling Performance of Earth-Tube Heat Exchanger in a Mechanical Ventilated Farrowing House

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Received: August 18, 2010 / Published: June 20, 2011.

Abstract: Earth tempering of stable air has attracted great attention as a sustainable air conditioning method in pig houses. At summer time air cooling of income air strongly reduces heat stress and required ventilation rate. At winter time heating costs can be reduced. The effect of air condition using geothermal energy was investigated in a farrowing house. Underneath the foundation of the farrowing house 88 non perforated ribbed tubes (diameter: 20 cm) were piped in a depth of 1.6-2.0 m. Over a period of 12 month following data were recorded at hourly intervals and analyzed: outside air temperature, as well as air temperature in the air supply duct and in the compartments. Incoming air (supply duct) was heated up to 20 °C during winter time and in summer time cooled by up to 15 °C compared to the outside air temperature. In contrast to the outside air diurnal variation, temperature fluctuations of the incoming air were reduced by 90%. Due to cooling of the incoming air at summer time the stable inside temperature could be limited to maximal 29 °C (maximum outside temperature was 35 °C). Earth-tube heat exchangers with non perforated ribbed tubes were very efficient for air conditioning in farrowing houses. They were a cost effective supplement for sustainable cooling and heating of farrowing houses.

Keywords: Earth heat exchanger, heat stress reduction, farrowing house, air conditioning.

1. Introduction

In recent years heat production by means of earth-tube heat exchanger has gained in importance in building construction in Europe [1]. In the course of further shortage of fossil resources and high oil prices sustainability of livestock houses gets more important than ever. In addition increasing animal performances due to advanced breeding as well as improved housing and management, leads to higher demands for an optimal stable climate. Therefore the earth-tube heat exchanger becomes more popular, on the one hand because of energy saving at winter times, also because of the cooling of the incoming air at summer times. Especially in farrowing houses a balanced climate throughout the whole year for sows as well as for piglets has to be provided. The different demands to the ambient air temperature for sows and piglets are well-known: Piglets, especially in the first two weeks p.p. need ambient air temperature of about 33 °C. Room temperature of 27 °C and additional heat sources are necessary [2]. However the optimal ambient air temperature for lactating sows is in the range of 10-16 °C [3]. Temperatures above the thermo neural range leads the negative effects on feed intake, milk production [4] as well as sow condition including fertility [5-7]. Therefore earth tempering of stable air has attracted great attention as a sustainable air conditioning method in pig houses. At summer time air cooling of income air strongly reduces heat stress and required ventilation rate. At winter time heating costs can be reduced. This paper will focus on the air cooling and air heating capacity of an earth-tube heat exchanger in a farrowing house.

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2. Material and Methods

The effect of air condition using geothermal energy was investigated in a farrowing house (21.8 × 23.0 m), built in 2007 in Northwestern Germany. This house included four compartments (19.80 m × 5.60 m) with 22 farrowing pens (2.25 m × 1.80 m) each. Each pen was equipped with one diagonal crate. Piglets' nests were heated with warm water. Additionally up to the 4th day p.p. heat lamps were hanged up above the nests. Beside the sensible heat production of sows themselves, no further heat sources were installed. Sows were fed dry via volume dosing feeder. On average sows' performance was 29.9 live-born and 24.0 weaned piglets.

2.1 Farrowing House

The farrowing house was built on podsol soil, which featured a groundwater level at a depth of 1 m below ground surface (Figs. 1 and 2). Underneath the foundation of the farrowing house (21.8 × 23.0 m) non perforated ribbed tubes (diameter: 20 cm) were piped in a depth of 1.6-2.0 m. Therefore 10 excavations were done. Into each excavation 9-8 tubes were piped directly next to each other (Fig. 3). The distance between each excavation amounted 0.5 m. Due to the high ground water level the excavations were filled without washing in of the tubes. In total 88 tubes were piped, which corresponds with the number of farrowing pens in the farrowing house. The inlet ports were directly next to the outer wall. Roof overhang and grids above the inlet ports provided protection from climate influences and contaminants. The outlet ports opened out into an air supply duct. From there, the delivery air reached the attic via 5 ducts (1.0 m × 1.0 m). From these attic ducts 88 tubes (diameter 0.2 m) were piped into the farrowing house, one for each farrowing pen. The tubes ended 2 m above the sow's head. This kind of air supply is called "nose aeration". The air reached the farrowing house by negative pressure ventilation. The outgoing air of the compartments was exhausted via one exhaust air duct each (Multifan 4 E 45 Q-R, power: 6,400 m³/h at 0 Pa, Vostermans Ventilation B. V., NL), which was placed in the middle of the room. Exhausted air rate was controlled via airflow and inside air temperature.

2.2 Data Collection

Over a period of 12 month following, data were recorded at hourly intervals and analyzed: outside air temperature and outside relative humidity, as well as air temperature and relative humidity in the air supply duct and in two compartments of the farrowing house. Outside air temperature and relative humidity were measured with a WS 2000 PC radio meteorological station (ELV-Elektronik AG, Germany) 20 m away from the farrowing house. At four measuring points air temperature and relative humidity in the air supply

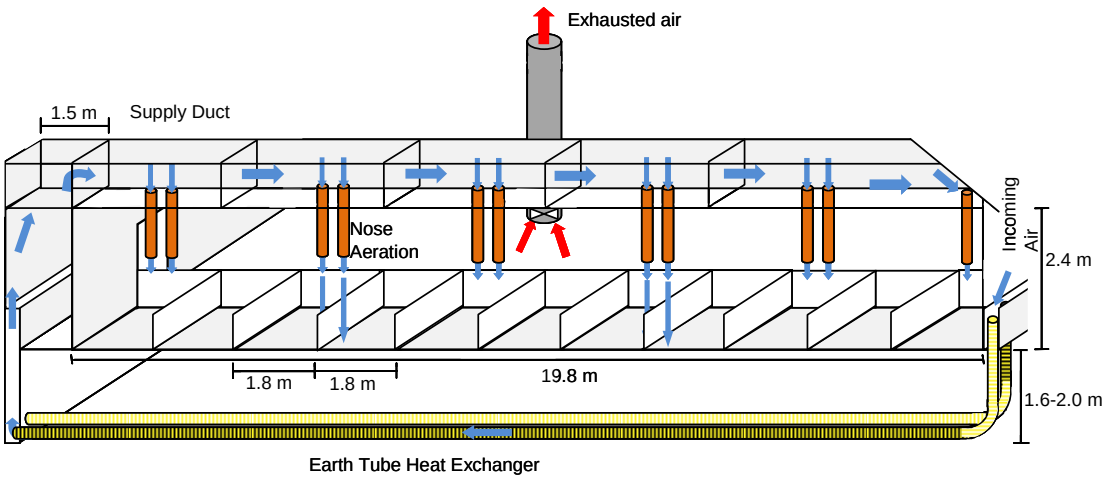

Fig. 1 Routing of air flow in the farrowing house, vertical section.

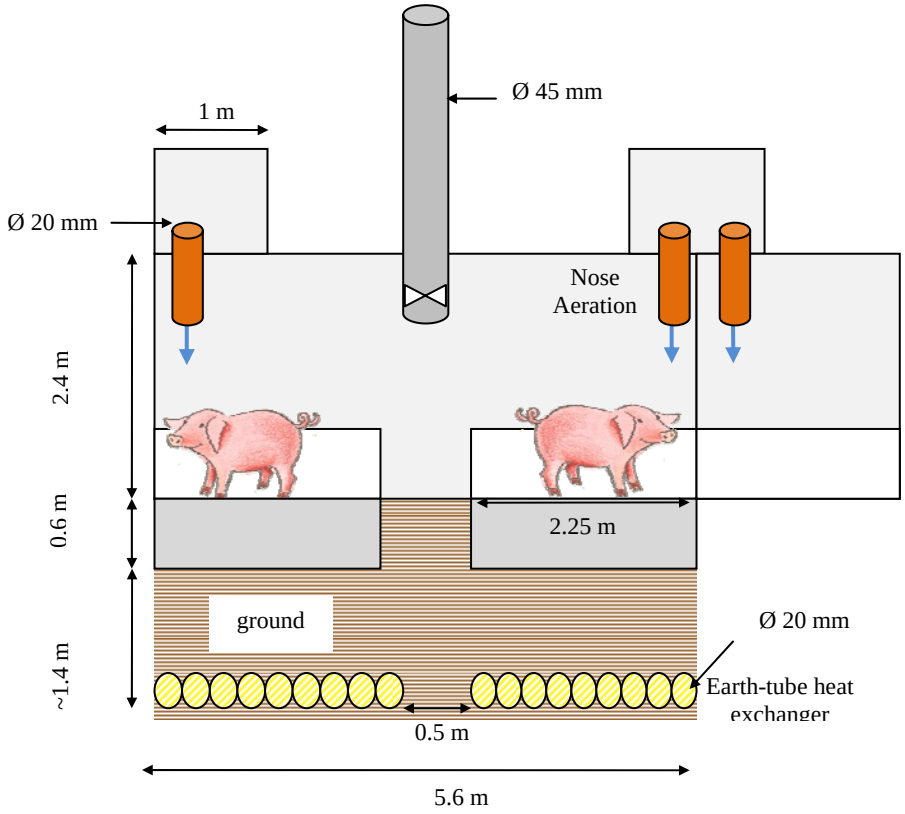

Fig. 2 Cross section of one farrowing compartment.

Fig. 3 Non perforated ribbed tubes (diameter: 20 cm) piped directly next to each other in a depth of 1.6-2.0 m.

duct were recorded with the aid of Micromec Loggers (Datenlogger + Messtechnik GmbH, Germany) and measuring sensors (Rotronic Messgeraete GmbH, Germany). Air temperature and relative humidity in two compartments were registered in the middle of the compartment at a height of 1.80 m using Tinytag Datalogger (Gemini Data Loggers Ltd., Great Britain). Because of malfunction of the measuring technique no data are available for outside climate from September 2nd to October 2nd 2009 and for the supply duct climate from 8th October to 4th November 2009. Measurements of the compartment air temperature started on June 6th 2009, however no data are available from August 31st-September 2nd 2009.

3. Results and Discussion

3.1 Seasonal Variation

The course of the hourly mean outside air temperature, supply duct air temperature (supply duct air temperature, mean of 4 measuring points) and compartment air temperature (compartment air temperature, highest value of two measuring points) are shown in Fig. 4. Outside air temperature showed a clearly higher variability during the year as well as the day than supply duct air temperature. Supply duct air temperature increased from January until July/August

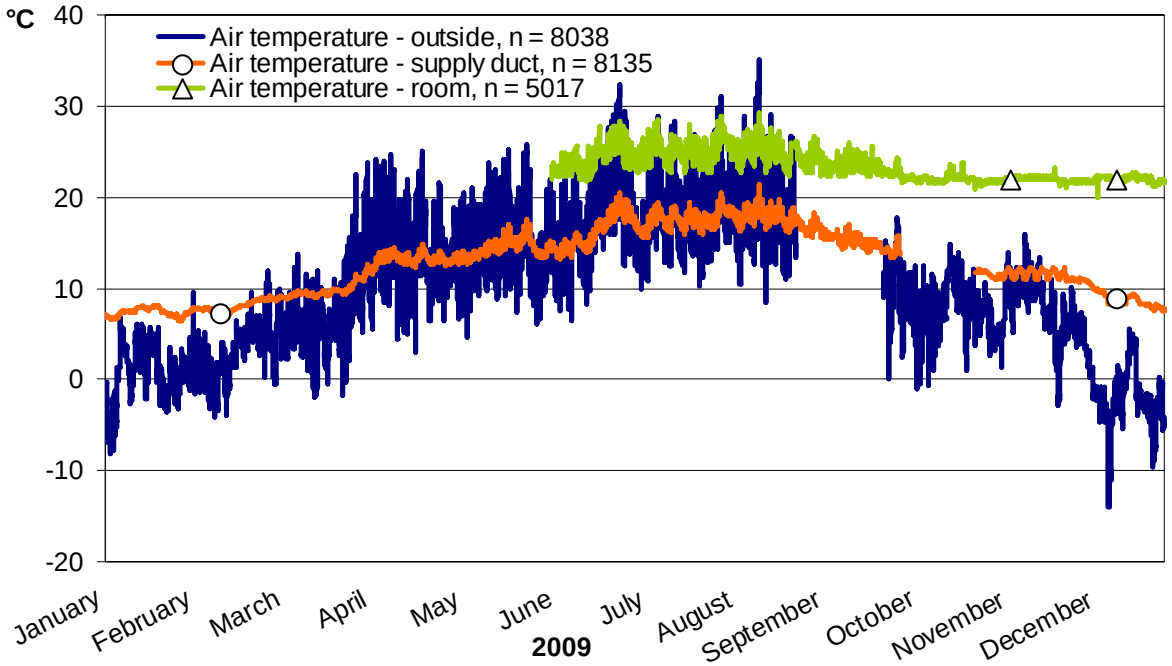

Fig. 4 Hourly means of outside (n = 8038) supply duct (n = 8135) and compartment (n = 5017) air temperature as a function of time of the year.

2009 from slightly lower than 10 up to slightly higher than 20 °C. Thereafter supply duct air temperature dropped until January 2010 to the same level as in January 2009. Compartment air temperature showed higher variability in midsummer compared to fall and winter. However this variability was far from those of outside air temperature.

On basis of hourly means average outside air temperature was only by 2.4 °C lower compared to the supply duct air temperature (Table 1). However standard deviation of outside air temperature was clearly higher compared to the one of supply duct air temperature. Highest measured outside air temperature was 35.1 °C, lowest outside air temperature -14.0 °C. The range for outside air temperature represented 49.1 °C. In contrast to outside air temperature supply duct air temperature featured a maximum of 21.5 °C and a minimum of 6.5 °C, which results in a range of only 14 °C throughout the whole year. During winter time incoming air (supply duct) was heated by up to 20 °C, which reduces additional heating costs. In summer time incoming air was cooled by up to 15 °C compared to the outside air temperature, which reduced heat stress of sows as well as required ventilation rates. Overall seasonal variation of incoming air temperature can be minimized by 70%.

Realized temperature differences between supply duct air temperature and outside air temperature on daily means (24 h average) basis are shown in Fig. 5. At a calculated outside temperature of 14.1 °C neither cooling nor heating of the earth-tube heat exchanger took place. With outside air temperature lower than 14.1 °C air in the supply duct was heated though the earth-tube heat exchanger. Maximal heating capacity on basis of daily means was 14 °C. If outside air temperature were above 14.1 °C the earth-tube heat exchanger cooled the incoming air. On basis of daily means the cooling effect amounted -6 °C. The reason that sometimes with outside temperatures between 12-18 °C no heating or cooling effect occurred might be due to seasonal effects. Different ground water temperatures (winter/summer) might influence the heating or cooling effect. Furthermore different volume air flow rates in summer and winter might also have an impact. This has to be investigated in further studies.

Table 1 Mean, Standard Deviation (SD), Maximum, Minimum as well as range of the air temperature outside and in the supply duct (n = 7,223 hourly means)*.

	Mean	SD	Maximum	Minimum	Range
Outside air temperature [°C]	10.0	8.3	35.1	-14.0	49.1
Supply duct air temperature [°C]	12.4	3.8	21.5	6.5	15.0

* Data from September 2nd to October 2nd 2009 and from 8th October to 4th November 2009 are not included.

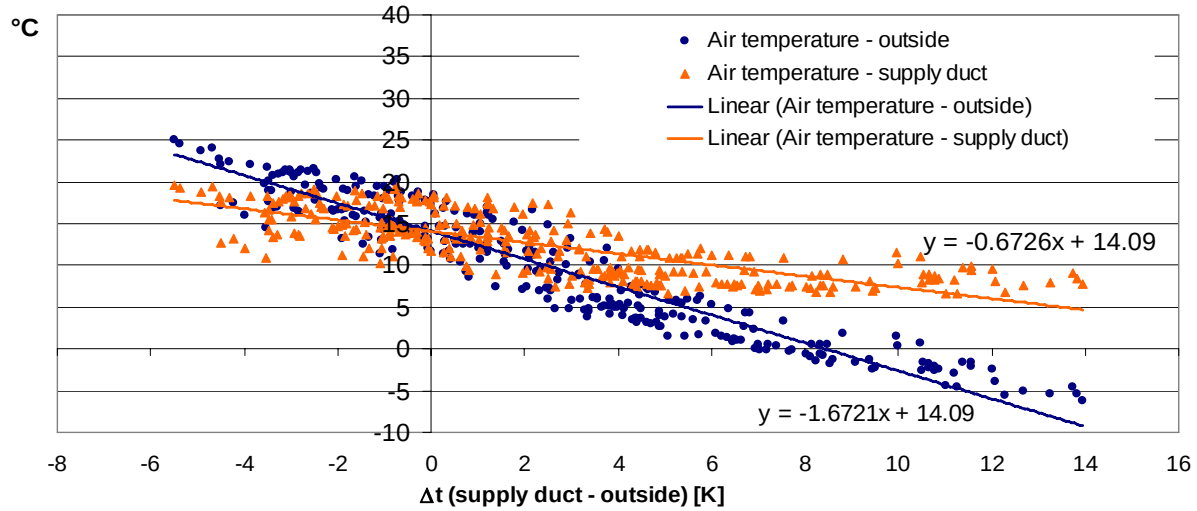

Fig. 5 Average daily supply duct and outside air temperature as function of the difference between both (n = 309), data from September 2nd to October 2nd 2009 and from October 8th to November 4th 2009 are not included.

3.2 Diurnal Variation

In contrast to the outside air temperature diurnal variation, temperature fluctuations of the incoming air were reduced in annual mean by 90%. Fig. 6 shows the diurnal variation of air temperature in summer (4 consecutive example days). Great diurnal variation (up to 14.9 K) of the outside air temperature has been measured. In contrast with the incoming air the diurnal variations have been strongly damped (less than 2.5 K). In the daytime incoming air was cold by about 10 °C, at night the effect of the earth-tube heat exchanger was closely to zero. Also compartment air temperature showed little diurnal variation (about 3.5 K) and fluctuated around 25 °C. Moderate diurnal temperature variations in the range of 5-8 K do not cause problems for pigs [8, 9]. However, high diurnal variation of for example 12 K has a negative effect on welfare and [10].

In winter time outside air temperature showed only low diurnal variations (Fig. 7) consecutive example days. However greater variations could be observed between the days. Outside air temperature ranged between -1 to -14 °C, whereas supply duct air temperature was found to be between 8 to 10 °C. Temperature differences between supply duct air temperature and outside air temperature amounted up to 22 °C. Also compartment air temperature stayed on a stable level of about 21 °C. For optimal performance pigs need constant air temperature. By reducing the temperature variations of incoming air constant air temperatures in the stable can be realized more easily without complex climate control.

3.3 Performance of Earth-Tube Heat Exchanger

The decisive factor for performance of an earth-tube heat exchanger is the type of soil and its conductivity in which it is integrated. Soils with high water holding capacity or soils with high groundwater level have best heat exchange efficiency [11]. Also the depth of the piped tubes is of importance in order to deliver a rewarding heating and cooling performance [12, 13]. Furthermore the heat exchange efficiency of

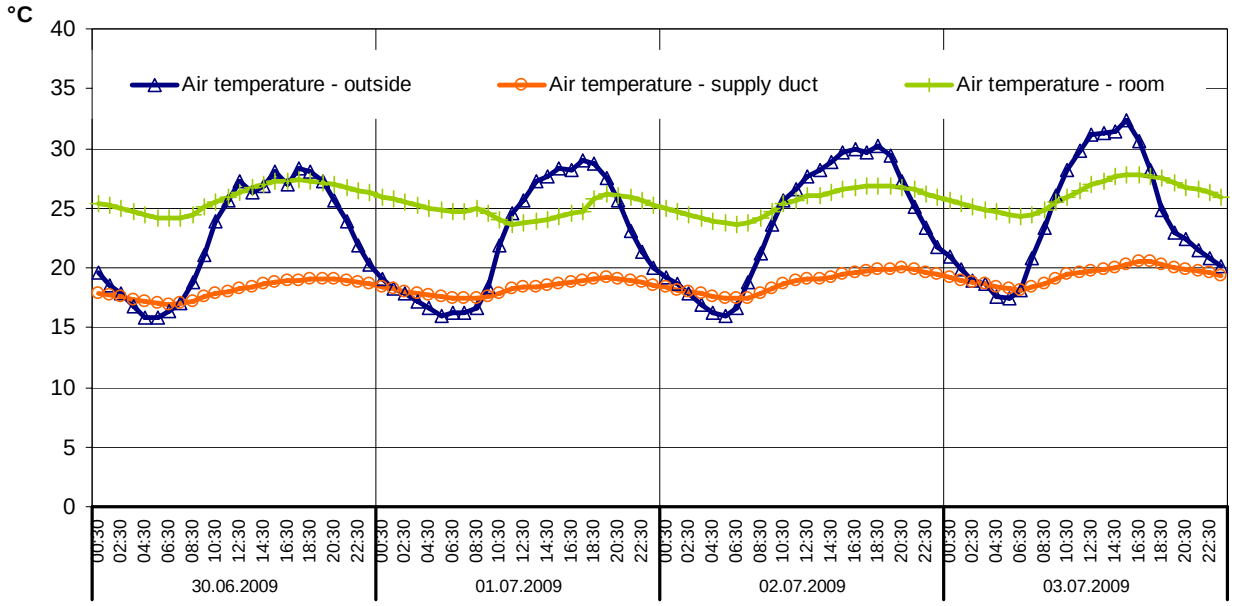

Fig. 6 Outside, supply duct and compartment air temperature on hourly basis for 4 consecutive example days during summer time (n = 96).

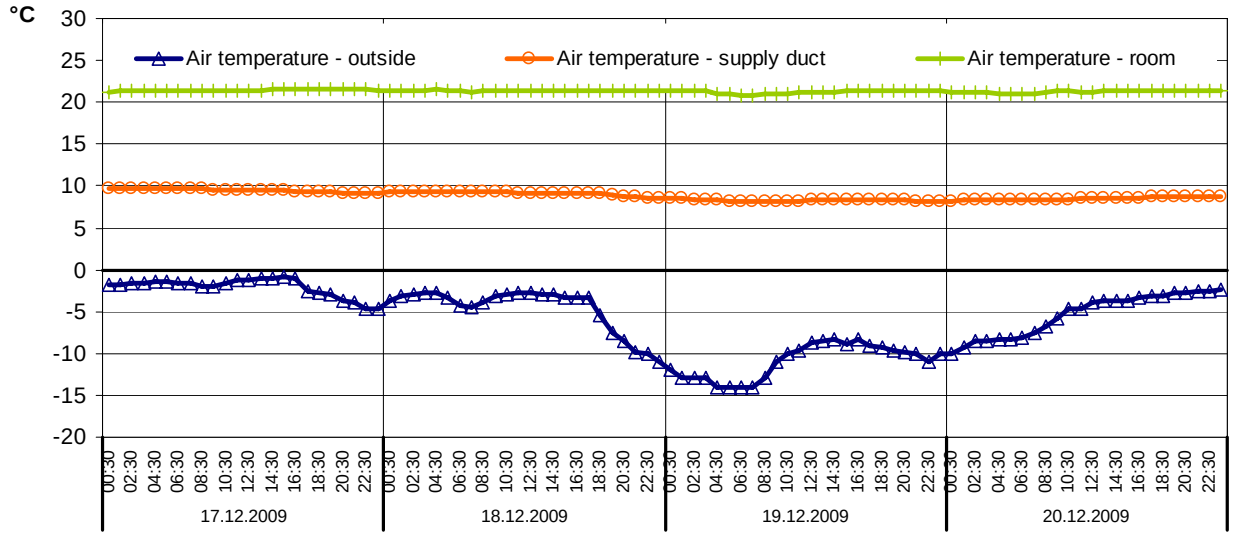

Fig. 7 Outside and supply duct and compartment air temperature on hourly basis for 4 consecutive example day during winter time (n = 96).

an earth-tube heat exchanger depends on the number, length and diameter of the used tubes as well as their distance between each other [11]. In this study the tubes of the earth-tube heat exchanger were piped rather closed to ground level and quite closed to each other. However due to the relatively high groundwater level at the location a quite considerable heat exchange efficiency could be established. The cooling performance on basis of daily means amounted maximal -6° k and corresponds with findings of neukermans and de schryvere [14] and huijben and hoofs [15] who report a cooling effect in pig houses of max. 6.5 K, 4 to 8 K, respectively. The maximal heating capacity (14 K) achieved in this study lies the same range established by Neukermans and De Schryvere [14]; Huijben and Hoofs [15] report lower heating performance of maximal 10 K. However, comparisons between diverse earth-tube heat exchangers at different location are questionable due the various basic conditions like soil type and climate.

4. Conclusion and Future Prospects

Earth-tube heat exchangers with non perforated ribbed tubes have proved to be very efficient for air conditioning in farrowing houses. They are a cost effective supplement for sustainable cooling and heating of farrowing houses. Seasonal variation of in incoming air can be minimized by 70%. Consequently additional heat cost can be reduced in winter. Heat stress of sows as well as required ventilation rates can be minimized in summer. Diurnal variation of the incoming is reduced by 90%. With this constant incoming air temperature can be realized without complex climate control. For evaluation of the *amount* of saved energy due to lower ventilation rates in summer time and reduced heating cost in winter time in subsequent studies further sensors are installed. Continuous online ventilation rate will be recorded using a measuring ventilator, furthermore pressure difference measurement will be done with online sensors, and the electric energy consumption of farrowing house as well as heat energy consumption of hot water floor heating system will be registered.

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Journal of Agricultural Science and Technology A 1 (2011) 194-198
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Impact of Soil Texture and Organic Matter Content on Methyl Isothiocyanate Volatilization from Soil Columns

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Received: August 31, 2010 / Published: June 20, 2011.

Abstract: Metam sodium (MS; sodium N-methyl dithiocarbamate) has emerged as a promising soil fumigant in the US to replace methyl bromide (MeBr). Metam potassium (MK; potassium N-methyl dithiocarbamate) and MS break down into the volatile gas methyl isothiocyanate (MITC) to control soil borne pests. Many studies have focused on MS, but MK has not been studied as thoroughly. The objective of this research was to determine the effect of increasing organic matter (OM) treatments and soil texture to minimize the off-gassing of MS and MK. Bench-scale soil column studies were performed to simulate organic matter treatments that may decrease the volatilization loss of MITC. Incorporation depth of OM simulated surface tillage (0-15 cm) practices. Soil was packed in steel columns and MS or MK was applied at a depth of 15 cm and MITC volatilization was measured using gas chromatography/mass spectroscopy. Volatilization of MITC behaved similarly for MS and MK with MITC movement impacted by soil texture. MITC volatilization was lower from a sandy clay loam than a sandy soil. Surface incorporation of OM did not significantly decrease MITC volatilization. These results suggest that soil texture is the dominant factor reducing MITC off-gassing and prolonging the time needed to control soil borne pests.

Key words: Metam sodium, metam potassium, methyl isothiocyanate, methyl bromide alternatives, soil columns.

1. Introduction

The phase out of MeBr in 2005 due to its contribution to ozone depletion [1] has increased the use of MS to control soil borne pests. However, inconsistencies in pest control from such alternative chemicals have made it difficult to replace MeBr as a soil fumigant. Furthermore, these alternatives are still highly volatile and lead to high concentrations of these chemicals off-gassing into the atmosphere. Increased efforts to reduce these emissions are needed in order to improve their efficacy and reduce chemical exposure. Since 2005, MS has emerged as the most widely used soil fumigant for the control of soil borne pests [2]. MS and MK degrade readily into the volatile gas MITC shortly after their injection into the soil [3]. MS and MK are applied in liquid form and through shank injection or chemigation practices.

Fumigant volatilization is inhibited by the rate of degradation of the chemical and by the transport within the soil. Fumigants are transported quickly throughout the soil by gas-phase diffusion. This gas-phase diffusion is dependent on the movement towards the soil surface and is affected by the soil bulk density and water content. Many chemical and biological factors can affect the degradation of MITC, however, temperature and organic amendments are thought to have the greatest impact [4, 5]. Historically, soil organic amendments have been used to control soil pathogens [6]. While more recently, organic amendments have been used in conjunction with soil fumigants in order to control soil borne pests and potentially reduce fumigant emissions [4, 7-9]. With the addition of organic matter to the soil, soil fumigant degradation can be significantly increased thereby decreasing volatilization loss [5, 9]. The purpose of this study was to better

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understand the impact of varying soil type and organic matter additions on MITC volatilization after MS and MK application in soil columns, similar to columns used by Zheng et al., 2006 [10].

2. Materials and Methods

2.1 Experimental Design

Two bench-scale studies were set up aimed at understanding different aspects of MITC fate under differing soil properties with varying treatments. The purpose of the first study was to analyze the MITC volatilization of MS and MK from a sandy soil and a sandy clay loam soil. The second study focused on the MITC volatilization from a sandy clay loam soil with varying organic matter amendment rates after MK application. The steel columns were packed with soil and the chemicals were applied at a constant rate typical for field use of the products, the volatilized chemical (MITC) was then collected and measured according to the methods detailed below.

2.2 Soil Preparation

Two soils were used; one, a sandy soil type containing 2.8% organic matter, 93.5% sand, 2.5 % clay and 4% silt, obtained from the upper 30 cm of a sandy alluviated outwash soil collected at Premont, Jim Wells County, TX, USA (longitude 27°20′, latitude 98°10′). The other an Orelia sandy clay loam (fine-loamy, mixed, hyperthermic Typic Ochraqualf) containing 2.3% organic matter, 55% sand, 33% clay, and 12% silt obtained from the upper 30 cm of farmland in Kingsville, Kleberg County, TX, USA (longitude W 097°53′, latitude N 27°33′). Both soils were air dried, sieved to 2-mm and moisture level adjusted to 8% by adding deionized water prior to packing soil into steel soil columns. Soil organic matter was obtained from Brownville, Texas yard waste compost, screened to 2.0 mm, and autoclaved for sterilization.

2.3 Chemical Application

In all studies MS (Vapam 42% MS [0.121 g MITC equivalent], Dow AgroSciences LLC, Indianapolis, IN) or MK (K-pam HL 54% MK) was injected at a depth of 15 cm from the soil surface to the center of each column at a rate of 356.8 kg Met-Na ha^{-1} in a total solution of distilled water (116.3 mL) to simulate a 1.125 cm water application event.

2.4 Soil Column Setup

Steel soil columns (similar to those used in studies done by Gan et al., 1999 and Zheng et al., 2006) [5, 10] were used to model gas flow through the soil profile. Columns were 60 cm high by 12.5 cm inside diameter with side sampling ports located at the 15, 25, 35, 45, and 55-cm depths. A headspace sampling chamber (4 cm high by 12.5 i.d.) was placed on top of the steel columns and sealed with airtight aluminum tape vacuum system was set up to measure the volatilization of MITC from the soil. Charcoal ORBO-32 filters (Supelco, Bellefonte, PA) were attached to a vacuum source set at 10 mm Hg that was pulling air from each of the 6 columns at an average of 1.5 mm Hg per column. Vacuum-side charcoal filters were used to trap any volatile chemical flowing out of the columns headspace chamber. On the inlet side of the column, another charcoal filter was attached to allow air to enter the column without allowing backflow loss of chemical out of the column.

In the first study, 7.5 kg of sand and sandy clay loam at 8% moisture was packed in triplicate replicated steel columns (as mentioned above) to a bulk density of 1.36 kg m^{-3}. In the second study, 3 columns were set up at varying OM rates (10.9, 17.0, and 32.6% OM) and packed to a bulk density of 1.35, 1.30, and 1.17 kg m^{-3}, respectively. The columns had OM mixed homogeneously in the upper 15 cm depth of the soil column to simulate surface tillage of the incorporated OM.

2.5 Chemical Analysis

For all studies, 500 μL air samples were extracted from the center of the columns from the side-ports and injected into the gas chromatograph after 6 hours. Analysis of MITC concentration within the soil-air

phase was performed via direct on-column injection of samples taken directly from the soil columns side ports. The gas chromatograph used was a SRI 8610C equipped with a flame ionization detector. It was equipped with an Rtx-624 wide bore capillary column (30 m × 0.53 mm × 3.0 μm; Restek Corp., Bellefonte, PA.). The oven temperature program was held at 65 °C for 1 min., then ramped 4 °C min^{-1} to 95 °C, then ramped 30 °C min^{-1} to 185 °C. Total run time was 11.5 min. Carrier gas was He, set at 5.05 mL min^{-1} flow rate. Peak area for MITC was measured by the area under the peak and integrated manually, with a MITC peak signal retention time at 8.7 min. MITC detection limit for this method was 10 mg L^{-1}. MITC peak retention time occurred at approximately 8.7 minutes.

Air samples were taken every 24 hours for a 7 day period. During this period ORBO-32 filters were changed every 4 hours during the day, and backup filters were attached to the vacuum source during the experiment and overnight for a period of 8 hours to ensure that no MITC was lost due to break through off the first filter. ORBO-32 charcoal filters used to collect MITC emissions were collected, end cap sealed and stored in the freezer at -20 °C until extraction procedure was done. Methanol solvent was used to extract MITC off of the charcoal filters. Methanol extraction efficiency was evaluated and found to be approximately 100%. Analysis of MITC from charcoal carbon filters and residual soil MITC levels was done using an Agilent 6890N gas chromatograph (GC) equipped with a 5973 Network mass selective (MS) detector (mass spectrometer). The GC/MS system was equipped with a DB-624 capillary column (30 m × 0.25 mm × 1.4 μm; J& W Scientific, Folsum, California, USA). Carrier gas was He, set at 5.0 mL min^{-1} flow rate. Solvent delay was 6.0 min., and oven temperature program was held at 65 °C for 1 min, then ramped 4 °C min^{-1} to 95 °C, then ramped 30 °C min^{-1} to 185 °C. Total run time was 11.5 min. with a MITC peak signal retention time at 10.45 min. MITC detection limit for this method was 1.0 mg L^{-1}.

2.6 Statistical Analysis

The first study was performed with three replicates per treatment and differences of the means between treatments were subjected to F tests and paired t-tests. All error bars shown are ± standard error of mean.

3. Results and Discussion

Results from the first study that focused on fumigant behavior from varying soil types indicate that volatilization loss of MITC occurred mostly within the first 72 hours after MS and MK injection and was highly dependent on soil texture (Fig. 1). MS and MK behaved similarly with respect to MITC volatilization loss. Lower MITC volatilization from the sandy soil compared to the sandy clay loam was due to further downward movement of MITC in the sandy soil (Fig. 1). Residual MITC was found at deeper depths in the sandy soil than in the sandy clay loam soil, providing further evidence that soil texture was a major contributing factor to MITC fumigant movement within the soil profile. This penetration of MITC deeper into the soil column profile shows that the larger pore spaces of sand facilitate the downward movement of the fumigant. This could increase fumigant efficacy by increasing the contact time of the fumigant with the soil and also by increasing the amount of soil that comes into contact with the MITC.

Inconsistent efficacy for pest control has been observed in several parts of the U.S. [11], and due to this problem many growers are hesitant to adopt the use of MITC generating compounds into a solid fumigant pest control program. Since MS is water soluble, it requires precise delivery to the treatment zone in the soil to enable more consistent and reliable pest control. This is commonly achieved using shank-mounted stainless steel tubes that are pulled through the soil and MS is injected at the desired depth (typically 25 cm deep) within the plow layer [12]. Because the vapor pressure of MITC is much lower than other soil fumigants, like methyl bromide, the movement of MITC in the soil pore space is limited, which diminishes

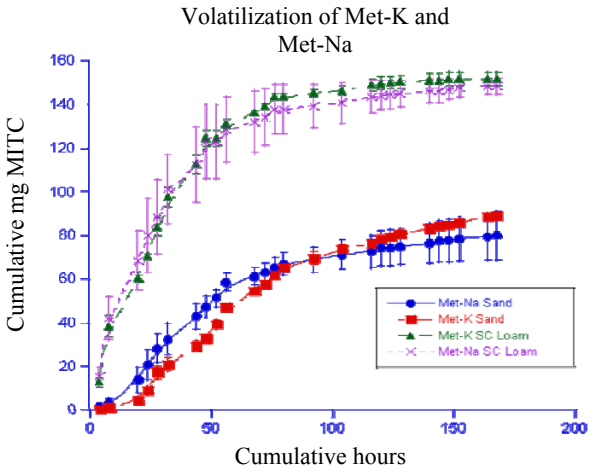

Fig. 1 Cumulative volatilization of MITC by MS and MK in sandy soil vs. sandy clay loam.

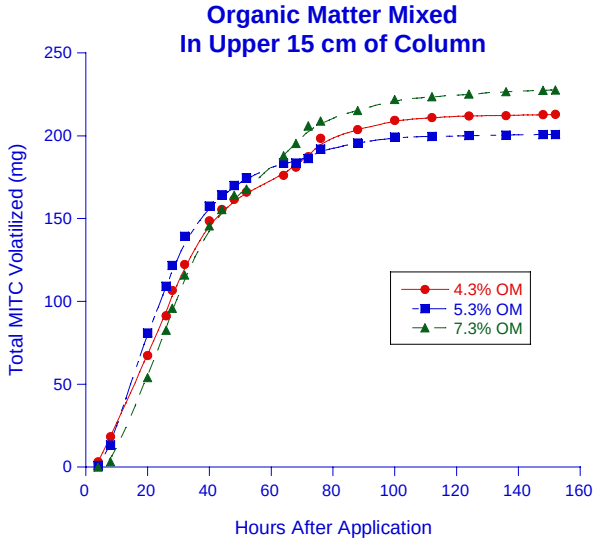

Fig. 2 Total volatilization of MITC from sandy clay loam soil mixed with high organic matter content.

the lateral movement of MITC in soils [13]. Poor lateral movement of MITC has been observed in sandy soils and the results of our study here that focused on MITC behavior in varying soil types provides further evidence that very targeted and direct application of both MS and MK is required when using these chemicals for fumigant pest control. The findings demonstrate that higher volatilization rate and low infiltration of MITC in the sandy clay loam soil would suggest that the smaller pore spaces and the higher affinity for water in the clay fraction of the soil prevent the chemical from moving downward in the soil column profile. As soil columns restrict lateral movement of fumigants, but provide access to vertical chemical movement, it is evident that downward movement is further restricted by soil type (Fig. 1), thus soil pore space plays a dominant role in MITC movement.

Results from the second study with increasing OM resulted in equivalent volatilization loss in all three treatments (Fig. 2). This occurred regardless of varying differences in soil bulk density or high OM incorporation ranging between 10.5-32.6% OM content. The results of this study indicate that MITC is not highly attracted to soil OM and incorporation of compost or another organic carbon source to try to mitigate MITC loss will most likely not be an effective means of reducing fumigant release to the atmosphere. This is an important factor when dealing with soil additives to reduce fumigant volatilization. The wide range of OM contents shows that while the fumigant may not be attracted to the OM in the soil profile, future studies should possibly focus on other means of suppressing the volatile gasses released during fumigation. The findings of this study are in contrast with that observed with other soil fumigants of high vapor pressure, where incorporation of organic matter, soil compaction and tilth all affected the dispersion of soil fumigants [14]. Furthermore, where studies performed with the fumigant 1,3-dichloropropene and compost additions led to decreased pest control efficacy for nematodes [15], our studies suggest that organic matter incorporation would not significantly alter pest control efficacy of MITC.

4. Conclusion

MITC emission into the atmosphere is highly dependent on soil texture, as sandier soils with larger pore space can lead to lower MITC volatilization as the fumigant penetrates deeper down in the soil profile, when compared finer textured soil with smaller pore space. Organic matter application to soils does not appear to be a productive method of suppressing MITC release from the soil as very high OM levels did not

result in improved fumigant retention within the soil. The results of these column studies provide evidence that bench-scale laboratory studies can be performed prior to large and costly field scale studies. The results of this study if taken into consideration will allow researchers to focus on other factors besides OM additions at suppressing MITC fumigant release from the soil. Future studies that may possibly improve MS and MK application and its subsequent suppression of MITC volatilization may be enhanced using surface irrigation applications after chemical injection to provide a water seal to the soil surface. Similar column studies like these can be performed to evaluate at a relatively low cost in the laboratory setting if such ideas will work, prior to implementation at the on-farm level.

Acknowledgments

This research was supported by the USDA-CSREES Methyl Bromide Transition Program Award # 2002-51102-1922, and the USDA-CSREES Hispanic Serving Institute Grant Award #2004-38422-14608.

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Journal of Agricultural Science and Technology A 1 (2011) 199-203
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Comparison of Mosses as Bioindicator of Heavy Metal Pollution in Aramoko-Ekiti and Are-Ekiti, Nigeria

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Received: April 26, 2010 / Published: June 20, 2011.

Abstract: The increasing global concern over the public health impacts attributed to environmental pollution led us to investigate and compare the deposition of some heavy metals in mosses from an urban area, Aramoko Ekiti and a rural area, Are Ekiti. Both towns are located in the SouthWest, Nigeria. Moss samples were collected at different sites in the two towns. These samples were then digested in acid and analysed for Cd, Cr, Ni, Pb and Cu, using a flame atomic absorption spectrometer. The results of the study show variations in the concentrations of the heavy metals among the different sites in each town as well as between the two towns. Apart from cd which was suspected to have originated from natural sources in the investigated samples, the relatively higher concentrations of the other metals in Aramoko-Ekiti suggests an important anthropogenic source which we suspect to be automobiles since there are little or no industrial or mining activities within the town. Furthermore, the relatively higher concentrations of the metals exhibited by moss samples collected around locations prone to higher traffic situations in the two towns such as roadsides, filling stations and garages stresses the significance of traffic density in heavy metal pollution of the environment. These places (filling stations and garages) should be sited far away from residential areas. Also, residences should be sited at considerably far distances from major roads. These will prevent the bioaccumulation of the heavy metals in residents. Though, the results show that Aramoko-Ekiti is more polluted with the heavy metals than Are-Ekiti, the concentration of the heavy metals were still within the permissible limits. Given the results of this work and similar ones, there is need to evaluate the pollution status of the environment from time to time especially the urban areas and high traffic areas.

Key words: Moss, bioindicator, heavy metal, pollution, traffic density, urbanisation.

1. Introduction

There has being a persistently daily increase in environmental pollution, posing a very serious problem for the flora and fauna. A large number of pollutants such as industrial wastes, poisonous gases and heavy metals are adversely affecting our environment. Thus, contamination of the environment by heavy metals have continued to receive attention not only in Nigeria but also all over the world. The significance of automobiles, fossil fuel combustion and incineration of domestic wastes as major sources of heavy metal pollution in the environment especially in the urban areas has been discussed and demonstrated in several studies [1-4].

Heavy metals are metallic chemical elements that have relatively high density. They are extremely toxic or poisonous at low concentration, and at high concentration, they can lead to poisoning [5]. Heavy metal poisoning could result from drinking-water contamination (e.g. lead pipes), high ambient air concentrations near emission sources or intake via food chain. Given the toxicological risks of heavy metals to human health, it becomes imperative to constantly monitor their content in the environment. The pollution status of the environment can be evaluated by physiochemical methods of analysis of the concentration of the pollutant in the air, soil, or water by the use of bioindicators [6]. Though, several materials have been used as bioindicators, including

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lichens, mosses, pine bark samples, pine needle litters, earthworms and moths [7], grasses [8], Kovacs et al. [9] observed that mosses are the most effective bioindicators. Other studies [10, 11] have already discussed the advantages of using mosses for monitoring heavy metal contamination from the atmosphere. Mosses retain heavy metals directly from precipitation, as well as from dry particulate matter owing to their lack of cuticle and true roots. Other advantages include perenniality, ease of sampling, wide distribution, and large capacity for cation exchange [12, 13].

Aramoko Ekiti is an urban area in the South West, Nigeria while Are Ekiti is a rural area in the same geo political zone. Thus, Aramoko Ekiti witnesses more road traffic situation and other anthropogenic activities than Are Akiti. Since there are no serious industrial and mining activities in these towns, the suspected major sources of heavy metals in the environment are automobiles. On the basis of the above, it was anticipated that Aramoko Ekiti will be more polluted with heavy metals from the mentioned source than Are Ekiti. Consequently, this study was designed to investigate and compare the deposition heavy metals in and between the two towns.

2. Materials and Methods

2.1 The Study Areas

The research was conducted in two towns in Ekiti State, Nigeria: Aramoko Ekiti, an urban town with relatively high traffic density; Are Ekiti, a rural town with relatively low traffic density. In the study, automobile was considered as the major source of heavy metal pollution in the towns since there are little or no mining and industrial activities within the towns and their environs.

2.2 Sample Collection, Digestion and Heavy Metal Analysis

Samples of mosses were collected in June 2009. These samples were collected from eight different sites, in each town i.e. Aramoko Ekiti and Are Ekiti. In each site however, three subsamples were collected. Moss samples were rinsed in deionized water and dried at 105 °C after each sample was sieved to remove sandcrete materials and soil particles. They were then milled and digested with digester block and extracts were separated from solid residue by centrifugation at 3500 rpm. Blank determination were carried out using the above procedures. The heavy metal determination in the specimen solutions were read by an air acetylene flame atomic absorption spectrometer (Bulk Scientific Model 210) fitted with a deuterium lamp for cooling of the floor. As a result, values have been counted as $\mu g\ g^{-1}$ dry weight (PPM).

2.3 Data Analysis

The means and the standard deviations were obtained and recorded.

3. Results and Discussion

The average concentrations of the heavy metals in moss samples collected at various sites in Aramoko Ekiti are given in Table 1. The highest level of Cr (132.142 $\mu g\ g^{-1}$) and Cu (150.209 $\mu g\ g^{-1}$) were recorded in mosses sampled from Aramoko Ekiti garage while the highest value of Ni (50.279 $\mu g\ g^{-1}$) and Pb (110.719 $\mu g\ g^{-1}$) were recorded in mosses sampled in Best Option Petroleum Station. These locations are prone to relatively higher traffic situation. The results also show that the concentrations of the heavy metals were relatively low in locations prone to relatively low traffic situation. One of such locations is Igemo, a remote residential area, which was where the least concentration of Cr (3.055 $\mu g\ g^{-1}$), Pb (0.841 $\mu g\ g^{-1}$), Ni (0.931 $\mu g\ g^{-1}$) and Cu (3.692 $\mu g\ g^{-1}$) were obtained. Other locations include Schools (Aramoko Community Grammar School and College of Education Sandwich Program Campus).

The foregoing results show that Cr, Pb, Ni and Cu in locations with relatively higher traffic situation were apparently from an important anthropogenic source

Table 1 Average concentrations ± S.d (μg g^{-1}) of some heavy metals in mosses sampled in Aramoko-Ekiti.

Location	Pb	Cu	Cd	Cr	Ni
Best option petrol station	110.719 ± 39.57	82.773 ± 0.42	1.056 ± 0.00	37.427 ± 0.76	50.279 ± 0.93
Aramoko garrage	71.476 ± 0.00	150.209 ± 0.42	1.760 ± 0.61	132.142 ± 1.53	27.623 ± 1.08
Roadside	40.363 ± 0.00	66.462 ± 0.00	3.169 ± 0.00	38.446 ± 1.59	4.034 ± 0.0.54
NURTW garrage	54.939 ± 2.70	72.062 ± 3.45	2.464 ± 0.61	81.729 ± 0.00	41.899 ± 0.00
Five O petrol station	84.370 ± 0.97	44.552 ± 0.00	1.056 ± 0.00	29.280 ± 0.44	26.071 ± 0.00
Aramoko community Grammar school	2.80 ± 0.49	23.371 ± 0.00	0.000 ± 0.00	10.948 ± 0.44	9.311 ± 0.93
College of education Sandwich program Campus	1.682 ± 0.00	23.615 ± 0.84	1.056 ± 0.00	14.513 ± 0.00	1.552 ± 0.54
Idemo	0.841 ± 0.00	3.652 ± 0.00	0.000 ± 0.00	3.055 ± 0.00	0.931 ± 0.00
X ± S.d	45.899 ± 41.90	58.337 ± 46.14	1.320 ± 1.15	43.442 ± 43.29	20.211 ± 15.53

which we suspect to be mainly the automobiles since there are little or no industrial and minning activities in the study area and its immediate environs. However, in other locations with relatively lower traffic situation, the low concentrations of the heavy metals in the investigated moss samples of the heavy metals in the investigated moss samples could be attributed to incineration of domestic wastes and occassional emmisions from vehicle and generators. Kakulu [1] identified the sources of heavy metals in the urban centres to include fossil fuel burning due to urbanization, automobile exhaust and incineration of domestic waste due to urbanization. The concentration of Cd in samples from all the sites are roughly similar regardless of traffic situation, thus suggesting that the metal was probably of natural (soil) origin. Wind blown mineral particles may also serve as a source of the heavy metals in mosses other than atmospheric deposition [14]. In comparison with data obtained from Ilorin, an urban centre in Nigeria, the levels of Cd and Ni are lower. Heavier road traffic with some industrial activities would probably explain the higher heavy metal burden in the mosses of Ilorin. The Cd, Cu and Pb contents were however comparable with the data reported for the mosses of Shillong, India [2].

Table 2 presents the mean contents of the heavy metals in mosses collected at different locations in Are Ekiti, Nigeria. Just like for the mosses of Aramoko Ekiti, it is worthy of note that the concentrations of Cr, Pb, Ni and Cu were higher in locations prone to higher traffic situation such as roadside, market and conprehensive health centre. However, the highest concentration of the heavy metals except Ni were recorded in moss samples collected around roadsides. Ho and Tia [8] reported that the major source of elevated levels of Cu, Zn, Fe and Cd in roadside plants was motor vehicle. The assertion is confirmed by the fact that these metals are important components of many alloy pipes, wires and tyres in motor vehicle. The metals are released into the environment as a result of mechanical abrasion and normal wears and tears. The results further reveal that mosses collected from remote residential areas such as Idemorun had relatively low concentrations of the heavy metals (Table 2). Little or no traffic and industrial or minning activities in these areas would probably explain this. The concentration of Cd in all the sites regardless of traffic situation are also roughly similar. This suggests that the metal was probably of natural origin. In Nigeria, most of the investigations [1, 3, 4, 15] have been largely confined to urban areas, with minimal information about the level of pollution in the rural areas. This will limit comparison of data in Are Ekiti with other rural areas in Nigeria.

Table 3 shows the comparison of average concentrations of the heavy metals in mosses sampled in Aramoko Ekiti and Are Ekiti. The concentration of Cd in the two towns is roughly similar regardless to traffic density and urbanization. This suggests that the metal is of natural origin (wind blown mineral particles). The concentration of the other metals are higher in Aramoko Ekiti than Are Ekiti. This suggest

Table 2 Average concentration (μg g^{-1}) of some heavy metals in mosses sampled in Are Ekiti.

Location	Cd	Cr	Pb	Ni	Cu
Comp. Health Centre	0.000 ± 0.00	14.513 ± 0.00	9.811 ± 0.48	10.242 ± 0.00	43.578 ± 0.84
Odo ode	0.000 ± 0.00	24.188 ± 0.60	3.363 ± 0.00	3.163 ± 1.41	24.832 ± 0.00
Idemorun	0.000 ± 0.00	4.328 ± 0.44	1.121 ± 0.49	0.931 ± 0.00	8.277 ± 0.42
Market	0.000 ± 0.00	36.664 ± 0.00	9.250 ± 0.00	1.241 ± 0.54	18.259 ± 0.00
Roadsides	3.521 ± 0.56	34.929 ± 2.21	15.416 ± 0.97	2.793 ± 1.47	28.240 ± 0.42
Oke aafin.	0.000 ± 0.00	11.457 ± 0.00	3.083 ± 0.97	1.862 ± 0.00	6.086 ± 0.84
Palace	0.000 ± 0.00	38.955 ± 2.65	1.121 ± 0.49	0.931 ± 0.00	6.573 ± 0.00
Are/Afao Pry Sch.	2.817 ± 0.61	6.874 ± 0.00	5.326 ± 0.97	3.414 ± 0.54	19.720 ± 0.00
X ± S.D	0.792 ± 1.38	21.489 ± 14.04	6.061 ± 4.90	3.072 ± 3.06	19.446 ± 12.87

Table 3 Comparison of average concentrations (μg g^{-1}) of some heavy metals in mosses sampled in Aramoko Ekiti and Are Ekiti.

Heavy metal	Aramoko Ekiti*	Are Ekiti**
Cd	1.320 ± 1.15	0.792 ± 1.38
Cr	43.442 ± 43.29	21.489 ± 14.04
Pb	45.899 ± 41.90	6.061 ± 14.04
Ni	20.211 ± 15.53	3.072 ± 3.06
Cu	58.337 ± 46.14	19.446 ± 12.87

*: Average traffic density of 472 Vehicle/hr; **: Average traffic density of 93 Vehicle/hr.

an important anthropogenic source which we suspect to be mainly automobiles since there is little or no industrial or minning activities in the town. Many researchers have shown that urban areas receive a load of contaminants usually greater than surrounding sub-urban areas to the concentration of anthropogenic activities of urban settlements [16-18].

Generally, the foregoing results reveal that the concentration of the heavy metals in the two towns are low and therefore still within permissible limits [19].

4. Conclusion

This result confirms the presence of heavy metals in the environment. It further stresses the significance of traffic density and urbanization in heavy metal pollution of the environment. The study also reveals that the level of the heavy metals are relatively higher in places prone to dense traffic situation within each town. Therefore to prevent possibleaccumulation of the heavy metals in residents, such places should be sited far away from residential areas. Also, residences should be sited at considerably far distances from the major roads. Finally, given the results of our present study and similar ones, it is important to evaluate the pollution status of the environment from time to time especially the urban areas and high traffic areas.

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Journal of Agricultural Science and Technology A 1 (2011) 204-210
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Effect of Dendranthema on *Cotesia plutellae* Parasitism in Brassicaceous Crops: Control of Diamondback Moth (DBM), *Plutella xylostella* (Linnaeus)

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Received: May 20, 2010 / Published: June 20, 2011.

Abstract: The parasitism rate and the caterpillar infestation were studied by intercropping Dendranthema flowering plants with some cruciferous crops. These flowering plants increased significantly the rate of *Cotesia plutellae* parasitism in the adjacent plots. It reached to 42.96% in treatment plot and 18.19% in the control. Although there was no significant difference in no. of diamondback moth (DBM) larvae per plant, 29.09% in population reduction was occurred in treatment plot. Abundance of cabbage looper and armyworm were also lower in adjacent plot. But, small white butterfly population (23.46%) was higher in adjacent plots as the result of Dendranthema plant. From the present experiment, Dendranthema flowering plants should be cultivated in Brasssicaceous crops for the control of DBM by providing the essential resources to larval parasitoid *C. plutellae* adults and then, this plant can be used as attractive plant in the control of small white butterfly. Serious elimination (Rating 4) of candidate insecticides to adult *C. plutellae* was observed at their recommendation doses. Among them, less toxic effect to *C. plutellae* was occurred in O,O-dimethyl S-methylcarbamoylmethyl which is the highest LC_{50} (4,765 ppm) to DBM by leaf-disc bioassay method and the lowest LC_{50} (2,903 ppm) was found in marlathion. It was occurred that the test strain have resistance to the recommended dose of marlathion (1,243 ppm) and O,O-dimethyl S-methylcarbamoylmethyl (3,750 ppm).

Key words: Dendranthema, *Cotesia plutellae,* biological control, diamondback moth, brassicaceous crops.

1. Introduction

Myanmar is an agriculture-based country with the vast land and aquatic based resources. Agriculture has an important role in Myanmar's economy. Myanmar has wide variety of climate and soils on which a range of vegetable crops can be grown. By having different agro-economical zones, it has been growing cereal crops, oilseed crops, pulses, industrial crops, vegetables, fruit and flowers under different cropping systems.

Among the vegetables, Cruciferous vegetables are the economically important crops in Myanmar and are grown on about 9,800 hectares in 1996-1997 and 12,000 hectors in 1998-1999 [1]. Cabbage is attacked by the different kind of insects. The diamondback moth (Lepidoptera: Plutellidae) is one of the most destructive *Brassicae* crop pest and widely distributed insects in the world [2].

In Asia, it has become resistant to nearly every class of insecticide [3, 4] even *Bacillus thuringiensis* [5, 6]. In order to reduce the damage by insects growers use several applications of different insecticides [7]. When new and effective insecticides become available, continuous and overdose use of insecticides force the diamondback moth to develop resistance and the product is no longer effective. This can occur within one or two growing seasons. The overuse/abuse of pesticides against diamondback moth has become a serious problem in many parts of Asia. This overuse causes not only health problems to farmers applying

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the pesticides, but also environmental degradation, and may result in excessive residues in vegetables [8]. In addition, Sandur [9] reported that natural enemies of diamondback moth and other pests are hazarded by this because of over-reliance on pesticides.

L. Guan-Soon [10] found that refuge or shelter plants will become important in the control of DBM as they provide food for the survival of parasitoids, particularly in avoiding excessive pesticide sprays. These plants include many species of wild flowering plants and cultivated legumes (e.g. beans and peas). Planting selected flowers into brassicaceous crops could enhance control of the diamondback moth by parasitic wasps [11].

Dendranthema, *Chrysanthemum* spp. is basal mounds of foliage bursting with 24″ stems of 3″ white daisies. These species are familiar as garden flowers, of which there are many varieties in cultivation, blossoming freely, the tufts of foliage of a dark green, and the flowers of all shades of rose and crimson. The objective of this study is to preserve the natural enemies by intercropping and selective application of insecticides for the control of DBM.

2. Materials and Methods

2.1 Field Experiment

This experiment was carried out in farmer's field, located in Pyin Oo Lwlin Township, Mandalay Division, Myanmar, in the beginning of winter season (September 2008, 25 ± 5 °C, 75 ± 5 RH). Larval sampling was taken during the midseason (4-8 weeks after transplanting) of cruciferous growing season. About 0.2 hectares of cabbage, cauliflower, and kale plots were used for each treatment in this experiment. Dendranthema flowering plants were cultivated at the top of them. CAB1, CAU1 and KAL1 were treatment plots and situated bordering to the Dendranthema flowering plot. CAB2, CAU2 and KAL2 were used as control and 20 meter away from the flowering plot as shown in Fig. 1. The remaining planted area between each sub-plot was served as buffer.

2.2 Caterpillar Infestation in Cruciferous Crops Affected by Dendranthema

Sampling was carried out before insecticide treatment. Average ten plants from each site were selected in random, avoiding the outer line of the plot and thoroughly scouted for any larvae. Samples from each sub-plot were taken to the laboratory where all live larvae were provided with cabbage leave and all caterpillars were identified and abundance of each was examined. Replication was two times with 14 days interval. Two sampled t-test at 95% confidence interval was used in order to compare the larval incident of treatment and control plots.

2.3 Rate of Cotesia plutellae Parasitism in Cruciferous Crops Affected by Dendranthema

In this experiment, parasitism rate of DBM larval parasitoid *C. plutellae* was studied two times with 14 days interval. Average 10 larvae of DBM were collected from each sub-plot and larva was placed individually in 4.5 cm diameter of vial, in which the larva was provided with fresh cabbage leaves. The vials were covered with paper for ventilation. The leave were replaced every second day, until larvae pupated or parasitoid cocoons were formed. Insects were held in the laboratory at 23 °C ± 2 °C, 70 ± 5% RH with natural light. Emergent parasitoids were identified and the incidence was calculated. Larvae that escaped or died of unknown causes were disregarded for calculating rates of parasitism. Parasitism rate was expressed in term of percentage for each plot and two

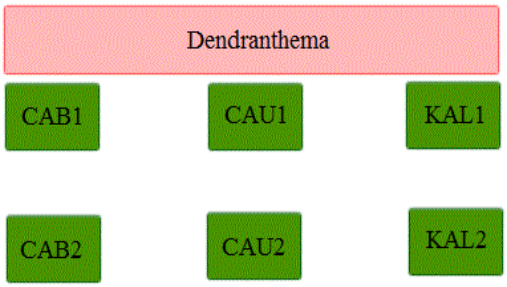

Fig. 1 Field layout for the experimental trial at farmer's field in pyin Oo Lwin, CAB-cabbage, CAU-cauliflower, KAL-kale.

sampled t-test was used in order to compare the treatment and control.

2.4 Elimination of Insecticides on C. plutellae Adults and Diamondback Moth Larvae

2.4.1 Parasitoid Colony Rearing

Potted common cabbage plants were used for rearing of DBM larvae. Freshly detached cabbage leave were placed around the cabbage potted plant on the floor of the cage. This served as food for those larvae of DBM which fell from the plant. About 100 larvae of 2nd and 3rd healthy instars were used as hosts for parasitoid rearing. A cabbage leave with DBM larvae was placed into the stock culture cage of *C. plutellae* to which 10% honey soaked cotton wool was provided to reduce their food stress. The larvae of DBM were withdrawn at 24 hours after introduction to the parasitoid cage. The parasitized larvae of DBM were observed and provided with fresh cabbage leaves and they were transferred to sterilized beaker until pupation of *C. plutellae* was observed. The cocoons of parasitoid were collected using fine brush and placed in the clean bottle for further study. The parasitoid culture was maintained under laboratory condition of 28 ± 3 °C, 80 ± 5 % RH with natural light.

2.4.2 Insecticides

Candidate insecticides used (malathion, chlorpyrifos and O,O-dimethyl S-methylcarbamoylmethyl) were widely used in Myanmar to control the DBM population in cultivation of cruciferous crops. The toxic effect of these insecticides on *C. plutellae* was tested with their respective recommended application doses of marlathion 1,243.75 ppm, chlorpyrifos 7,500 ppm, and O,O-dimethyl S-methylcarbamoylmethyl 3,750 ppm.

2.4.3 Toxicity of Insecticides to *C. plutellae* Adults

The dry film method was employed as a test method [12, 13]. Elimination of these insecticides on *C. plutellae* adults was studied at their respective recommended doses and distilled water was provided as a control. All insecticide solutions were diluted with distilled water to get the required concentration. 2.3 cm squares of cabbage leave were prepared and dipped in each insecticide solution for 5 sec. These cabbage leave were allowed to air-dry for 2 hours and pesticide-treated and control cabbage leave was added individually to bottle of 6 × 3 × 3. Adult *C. plutellae* (2-3 day old) was released into bottle. Then, it was covered with cotton cloth for good ventilation and parasitoid wraps were fed with honey soaked cotton wool. Ten adults of *C. plutellae* were used for one treatment and all tests were replicated two times. Mortality was determined 24 and 48 hours after treatment. Any adult that did not move in a 10 sec period, even though touched with fine brush, was recorded as death. The experiment was carried out at 28 ± 3 °C, 80 ± 5 RH with natural light.

2.4.4 Toxicity of Insecticides to Diamondback Moth Larvae

A leaf-disc bioassay was carried out to estimate the levels of resistance to candidate insecticides. The test DBM strain was collected from Pyin Oo Lwin Township, Mandalay deviation, Myanmar in September 2008. This field strain was taken to the laboratory and the larvae were reared on potted kale young plants and adults were released into cages of 15 × 15 × 15. The 2nd instars of 3rd generation were exposed to the insecticides. As a rule, five concentration doses with 30 larvae (10 larvae/ replication) were used for each insecticide. Leaf tissue discs of 4 cm diameter were cut from cabbage leaves and submerged in each solution for five seconds and than allowed to air dry in the environment for one and half hours. After this period, the individual disc was placed in 4.5 cm vials and the larvae were transferred to the leaf disc. Each vial was covered with filter paper and kept at 25 ± 3 °C, 70 ± 5% RH. Mortality was examined 24 hours after treatment. Larvae were considered dead if they did not move or twitch after 10 sec. of being probed with a hairbrush. If mortality in control was higher than 10%, experiment was carried out again. In order to determine percent mortality, lives

and deaths of each concentration were accumulated and LC_{50} for each insecticide was estimated from linear regression line and equation with the help of MS Excel.

3. Results and Discussion

3.1 Effect of Dendranthema on C. plutellae Parasitism and Larval Infestation

The performance of natural enemies in agricultural systems is often limited by the absence or scarcity of essential resources [14] and they need a source of sugar to realize their maximum longevity and fecundity [15]. Keller and Baker [16] reported that the flowering plants Alyssum (*Lobularia maritime* [L.] Desv, Brassicaceae) and *Diadegma insulare* (Cresson) failed to improve biological control of diamondback moth. But, in this study, it was occurred that parasitism rate of *C. plutellae* was significantly higher in treatment plot and it reached to 42.96% in plots adjacent to the flowering plants but 18.19% in away plots from the flowering plants. In cauliflower, the parasitism rate (61.11%) was occurred in treatment plot and it was three-fold higher than the control. The parasitism (20.56%) was occurred in kale and 47.22% of DBM larvae were parasitized by *C. plutellae* in cabbage plot. From the data shown in Table 1, the parasitism rate on DBM was higher in treatment plots than the control. It can be assumed that the higher parasitism of *C. plutellae* was the result of sufficient nectar from this flowering plant to increase their longevity and fecundity. N. J. Mills and É. Wajnberg [17] supported that the parasitoids could provide enhanced pest suppression in the presence of a nectar subsidy as time spent searching for food. However, comparison between parasitism rates in different cruciferous crops was not conducted in the present work.

Effect of Dendranthema flowering plot on larval population was also studied in both adjacent and away plots of cruciferous crops and the data was shown in Table 2. Three main caterpillar infestations, DBM, small white butterfly, and cabbage looper, were occurred in this mid-growing season of cruciferous crops in Pyin Oo Lwin Township and the other caterpillar, armyworm, was rarely occurred. The caterpillar abundance was lower in treatment plot. The number of DBM larvae was lower in adjacent plots (0.39 larvae per plant) than the away plots (0.55 larvae per plant) but not significant. However, it was noticed that Dendranthema flowering plant could reduce the density of DBM larvae by 29.09%. Moreover, 57.4% reduction of cabbage looper infestation was occurred in treatment plot. Armyworm was the lowest infestation

Table 1 Effect of dendranthema flowering plot on DBM larvae infestation per plant and *Cotesia plutellae* parasitism rate at different cruciferous crops.

Treatment	Kale		Cauliflower		Cabbage		MEAN±SD	
	P	N	P	N	P	N	P	N
Adjacent (Mean)	20.56	0.45	61.11	0.55	47.22	0.15	52.96 ± 19.77^{b}	0.39 ± 0.21^{a} *29.09%
Away (Mean)	14.29	0.68	16.67	0.73	23.61	0.25	18.19 ± 4.42^{a}	0.55 ± 0.26^{a}

Means followed by the same letter within a column for each treatment are not significantly different ($P > 0.05$; two sample t-test); P: % parasitism, N: No. of larvae; *: the percent decrease in population = (Control - Treatment)/ Control × 100.

Table 2 Effect of dendranthema flowering plot on caterpillar infestation per plant in cruciferous crops.

Insect	Kale		Cauliflower		Cabbage		Average (MEAN±SD)	
	Adjacent	Away	Adjacent	Away	Adjacent	Away	Adjacent	Away
D	0.45	0.68	0.55	0.73	0.15	0.25	0.39 ± 0.21^{a} *29.09%	0.55 ± 0.26^{a}
S	9.58	1.91	4.18	2.3	4.5	2.2	6.18 ± 3.17^{a}	2.37 ± 0.21^{a} *23.46%
C	0.19	0.55	0.1	0.05	0.1	0.25	0.16 ± 0.1^{a} *57.14%	0.33 ± 0.33^{a}
A	0.0	0.05	0.0	0.0	0.0	0.0	0.0^{a} *1%	0.03 ± 0.05^{a}

Means followed by the same letter within a row for each treatment are not significantly different ($P > 0.05$; two sample t-test) D-DBM; S: small white butterfly, C: cabbage looper, A: armyworm, *: the percent decrease in population.

during this season. But, small white butterfly was higher in treatment plots (23.46%) as an exception against the control and reached to 6.96 per plant. From this experiment, Dendranthema flowering plant has the ability to attract the small white butterfly. Therefore, this flowering plant can be used in DBM control by preserving parasitoid *C. plutellae*, as well as, the attractive plant in the control of small white butterfly.

3.2 Effect of Insecticides on C. plutellae Adults and Diamondback Moth Larvae

Marlathion, chlorpyrifos, and O,O-dimethyl S-methylcarbamoylmethyl were mostly used to control the insect pests in this cultivation area as the recommended doses. Based on the criteria suggested by Miyata et al. [18], insecticides were rated into four groups based on their harmful potential to parasitoids. From the experimental data (Table 3), mortality of *C. plutellae* reached to rating 4 after 48 hr incubation. It was examined that the candidate insecticides were seriously eliminating the *C. plutellae* at their respective application doses. Thus, it was stated that introducing selective insecticides is one of the most important strategies to preserve natural enemies and it can retard or avoid the development of insect resistance to insecticides [19].

In parallel, the relationship between larval mortality of DBM and various concentrations of each insecticide was estimated by simple regression analysis (Figs. 2-4) and it was used to compare the potency of insecticides on the selected DBM strain. In this experiment, 2nd instars larvae of 3rd generation were used as test insect and they were chosen to be homogenize as resistance to insecticide can vary depending on age, sex, stage, condition of nutrition, etc. The lowest concentration of LC_{50} was occurred in malathion whereas it was about 2,903 ppm of AI and the highest LC_{50} (4,765 ppm) was recorded in O, O-dimethyl S-methylcarbamoylmethyl. This experiment showed that this test strain has developed resistance to the field application doses of O, O-dimethyl S-methylcarbamoylmethyl (3,750 ppm) and marlathion (1,243 ppm). On the other hand, it was found that the application dose of chlorpyrifos (7,500 ppm) was higher than its LC_{50} (3,312 ppm) by leaf-disc bioassay method. The overdose of insecticides can lead to resistance development of insect and the adverse effects to human and environment. Less toxic effect was occurred in O, O-dimethyl S-methyfcarbamoylmethyl to *C. plutellae* by thin film method. However, all these candidate insecticides were seriously eliminating the natural enemie, *C. plutellae.* Thus, insecticides selection is important in preservation of natural enemies and resistance management for the control of DBM.

4. Conclusion

Based on the results, it can be pointed out that planting Dendranthema flowering plants into brassicaceous crops enhance the control of the diamondback moth by attracting parasitic wraps *C. plutellae*. Therefore, it can be used for preservation of natural animals. And, it is important as an alternate in resistance management of DBM. Moreover, it can be used in the control of other caterpillar infestations. In turn, it can be used as attractive plant for small white

Table 3 Elimination of various insecticides at their application doses to adults of *Cotesia plutellae* after 24 and 48 hours.

Treatment		% Mortality after treatment		Rating
Active ingredient	Concentration (ppm)	24 hours	48 hours	
Marlathion	1,243.75	100	100	4
Chlorpyrifos	7,500	90	100	4
O,O-Dimethyl S-methylcarbamoylmethyl	3,750	60	100	4
Control		0	0	1

1 = harmless (< 50%), 2 = slightly harmful (50-79%), 3 = moderately harmful (80-90%), 4 = harmful (> 99%).

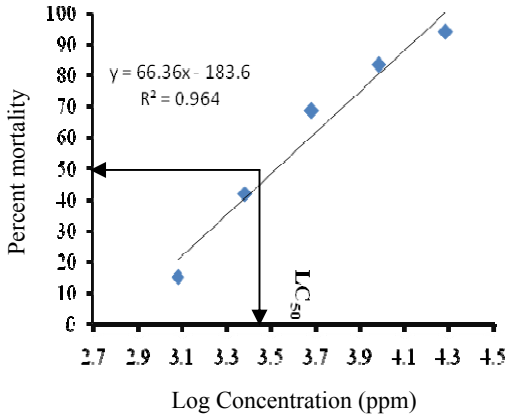

Fig. 2 Relationship between larval deads and various concentration of chlorpyrifos by leaf-disc bioassay method.

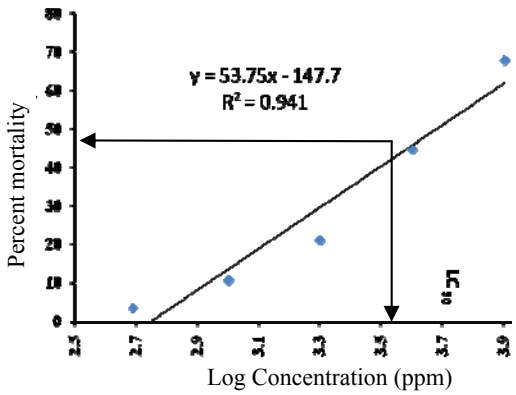

Fig. 3 Relationship between larval death and various concentration of O, O-dimethyl S-methylcarbamoylmethyl (ppm) by leaf-disc-bioassay method.

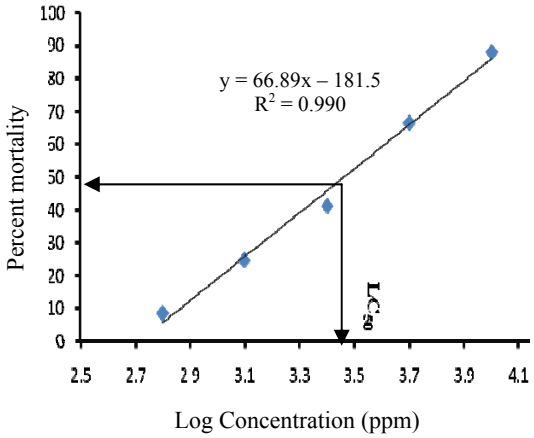

Fig. 4 Relationship between larval deads and various concentration of marlathion by leaf-disc bioassay method.

butterfly. Therefore, intercropping of Dendranthema flowering plants is effective in cruciferous crop cultivation.

From this experiment, the insecticides used to control the diamondback moth were seriously eliminating the wrap *C. plutellae* but DBM larvae have been resistance to the field application dose of these candidate insecticides except O, O-dimethyl S-methylcarbamoylmethyl. It can be pointed out that not only toxicity of insecticides to pest insects but also natural enemies should be taken into consideration in the control of DBM.

Acknowledgements

The authors are grateful to Myo Myint and Zaw Khing Oo (Department of Biotechnology, Mandalay Technological University) for critical review of the manuscript. Special thanks are extended to San San Lwin, Biological Control Section, Ministry of Agriculture and Irrigation for providing technical assistance.

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Journal of Agricultural Science and Technology A 1 (2011) 211-216
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Pathogenecity of Avian Influenza Virus H5N2: A Report from Western Iran

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Received: July 19, 2010 / Published: June 20, 2011.

Abstract: Influenza type A, is an avian disease with a complicated ecology and transmission routes in verity of avian and mammalian species. The present study aimed to demonstrate the characteristic, clinical and experimental features as well as pathogenecity of Avian Influenza Virus H5N2 through a laboratory-based experiment in western Iran. A post-mortem examination of experimentally chickens was undertaken in 2007. Overall 25 local native chickens including 15 layers and 10 roosters suspected with AI infection as well as 50 experimental chickens were studied. The virus was isolated from the embryonated specific pathogen-free (SPF) chicken eggs. There was an embryo mortality rate of 71% within 48 hours post inoculation (PI). Hemagglutinin (HA) inhibition titres against AIV subtype H5N2 in the layers ranged from 4.20 to 4.75 (acute) and 6.21 to 7.82 (convalescent). Accumulated mucous in trachea of the dissected birds, congested lungs, atrophied bursa, haemorrhagic cecal tonsils and inflamed thymus were the main clinical symptoms. Thickened and infected air sacs, pre hepatitis and enteritis signs were also observed. In experimental birds, the eyes' colour became red and the eyelashes were almost double in diameters after being infected. The AI virus found in the present study was classified as a highly pathogenic avian influenza.

Key words: Avian, influenza, chicken, H5N2 virus, Iran.

1. Introduction

Avian influenza virus (AIV) is an 8-segmented RNA belongs to Orthomyxoviridae family and cods 10 proteins including Hem-agglutinin (HA) or Neuraminidase (NA) [1, 2]. The RNA has a high frequency of mutations inducing antigenic changes in either H or N molecules. Currently, 16 HA subtypes (H1, H16) and 9 NA subtypes (N1, N9) are known to allow 144 various combinations potentially.

H5N2 as a subtype of the influenza virus A can infect a wide range of avian species including poultry [3]. The highly pathogenic avian influenza is determined if an intravenous pathogenic index in a 6 weeks old chicken was greater than 1.2 or caused at least 70% mortality in 4-8 weeks old infected chickens.

Recently an outbreak of H5N2 avian influenza was reported in Texas, the first highly pathogenic strain in the United States in 20 years [4]. Nowadays the virus has been spread worldwide [5-12], resulted in depopulation of more than 140 million birds [1, 13]. From late 2005 to 2006 the virus H5N2 spread through central and western Asia to Europe [1, 14]. In 2006, a series of HPAI outbreaks was reported in Iraq, Azerbaijan, Pakistan and Afghanistan [14, 15].

In Iran, in 1998 an AI outbreak was occurred and affected poultry industries seriously [16]. In 2006 in order to stop the AI outbreak, thousand birds were slaughtered by the Iranian Veterinary Organization in Ilam province western Iran.

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The present study as the first report of a highly pathogenic AIV H5N2 in this region, aimed to demonstrate the characteristic, clinical and experimental features as well as pathogenecity of AI through a laboratory-based experiment from western Iran, which helps health workers and policy makers to improve preventive veterinary programs.

2. Materials and Methods

2.1 Study Settings

The study was undertaken in Mehran and Dehloran districts located in western Iran (Ilam province). The map is provided in Fig. 1. The experimental design and laboratory tests were carried out in the School of Veterinary Sciences Ilam University.

2.2 Virus Isolation

Tissue samples were collected from 25 local native chickens flocks including 15 layer and 10 roosters suspected of AI infection. A 10% (w/v) suspension of mixed homogenized different organs including trachea, air sacs, kidneys, hearts, liver, bursa and spleens was prepared in phosphate buffered saline containing 1000 I/mL Mycostatine and 2,000 µg/mL Streptomycin. The suspension was centrifuged at 1,500 rpm for 20 minutes. Through two passages attempted, the supernatant was inoculated in 9-day-old embryonated specific pathogen free (SPF) chicken eggs (n = 50) via allantoic sac route. The eggs were incubated at + 37 °C and daily checked for embryo mortality. The allantoic liquid (fluid) collected from embryos that died between 24 and 96 hours post inoculation and tested by hemagglutination (HA) and hemagglutination inhibition (HI) using specific antiserum to H5 AIV subtype.

Fig. 1 Geographical map of study settings.

2.3 Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

The supernatant liquid (fluid) of homogenised tissue suspension of natural and experimental AIV infection was tested for the presence of AIV. The H5N2 is a monoclonal virus and mRNA was collected from blood serum for its detection. The RNA isolation process was done using RNX-plus kit (Cinagen Co. Tehran/Iran). The first strand cDNA for RT-PCR was synthesized using a Random-Hexamer primer and a Revert Transcriptase Enzyme (Revert Aid First strand cDNA synthesis kit, K1622, Feramentase, EU). The PCR primers were designed by Gene Runner software (version 3). The primer sequences were; Forward: 5′3′ and Reverse: 3′5′. The PCR reaction was conducted according to the following program: 5 minutes at 95 °C, 45 seconds at 95 °C, 45 seconds in annealing temperature of 58 °C, 45 seconds at 72 °C for 35 cycles and 10 minutes at 72 °C.

2.4 Animal Experiment

The H5N2 lyophilized antisera and antigen to use in HA/HI antigens and genotyping of the isolates was obtained from the Laboratory of Microbiology Department, Veterinary University in Lahore/ Pakistan. The hemagglastinating new isolate from the infected chickens was repeatedly inoculated at 3 days intervals to rabbit (n = 6) to obtain hyper-immune serum. The

acute and convalescent period sera were obtained from experimental local breed chickens including 15 layer and10 roosters whom were infected intravenously with $10^{4.9}$ ELD_{50} using viruses collected from allantoic liquid of dead embryos eggs after second passage. Chickens were also tested for HI antibody using the H5N2 AIV antigen as recommended earlier [14].

2.5 Hemagglutination Inhibition Test

The HI antibody titre was determined [14]. The HA titre of the test antigen was reciprocal to the highest dilution when the RBCs had settled down at the bottom of well 12 and HA activity became clear in well 1.

The HI test for each serum sample was serially diluted in PBS, pH 7.2 (dilutions 1:2 to 1:1024) from micro-wells 1 through 10. Wells 11 and 12 were used as the antigen and erythrocyte controls respectively. A 25 uL amount of 0.5 % chicken erythrocyte suspended in PBS, pH 7.2 was added to each well and the results were recorded after incubating the plates at room temperature for 30 minutes.

2.6 Post-Mortem Procedure

The post-mortem procedures of morbededent birds were performed under a very high bio-security measurement in the post-mortem examination hall at the School of Veterinary Sciences, Ilam University where clinical symptoms of each organ including air sacs, trachea, liver, kidney, spleens, thymuses, nasal discharged, bursa, preventiculer were obtained and recorded. All procedures were undertaken under supervision of Veterinary Department Animal Care Unit.

2.7 Serology Sera Sampling

Serology sera samples of 25 local chickens which had experienced sickness during the past 2-3 weeks were examined for HI antibody to IBV (M, 41, D. 274, D.1466 types), NDV, AIV (H5N2) and Mycoplasma Gallisipticum. MG antibodies were also examined using serum plate agglutination tests. The salmonella pullorum (SP) antibodies were detected using serum plate tests. The SP antigen was obtained from the Cinagen Laboratory, Tehran- Iran. The antibody to MG was also determined using the serum plate tests as previously recommended [14].

3. Results

3.1 Virulence of Virus in ECE and Experimental Chickens

The maximum embryo mortality was observed after 48 hours post inoculation (PI) and all experimental embryos were died after 96 hours PI (Table 1). All the birds died 120 hours PI (Table 2).

The allantoic liquid (fluid) AF and amino allantoic liquid (fluid) AMN-F indicated well HA activity upon with chicken RBCs. The HA titre in pooled AF and AMN-F on first passage was 8; and at the second passage was 32, indicating that the virus titre increased upon further passaging. The AF had higher HA activity than AMN-F. The layer chickens in acute phase of illness had a decreased geometric mean (GMT) HI titre against AIV. The GMT HI titre in acute illness was 4.75 and the highest GMT HI titre in convalescent sera noted in layer birds was 7.69. The maximum GMT HI titres in acute and convalescent phases in roster were 4.75 and 8.01 respectively (Table 3).

3.2 Clinical Symptoms Observed

Clinical symptoms were found in experimental chickens after post-mortem examinations with mucous accumulated in trachea of the dissected birds. Lungs were congested, bursa was atrophied, cecal tonsils were haemorrhagic and thymus was inflamed. Thickened and infected air sacs, pre hepatitis and enteritis were also observed. In experimental birds, the eyes' colour became red and the eyelashes were almost double in diameters after being infected. The clinical symptoms of experimental chickens were similar to those observed during pot-mortem examination in naturally infected birds.

Table 1 Cumulated frequency of mortalities due to H5N2 virus in experimental chickens*.

Controlling times (hours)	12	24	36	48	60	72	84	96
Number of mortalities (n = 50)	2	7	9	18	5	5	2	2
Cumulated frequency (%)	4	18	36	72	82	92	96	100

* Dose 10 4.9 ELD 50 by intravenous rote.

Table 2 Cumulated frequency of mortalities of H5N2 virus in embryonated chicken eggs PI*.

Controlling times (hours)	12	24	36	48	60	72	84	96	108	120
Number of mortalities (n = 25)	0	0	0	1	2	3	6	10	2	1
Cumulated frequency (%)	0	0	0	4	12	24	48	88	96	100

* Post inoculation, with dosage of 0.1 mL/egg.

Table 3 AIV HI antibody titre in sera of chicken layers and rosters.

Flock	Type of birds	Phase	G. Mean*
A	Layer	Acute	4.24
B	Layer	Acute	4.41
C	Layer	Acute	4.31
D	Layer	Acute	4.20
E	Layer	Acute	4.35
F	Layer	Acute	4.28
G	Layer	Acute	4.37
H	Layer	Acute	4.75
I	Layer	Acute	4.21
J	Layer	Convalescent	6.94
K	Layer	Convalescent	6.21
L	Layer	Convalescent	6.99
M	Layer	Convalescent	7.39
N	Layer	Convalescent	7.26
O	Layer	Convalescent	7.69
P	Rooster	Acute	4.62
Q	Rooster	Acute	4.85
R	Rooster	Acute	4.29
S	Rooster	Acute	4.48
T	Rooster	Convalescent	8.01
U	Rooster	Convalescent	7.35
V	Rooster	Convalescent	7.48
W	Rooster	Convalescent	7.80
X	Rooster	Convalescent	7.61
Y	Rooster	Convalescent	7.44

Convalescent sera was collected between 7-13 days of post initial signs of illness.

* Geometric mean.

4. Discussion

The present study describes H5N2 virus inoculated from local native chickens and roosters from bordering areas between Iran and Iraq in 2006, where more than thousands birds were slaughtered through a successful policy and mission by the Iranian Veterinary Services to stop spreading the infection inside poultry industries [16].

AIV H5N1 emerged in 1996 in china [5], and has expanded its geographical distribution to affect poultry and human populations in Asia, Europe and Africa [11, 17]. By the end of 1997, the bird flu was increasingly started to spread in major parts of Asia where affected within a few month [18, 19]. In Iran during 1998-01, influenza (AI) occurred in broiler chickens with a mortality rate of 20% [20], using AIV experimental inoculation of H5N2 to 4 week old chickens [21].

The AIV H5N2 isolated in the present study induced 71% death in chicken embryos within 48 hours and 100% after 96 hours PI, indicating an extremely virulent. AIV has already been propagated in 9-11 days old chicken embryos and induced embryonic death at varying time intervals following inoculation [22]. The embryonic mean death time depends on the AIV subtypes [23]. There is evidence that AIV serotype H5N2 induced embryonic death within 48 hours [24]. The isolation of AIV types such as H5N2 [25], and H5N1 [26-27], from various avian species in a variety of countries have already been documented. A highly pathogenic strain of H5N2 has caused flu outbreaks, resulting in great economic losses in 1983 in chickens and turkeys in Pennsylvania and in chickens alone in Mexico [28]. A number of epidemiological assumptions such as increased number of wild migrated birds, as well as heavily air pollution due to dusty and dry whether in this area through recent years could be suggested to

justify this situation, which needs further investigations. The clinical symptoms observed in the present study were similar to those previously reported [25, 29, 30].

5. Conclusions

The present study described the characterization of H5N2 influenza virus in local native breed chickens from western Iran. The AI virus found in the present study was classified as a highly pathogenic avian influenza (HPAI) by producing a mortality rate of 71%. Further laboratory-based studies and considerations in terms of diagnostic procedures using more accurate equipments and methods are recommended.

Acknowledgments

The Iranian Ministry of Higher Education financially supported the present study through a research grant awarded to Dr A. M. Bahrami. The cooperation of the Laboratory of Microbiology Department, Veterinary University in Lahore/ Pakistan and the editing assistance of Mr. Mostafa Delpisheh are gratefully appreciated.

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Journal of Agricultural Science and Technology A 1 (2011) 217-220
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Comparative Study on the Antibacterial Activity of Fresh and Fallen Leaves of *Terminalia catappa* L.

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Received: July 16, 2010 / Published: June 20, 2011.

Abstract: In the work reported here, the potential of different methods (cold and hot water, and ethanolic) of extraction of Indian almond (*Terminalia catappa* L.), fresh and fallen leaves as an anti-bacterial agent was investigated. The hot water extract did not show any spectrum of activity against the selected bacteria while the cold water extract showed slight antibacterial activity suggesting that the effective components are heat labile. The ethanolic extracts of the leaves have higher antibiotic spectrum of activity than the cold water extract showing ethanol to be a better solvent in extracting the effective component. The fallen leaves seem to have a higher concentration of the effective component against the bacteria while ethanolic extract of the fresh leaves have similar spectrum of activity to Ciprofloxacin and Nitrofuratoin. These suggest that some effective components are lost when leaves fall off while some seem to increase in concentration. Fresh and fallen leaves contain tannin and flavonoids. In addition, the fallen leaves contain flavones. This might be responsible for the higher activity of fallen leaves extract observed against Pseudomonas and Staphylococcus spp. in our study. Combined use of extracts from fresh and fallen leaves broadened the spectrum of activity.

Key words: Natural resources management, *Terminalia catappa* leaves, extraction, antibacterial activity.

1. Introduction

The Indian almond tree, *Terminalia catappa* L. is a Combretaceous plant (tropical almond family). The plant is a large deciduous stately tree, originally from India, growing up to 30 m height with a thick broad trunk; the leaves cluster toward the end of the branches with glossy, obovate blades mostly 8-30 cm in length and turn red before turning brown and falling [1]. It thrives as an ornamental tree in many tropical cities in the world. It is found in almost every town and village in Southern Nigeria. It is also known as Malabar Almond, Tropical Almond, and Fruit (by some Nigerians).

In Southeast Asia, leaves and barks of Indian almond tree are widely used in humans as a folk medicine to treat dermatosis, hepatitis, thrush and other oral infections, and intestinal ailments in children. In modern medicine, many pharmacological studies on various extracts of the leaves and barks have been reported to possess anti-cancer [2], antioxidation [3, 4], anti-HIV reverse transcriptase[5], hepatoprotection [4, 6], antifungal properties against *Pythium ultimum*, *Sclerotium rolfsii*, and *Aspergillus fumigatus* [7] , and antibacterial properties against *Staphylococcus epidermidis*, *S. aureus*, *Bacillus cereus*, *B. Subtilis* and *Pseudomonas aeruginosa* [8].

Indian almonds leaves are rich in compounds produced by the tree to protect itself against bacteria, fungi and similar organisms. Compounds found in Indian almonds leaves have however been researched for their potential health benefits for the human body. Indian almond has strong antibacterial properties and works against Gram positive and Gram negative microorganisms.

In Nigeria fallen leaves are used as an herb to treat liver diseases. The leaves also have potential in the

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management of sickle cell disorders. Dried leaves are used for fish pathogen treatment, as an alternative to the use of chemicals and antibiotics. In the work reported here, the potential of different methods of extraction of Indian almond, fresh and fallen leaves as an anti-bacterial agent was investigated.

2. Materials and Methods

2.1 Plant Material

The leaves of Indian almond tree were collected from the Botanical garden of the University of Ibadan, Ibadan, Nigeria. The leaves collected were fresh leaves and fallen leaves.

2.2 Microorganisms

The bacterial isolates used in this study were obtained from the Microbiology department of the University of Ibadan, Ibadan, Nigeria. The isolates include:

Enterobacter species, Proteus species, Klebsiella species, Escherichia coli, Pseudomonas species, Streptococcus species, Staphylococcus species.

2.3 Preparation of Extract

Harvested leaves were rinsed with sterilized water. 50g of the leaves were then chopped and blended with sterilized blender separately, that is, the fresh leaves and fallen leaves were chopped and blended separately. Then the chopped and blended leaves were soaked in 100 mL each of hot water, cold water and ethanol separately, for 12 hours as the extraction solvents. The extract was then filtered with sterilized Whatman filter paper No 1 125 mm Cat. No 1001125.

2.4 Antibacterial Activity of the Extracts of Fresh and Fallen Leaves of Terminalia catappa

The antibacterial activity tests were carried out using the agar-disc diffusion method (Kirby-Bauer Method) [9, 10]. Briefly, the filtrates obtained from the extraction was applied to the plates and incubated for 48hrs.Thereafter; 6mm diameter filter paper discs were impregnated with the extracts and placed on the test bacteria seeded plates. After 24h of incubation, the areas of inhibition were noted. The antibacterial activity of the selected antibiotics were likewise carried out using commercial antibiotic sensitive disc, Arternate multi-susceptibility disc manufactured by Alternate Laboratories, Emene, Enugu, Nigeria.

3. Results and Discussion

Table 1 revealed that the fresh and fallen leaves contain tannin and flavonoids. In addition, the fallen leaves contain flavones. This might be responsible for the higher activity of fallen leaves extract observed against Pseudomonas and Staphylococcus spp. in our study. Nwosu et al. and Nantarika and Nongnut [13, 15] reported that tannins and flavonoids are responsible for antibacterial activity.

The results in Table 2 showed that the ethanolic extracts of the leaves have higher antibiotic spectrum of activity than the water extracts. It can be inferred that extraction with ethanol yielded more effective components. This possibly justifies the use of alcohol (local gin) for preparation of plant parts for medicinal uses in Nigeria [12].

The hot water extract did not show any spectrum of activity against the selected bacteria while the cold water extract showed slight antibacterial activity. It can be deduced that hot water destroyed the effective components in the extract, which implies that the effective components are heat labile.

Table 2 still showed that the ethanolic extract of the fallen leaves had high activity against *Pseudomonas* spp. and *Staphyloccus* spp. while the ethanolic extract of the fresh leaves showed low activity against

Table 1 Review of the qualitative phytochemical screening of fresh and fallen leaves of *Terminalia catappa.*

Component	Fresh leaves	Fallen leaves
Alkaloids	Present	Present
Cardiac glycosides	Trace	Abundant
Saponin	High	Absent
Tannin	Present	Present
Flavonoids	Present	Present (contains flavones in addition)

Source: Ref. [11].

Table 2 Antibacterial activity of cold & hot water and ethanol crude extracts of fresh & fallen leaves of *Terminalia catappa.*

Extract	*Enterobacter* spp.	*Proteus* spp.	*Klebsiella* spp.	*Escherichia* spp.	*Pseudomonas* spp.	*Streptococcus* spp.	*Saphylococcus* spp.
Cwgl	-	-	-	-	+	-	-
Hwgl	-	-	-	-	-	-	-
Egl	+	-	-	+	+	-	-
Cwfl	-	-	-	-	-	-	-
Hwfl	-	-	-	-	-	-	-
Efl	-	-	-	-	++	-	++

Cwgl-Cold water fresh leaves extract; Hwgl-Hot water fresh leaves extract; Egl-Ethanolic fresh leaves extract; Cwfl-Cold water fallen leaves extract; Hwfl-Hot water fallen leaves extract; Efl-Ethanolic fallen leaves extract; - Not sensitive; + Sensitive.

Table 3 Antibacterial activity of selected antibiotics.

Antibiotic	*Enterobacter* spp.	*Proteus* spp.	*Klebsiella* spp.	*Escherichia* spp.	*Pseudomonas* spp.	*Streptococcus* spp.	*Staphylococcus* spp.
GN(10MCG)	-	+	++	-	-	-	-
CIP(5MCG)	++	-	-	+	-	-	-
C(10MCG)	-	-	-	-	-	-	-
AM(25MCG)	-	++		+	-	-	-
AN(30MCG)	++	+	-	-	-	-	-
CF(20MCG)	-	+	-	+	-	-	-
N(100MCG)	-	-	-	-	-	-	-
AG(30MCG)	-	+	-	-	-	-	-
NB(10MCG)	-	-	-	-	-	-	-
T(50MCG)	-	-	-	-	-	-	-

GN-Gentamycin, CIP-Ciprofloxacin, C-Chloramphenicol, AM-Ampicillin, AN-Nalidixic acid, CF-Cefuroxime, N-Nitrofurantoin, AG-Augmentin, NB- Norfloxacin, T-Tetracycline.

Enterobacter spp., E coli and *Pseudomonas* spp. The fallen leaves seem to have a higher concentration of the effective component against the bacteria.

Comparing Tables 2 and 3, the ethanolic extract of the fresh leaves had similar spectrum of activity to Ciprofloxacin and Nitrofuratoin which acts by preventing DNA synthesis and as metabolites respectively in susceptible bacteria [13, 14].

Table 2 showed that the ethanolic extract of the fallen leaves had higher activity on Pseudomonas spp. compared to the ethanolic fresh extract. In addition to this, the ethanolic fallen leaves extract had high activity against *Staphylococcus* spp. while the ethanolic fresh leaf extract did not show any activity. It was also observed that the ethanolic fresh leaf extract had low activity against *Enterobacter* and *Escherichia* spp., while the ethanolic fallen leaf extract did not show any activity against these two microorganisms. It can therefore be inferred that some effective components are lost when the leaves fall off the tree while some seem to increase in concentration.

4. Conclusions

In conclusion, we propose that a combination of both fresh and fallen leaves be used to broaden the spectrum of activity as observed in our study. Furthermore, alcohol proved to be a better solvent for the extraction of the active components and is advocated for use for the extraction. Our study also revealed that some active components were destroyed by heat. Therefore any method that involves heat during extraction should be avoided.

Further work is suggested for the fractionation of the extracts to ascertain the real active components.

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Journal of Agricultural Science and Technology A 1 (2011) 221-224
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

National Policy Change's Influence on the Landscape Pattern in Maoxian County

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Received: July 26, 2010 / Published: June 20, 2011.

Abstract: Landscape pattern analysis is becoming the core to study global or local change. Incorporated with fast, dynamic and precise spatial information technology, Landscape pattern analysis has been a foundation for the governments to make decision. In this paper, Maoxian county, located in the northwest of Sichuan Province, was selected as the study area. Landsat TM data in 1994 and Landsat ETM data in 2002, with dates are just same as the time of national policy changes, are classified to make the landscape patterns change. The result indicates that, from 1994 to 2002, owing to the policies of the project of wild wood resource protect and the project of returning land for farming to forestry (grass), the forests has increased about 2.68%, Natural meadow has increased about 0.83%, Shrubby has increased about 0.63%, Farm land has decreased about 4.10% and the fragment of forests in 2002 is lower than in 1994. This states that the national policy actioned during 1990s have much positive influence on the landscape patterns in Maoxian county.

Key words: Landscape pattern, national policy change, maoxian, remote sensing.

1. Introduction

Due to the growth in living standard and social increase in population, people have a higher demand of resource in quality and quantity. But with the heavy consumption of resource, the ecological environment around us juddered, the frequently occurred scourges such as desertification, desertification, environment pollution, flood and soil erosion are the result of this, so it urged for us to use resource frugally, to mask the current situation and development trend as soon as possible and to control and keep away the scourge, and then to improve the ecological environment in any way [1, 2]. One of the best ways to improve ecological environment is to draw up right policies and carry out them, but how to measure the actions and the rightness of the policies? As we know, the good and bad of ecological environment can be measured by landscape pattern analysis. Landscape pattern analysis is the main research field of landscape, the main object of it is to explain and understand the ecological process and ecological phenomenon of landscape pattern, so to obtain landscape pattern is the focus [3-10]. There are many ways to obtain landscape pattern today, and remote sensing is the best way when to handle large area.

Remote sensing is the science (and to some extent, art) of acquiring information about the Earth's surface without actually being in contact with it. This is done by sensing and recording reflected or emitted energy and processing, analyzing, and applying that information. With remote sensing, people can acquire large area's landscape pattern in short time to analyze the landscape pattern in time, and acquire the same area's landscape pattern in different time to do comparative analysis, also map the landscape pattern to visualize it [11].

In this paper, the authors attempt to analyze the policies' effect on Maoxian county's landscape with

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remote sensing and to provide a direct viewing of policy's actions on ecological environment.

2. Methods

2.1 Study Area

Maoxian County, with a scope of north latitude from 31°24′ to 32°17′ and east longitude from 102°56′ to 104°10′, is located in the southeast of Aba autonomous prefecture of Sichuan, bordered upon Beichuan county, Anxian county, Mianzhu county, Shifang county, Pengxian county, Wenchuan county, Lixian county, Songpan county. Fig. 1 shows the location of Maoxian county. Maoxian county is featured by complex geological structure and large-span elevation, which leads to a multi-type land cover.

2.2 Data Collection

Before 1995, there are few policies drawn up to preserve the landscape in Maoxian county. But from 1996 to 2000, with the national policy of the project of wild wood resource protect and the project of returning land for farming to forestry (grass) carried out, Maoxian county starts to emphasize on the ecological environment recovery. So, TM image of July 28, 1994 and ETM image of august 24, 2002 was collected to study in this paper, and bands 4, 3, 2 of both images was selected to do the landscape pattern analysis [12, 13].

2.3 Technical Flowchart

The technical flowchart used in this paper is shown as Fig. 2. In this paper, the landscape patterns of 1994 and 2002 extracted first, and then the change was detected to analyze the policy change effect on the landscape pattern with the weather effect ignored.

2.4 Landscape Classification System

A landscape classification grades like Table 1 was used in this paper. Including: (1) Water: including Rivers, marsh, lake and other place where have water on it; (2) Forest land: Including nature forest and manned forest; (3) Shrubby: Including the trees with

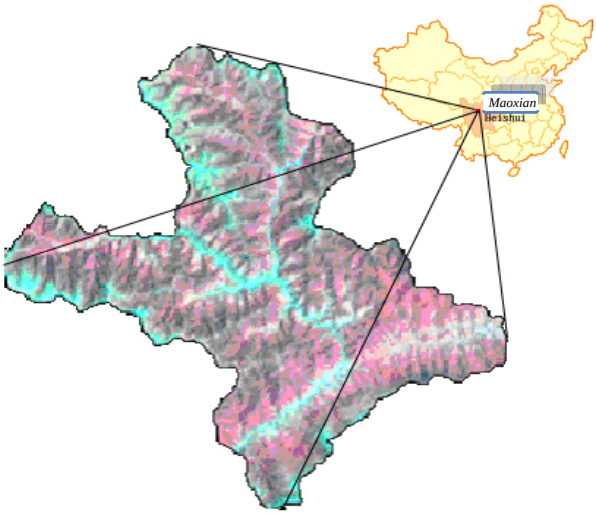

Fig. 1 Location of the study area.

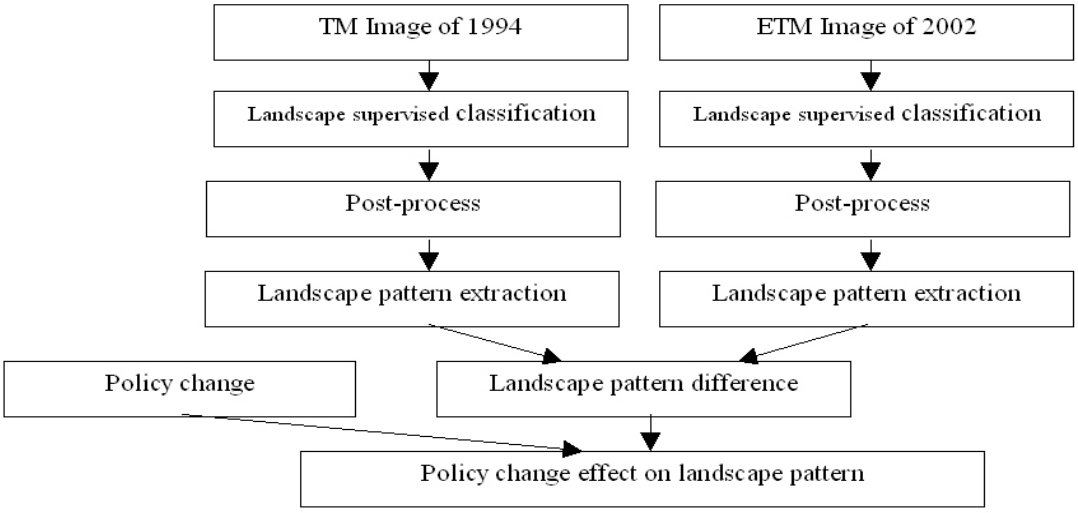

Fig. 2 Technical flowchart.

Table 1 Landscape classification table.

First grade landscape	Second grade landscape
	Water
	Forest land
Nature landscape	Shrubbery
	Natural meadow
	Farm land

unobvious stem; (4) Natural meadow and (5) Farm land.

3. Results

Fig. 3 and Fig. 4 are the landscape pattern of 1994 and 2002 of Maoxian County derived from land use classification, and Table 2 is the corresponding statistics data derived from Fig. 3 and Fig. 4 by ERDAS Imagine 8.5.

From Table 2, we can see that Farm land decreases sharply but forest land, shrubby and natural meadow increase heavily. This state that, in the last eight years, policies have much influence on Maoxian county's landscape to make the farm land changed to forest land, shrubby and natural meadow.

4. Discussion and Conclusions

In the past years, the Chinese government and Chinese local government at different levels have drawn up several policies to protect ecological environment, which bring notable results. But how to measure the results is a challenge work. The authors do this by using remote sensing and landscape pattern analysis technology in this paper, and get a result corresponding to the literatures about Maoxian County.

But some problems need to be put more attentions:

(1) Other factors: Policy is the main driving power to protect ecological environment, but other factors such as soil erosion, hew, graze, etc. should be taken into account when analyze landscape pattern. Meanwhile, deforestation, agricultural production and transportation construction are the main reasons for the decline in the ecological environment were still factors affecting the landscape change in the study area [12, 13].

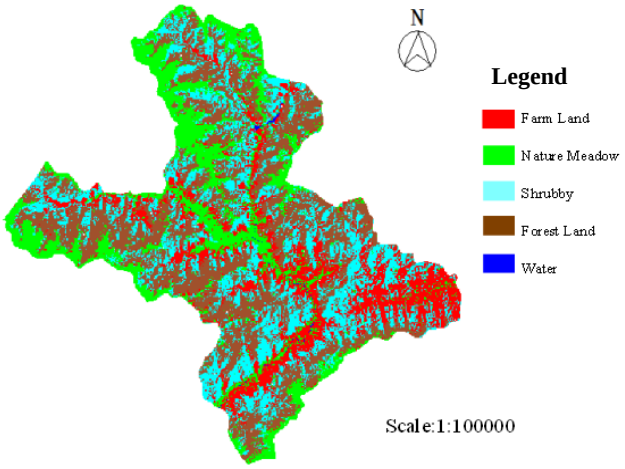

Fig. 3 Landscape pattern of Maoxian county in 1994.

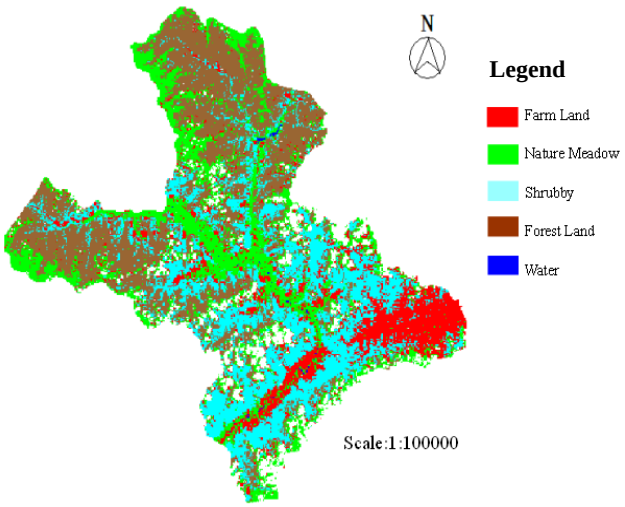

Fig. 4 Landscape pattern of Maoxian county in 2002.

Table 2 Statistics data of landscape pattern of Maoxian county in 1994 and 2002.

Landscape type	Percent (%)		Difference (%)
	1994	2002	
Farm land	18.5567	14.4613	-4.0954
Water	0.1844	0.1405	-0.0439
Natural meadow	20.8557	21.6866	0.8309
Forest land	33.5571	36.2375	2.6804
Shrubby	26.8461	27.4741	0.6280
Total	100	100	

(2) Image resolution: The resolution of image will affect the classification precision, in the following work, the authors plan to employ higher resolution image.

(3) Climate change: Climate change is a major

global issue of common concern to the international community. It is an issue involving both environment and development, but it is ultimately an issue of development [12, 13]. With the global warming, ecological environment have changed much, so this factor should also be considered when analyzing landscape pattern.

Acknowledgments

Funding support was obtained partially from the Science and Technology Bureau of Sichuan Province of China, NSF-China Project (40671132).

The authors would like to thank Professor Hong Jiang, from Nanjing University, for his many important suggestions and discussions.

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Journal of Agricultural Science and Technology A 1 (2011) 225-230
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Integrated Control of *Meloidogyne Incognita* Infesting Tomato Plants Using Callus Powders Derived from Certain Plants Either Alone or in Combination with Oxamyl under Out-Door Conditions

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Received: July 31, 2010 / Published: June 20, 2011.

Abstract: The effect of four dried callus powders derived from certain plants, i.e. tomato, *Lycopersicon esculentum*; periwinkle, *Catharanthus roseus*; thorne apple, *Datura stramonium* and oleander, *Nerium oleander* applied separately or concomitantly with oxamyl at a half of the recommended dose each on tomato seedlings grown in soil naturally heavy infested with *M. incognita* under field conditions, indicated that dried callus powder derived from *N. oleander* either alone or mixed with oxamyl significantly surpassed the other tested treatments in improving percentages of increase in whole plant fresh and shoot dry weights as well as increasing numbers and weights of fruits. Concerning other treatments including oxamyl at a half of recommended dose with each tested callus powder, similar results were evident regarding tomato plant growth parameters. *C. roseus* plus oxamyl achieved the highest percentage of increase in whole plant fresh weight (141.4%) and shoot dry weight (189.7%) and ranked second to *N. oleander* + oxamyl in this respect over the untreated plants, respectively. One the other hand, among all tested materials single application of either *L. esculentum* or oxamyl gave the least values of such criteria which averaged 34.4% and 61.7% or 39.5% and 11.2%, respectively. All tested treatments significantly reduced the total number of galls, egg-masses and eggs in egg-masses especially, dried callus powder of *N. oleander* which applied singly or combined with oxamyl. The least values of reduction percentages of galls and eggs in egg-masses among all treatments were recorded from tomato plant received dried callus powders of *L. esculentum* applied alone or concomitant with oxamyl.

Key words: Tomato, *Meloidogyne incognita*, MS medium, callus powders.

1. Introduction

Tomato, *L. esculentum* Mill. is considered to be one of the most important commercial and dietary vegetable crops all over the world including Egypt. In 2005, Egypt ranked the fourth among the five top producers of tomatoes in the world.

Plant parasitic nematodes caused significant damage and losses to most agricultural crops in the tropical and sub-tropics [1]. The root-knot nematodes, *Meloidogyne* spp. are among the most economically important parasites of tomato cultivars in Egypt. Of the root-knot nematodes, *M. incognita* (Kofoid&White) Chitwood, *M. javanica* (Treub) Chitwood, *M. arenaria* (Neal) Chitwood and *M. hapla* Chitwood are considered to be the most popular species which affect major field and vegetable crops and cause more than 90% of the estimated damages.

Today, plant parasitic nematodes are successfully controlled by chemical nematicides. With an increase awareness of the harmful effects of chemical pesticides and the changing public attitude towards environmental pollution, chemical nematicides are

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losing their popularity among farmers. It has now become essential to search for alternative cheap and environmentally friendly ways for the management of phytonematodes in various agricultural crops. Some safe procedures for nematode control have been developed based on biological control agents and organic amendments; however, there is still a need for alternative compounds for effective nematode control to be developed [2].

The practice of plant tissue culture has contributed towards the propagation of large number of plant from small pieces of stock plants in relatively short period of time [3]. As for nematologists, tissue culture technologies have been widely used for monoxenic culture of various plant parasitic nematodes [4]. However, resistance induction for susceptible plants to nematode infestation through the addition of certain components to callus or root explants medium has not yet undertaken.

In recent years, there has been an increased interest in the application of antioxidants, vitamins, amino acids, mineral salts, growth hormones and natural products to medicinal and agricultural treatments [5-7]. Moreover, little interest has been given to callus tissues of various plant parts that showed antimicrobial and nematicidal properties. Therefore, the objective of the present investigation was to assess the nematicidal activity of callus dried powders derived from certain plants against *M. incognita* infesting tomato plants under field conditions.

2. Materials and Methods

2.1 Plant Materials and Site of Experiment

A micro-plot field experiment was conducted at the Nematology Research Unit of the Agricultural Zoology Department, Fac. of Agric., Mansoura University in order to determine the influence of dried-powdered callus derived from certain plants integrated with oxamyl at half of the recommended dose (0.15%) on *M. incognita* reproduction and the resulting effect on tomato plant growth.

2.2 Callus Induction

Three-fourth strength MS Murashige and Skoog [8] medium were prepared. The media were solidified with 6 g/L agar and sucrose at 30 g/L. The media were distributed into four 1 liter clean flasks, contained 500 ml of nutrient media each. The following four plants, i.e. resistant tomato cv. Flora Dade (*L. esculentum*), oleander (*N. oleander*), thorne apple (*D. stramonium*) and periwinkle (*C. roseus*) were used to induce calli. Each flask containing 500 mL MS medium was supported with different growth hormones for calli induction. For induction of tomato callus, media were supplemented with 2 mg/L (1 mg/500 mL medium) naphthaline acitic acid (NAA) plus 1 mg/L (0.5 mg/500 mL medium) benzylaminopurine (BAP). MS medium supported with 1 mg/L (0.5 mg/500 mL medium) 2,4-D was used for induction of calli derived from periwinkle or oleander or thorne apple plants. Each flask of 1 L containing 500 mL medium was received 4.5 mL coconut water, then divided into twenty 250 mL sterile jars, contained 25 mL of medium enriched with the previous components. After three days, the contaminated jars were disregarded. Tomato callus was obtained from tomato plantlets cv. Flora Dade (resistant cultivar) which seeded on autoclaved watered-cotton as a liquid medium in 250 mL jars under aseptic conditions after surface sterilizing. Ten seeds were distributed randomly in each jar, kept in growth chamber at 25 °C with 16 h. light/8 h. dark cycle and left for germination for three days.

On the other hand, thorne apple, periwinkle and oleander calli were obtained from nodes of these plants growing in greenhouse. The nodes of the previous plants were shacked for 10 min. in sterilized distilled water provided with some drops of soap, then surface sterilized by immersing in 50% ethanol for two min., then rinsed three times in sterilized distilled water. Thereafter, these nodes were poured in 10% sodium hypochlorite solution plus a drop of tween 20 for 15 min., then these soaked nodes were rinsed three

times in sterilized distilled water and cultivated on the previous prepared media for each plant. After one month, the calli for each plant were collected, dried and powdered.

2.3 The Experiment

Heavy naturally infested experimental area (30 m^2) with root knot nematode, *M. incognita* at the level of approximately 2000 J_2s/250 g soil was prepared by following the Egyptian agricultural conventional processes. The experimental area was divided into 30 plots each of which was 90 × 90 cm.

Seedlings of susceptible tomato cv. TY-3017, one month old were transplanted in the soil. Each plot cultivated with four seedlings. Two weeks later, each seedling was treated with 1.0 g of each callus incorporated into the soil around plants and then watered to allow decomposition. Oxamyl 24 % L as a nematicide was applied as recommended dose (0.3%) in a single and at half one (0.15%) in mixed application along with the powders under study. Three untreated plots were served as control. Therefore, the treatments were as follows:

1-Tomato, 2-Thorne apple, 3-Periwinkle, 4-Oleander, 5-Oxamyl, 6-Tomato + Oxamyl, 7-Thorne apple + Oxamyl, 8-Periwinkle + Oxamyl, 9-Oleander + Oxamyl, 10-Untreated plants (Ck).

Each treatment was replicated three times. Plants were harvested 45 days after treatment.

2.4 Measurements

Data dealing with length and weights of fresh shoot and root were measured and recorded. Shoot dry weights, number of fruits and fruits weight were also recorded. Infected tomato roots were stained in hot lactophenol in acid fuchsin [9], and examined for the number of galls, egg-masses and number of eggs. The obtained data were subjected to statistical analysis of variance (ANOVA) [10], followed by Duncan's multiple range test to compare means [11].

Regarding ammonium (N), phosphorus (P), potassium (K) and organic carbon (C) determination 0.1 g of dry weight of each callus powder was subjected to chemical analysis according to Jackson [12] and Hesse [13].

3. Results and Discussion

Data in Table 1 present the effect of four dried callus powders derived from certain plants, i.e. tomato (*L. esculentum*), periwinkle (*C. roseus*), thorne apple (*D. stramonium*) and oleander (*N. oleaneder*) applied separately or concomitantly with oxamyl at a half of the recommended dose (0.15%) on tomato seedlings grown in soil naturally heavy infested with *M. incognita* under field conditions (30 ± 5 °C). Results indicated that all tested materials improved growth of tomato plants infested with *M. incognita* to various degrees. Dried callus powder derived from *N. oleander* either alone or mixed with oxamyl significantly surpassed the other tested treatments in increasing percentages of increase in whole plant fresh and shoot dry weights as well as increasing numbers and weights of fruits with values of 137.1 and 140.9%; and 54.8 and 313.7% or 147.1 and 212.3%; and 91.8 and 412.0%, respectively. Concerning other treatments including oxamyl at a half of recommended dose with each tested callus powder, similar results were evident regarding tomato plant growth parameters.

C. roseus plus oxamyl achieved the highest percentage of increase in whole plant fresh weight (141.4%) and shoot dry weight (189.7%) and ranked second to *N. oleander* + oxamyl in this respect over the untreated plants, respectively. One the other hand, among all tested materials single application of either *L. esculentum* or oxamyl gave the least values of such criteria which averaged 34.4 and 61.7% or 39.5 and 11.2%, respectively. Moreover, mixed treatments generally gave better results in these two plant growth criteria than single ones did.

Data presented in Table 2 show the impact of four dried callus powders applied singly or in combination

Table 1 Effect of dried callus powders derived from certain plants applied singly or integrated with oxamyl on the growth of tomato cv. TY-3017 naturally infested with *Meloidogyne incognita* under field conditions.

Treatments	*Plant growth response									
	Length(cm)		Fresh weight (g)		Fresh wt. of the whole plant (g)	Increase %	Shoot dry wt. (g)	Increase %	No. of Fruits	Wt. of Fruits
	Shoot	Root	Shoot	Root						
1	73.3 d-f	28 b-e	70.9 c	6.28 ab	75.07 b	34.4	15.30 de	61.7	10.7 ab	52.9 bc
2	87.3 a-c	32.7 ab	92.1 a-c	7.27 a	99.36 ab	77.9	19.07 cd	101.6	13 ab	51.3 bc
3	69.3 ef	31.7 ab	93.95 ab	4.31 bc	98.26 ab	75.9	17.32 cd	83.1	9 ab	70.3 ab
4	89.7 ab	25.7 de	126.7 ab	5.75 a-c	132.40 a	137.1	22.79 bc	140.9	11.3 ab	75.7ab
5	93 a	28 b-e	104.9 ab	6.05 ab	110.95 ab	98.7	22.51 bc	137.9	9.7 ab	44.7 bc
6	79 c-e	34 a	129.5 a	5.34 a-c	134.84 a	141.4	27.41 ab	189.7	11.7 ab	67.9 ab
7	80 b-d	30 a-d	96.4 a-c	7.29 a	103.72 ab	85.7	18.15 cd	91.9	11 ab	73.7 ab
8	88 a-c	27.3 cd	131.72 a	6.29 ab	138.01 a	147.1	29.54 a	212.3	14 a	93.7 a
9	71.3 d-f	24.7 ef	74.62 bc	3.27 c	77.89 b	39.5	10.52 e	11.2	10.3 ab	27.4 c
10	64 f	22 f	51.60 c	4.20 bc	55.85 b	--	9.46 e	--	7.3 b	18.3 c

1. *L. esculentum*; 2. *C. roseus*; 3. *D. stramonium*; 4. *N. oleaneder*; 5. *L. esculentum* + oxamyl; 6. *C. roseus* + oxamyl; 7. *D. stramonium* + oxamyl; 8. *N. oleaneder* + oxamyl; 9. Oxamyl (24% L); 10. Check (Untreated plants).

*Means in each column followed by the same letter (s) did not significantly differ at ($P < 0.5$) according to Duncan's multiple range test.

Table 2 Effect of dried callus powders derived from certain plants applied singly or integrated with oxamyl on *Meloidogyne incognita* infesting tomato cv. TY-3017 under field conditions.

Treatments	*No. of galls/root system	Reduction %	RGI	*No. of egg masses/root system	Reduction %	EI	*No. of Eggs/g root
1	75 b	56.4	4.0	20 bcd	67.6	3.0	11345.7 ab
2	50.3 bc	70.8	4.0	15.3 cd	75.2	2.7	2579 b
3	55.7 bc	67.6	4.3	30 b	51.4	3.7	2293.3 b
4	40.7 bc	76.3	3.0	13.3 d	78.4	2.3	1295 b
5	61.3 b	64.4	4.7	16 bcd	74.1	3.0	8204.7 ab
6	45.3 bc	73.7	3.7	15.3 cd	75.2	3.0	1993.3 b
7	45 bc	73.8	3.3	14.3 cd	76.8	2.7	1625 b
8	25 c	85.5	3.0	12.7 d	79.4	2.0	1090 ab
9	45 bc	73.8	3.0	28.7 bc	53.5	3.7	1828.7 b
10	172 a	--	5.0	61.7 a	--	4.0	19050 a

1. *L. esculentum*; 2. *C. roseus*; 3. *D. stramonium*; 4. *N. oleaneder*; 5. *L. esculentum* + oxamyl; 6. *C. roseus* + oxamyl; 7. *D. stramonium* + oxamyl; 8. *N. oleaneder* + oxamyl; 9. Oxamyl (24% L); 10. Check (Untreated plants).

*Means in each column followed by the same letter (s) did not significantly differ at ($P < 0.5$) according to Duncan's multiple range test.

with oxamyl on root galling, egg-masses number and number of eggs in egg-masses of *M. incognita* infesting tomato cv. TY-3017 under microplot field conditions.

Results also indicate that all tested treatments significantly reduced the total number of galls, egg-masses and eggs in egg-masses. Among the tested materials, dried callus powder of *N. oleander* applied singly or combined with oxamyl overwhelmed all treatments in reducing number of galls, egg-masses and number of eggs with values of (76.3% and 85.5%), (78.4% and 79.4%) and (93.2% and 94.3%), respectively. Application of oxamyl either alone or mixed with *D. stramonium* gave equal results in reducing the number of galls with reduction percentage (73.8%) each. The least values of reduction percentages of galls and eggs in egg-masses/g root among all treatments were recorded from tomato plant received dried callus powders of *L. esculentum* applied alone or concomitant with oxamyl

which amounted to (56.4% or 64.4%) and (40.4% or 56.9%), respectively. As a whole it can be concluded from the previous results that callus powder derived from *N. oleander* applied either singly or integrated with oxamyl at its half recommended dose considered the best treatment in improving growth of tomato plants, increasing yield of fruits and suppressing *M. incognita* in naturally infested soil.

Nutrient constituent analysis (Table 3) showed that different dried callus powders derived from certain plants, i.e. tomato, thorne-apple, periwinkle and oleander varied significantly in amounts of potassium, phosphorus, ammonium (in form of nitrogen), organic carbon and carbon/nitrogen ratio. Amounts of these nutrients influenced root-gall nematode damage on tomato plant cv. TY-3017.

As a whole it can be concluded from the present findings that the callus powder derived from *N. oleander* as a novel biocide applied either singly or integrated with oxamyl at a haf of recommended dose was the best applications in improving growth of tomato, increasing yield of fruits as well as suppressing *M. incognita* development and reproduction in the naturally infested soil. This is not surprising results, since literature revealed that the leaves of this ornamental plant *N. oleander* have two principles toxic glucosides [14] with nematicidal activities against such pathogenic nematodes.

Moreover, oleander dried callus powder with high (4.2%) ammonium and low potassium (1.98%) contents gave relatively few root gall damage on tomato cv. TY-3017 in the present work. These observations agree with that of Oteifa [15] who stated that root-gall nematode damage on cabbage increased with amounts of potassium available to the host plant because potassium increased the rate of reproduction of nematode. Huber [16] also recorded that root gall nematode damage on lima bean decreased with increased ammonium supplied to the plant. Plant treated with this application alone or mixed with oxamyl gave significantly higher tomato yields than

Table 3 Approximate percentage of organic carbon, ammonium (nitrogen form), potassium, phosphorus, carbon/nitrogen ratio of organic dried callus powders derived from certain plants (dry-weight basis).

*Constituents	Tomato %	Thorne-apple %	Periwinkle %	Oleander %
(C)	46.75	53.57	39.94	25.81
(N)	1.36	2.78	4.2	4.2
(C/N)	13.9:1.0	14.2: 1.0	9.5: 1.0	6.1: 1.0
(K)	3.06	2.16	1.53	1.98
(P)	1.0	0.46	1.06	1.14

*Organic carbon (C), Ammonium (N), C:N ratio (C/N), Potassium (K), Phosphorus (P).

those of other tested dried callus powders. This was because relatively few root galls occurred at oleander dried callus powder. Plants with fewer root-galls would translocate more nutrients to vegetative organs than heavily galled-roots [17]. The C: N ratios of this compound was also very narrow (6.1:1.0) and agree with the findings of Miller and Donahue [18] who reported that organic residues with C: N ratio of 20:1.0 or narrow have sufficient nitrogen to supply the decomposing microorganisms and also to release for plant use.

4. Conclusions

Undoubtedly, tissue culture technology used in the present investigation through the tested components and chemicals succeeded to generate a sort of biochemical alterations in *M. incognita*-infected plants enabling them to overcome nematode damage. Conclusively, utilization of such technique in nematode control by producing such callus tissue powders of certain ornamental or medicinal plant parts provided with such tested chemical could gain new trend, safe and effective nematode management program(s).

Acknowledgments

The authors thank the support of grants from Mansoura University, Egypt for running such research.

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Journal of Agricultural Science and Technology A 1 (2011) 231-234
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

The First Record for Two Species of the Soldier Flies (Diptera: Stratiomyidae) from Turkey

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Received: January 12, 2011 / Published: June 20, 2011.

Abstract: Known data on the distribution of *Alliocera graeca* Saunders, 1845 and *Beris chalybata* (Forster, 1771) in Europe are summarized. *A. graeca* has been reliable recorded from Albania, Croatia and Greece. *B. chalybata* has been reliable recorded from Austria, Belgium, Bulgaria, Czech Republic, Denmark, England, Finland, France, Germany, Hungary, Ireland, Italy, Netherlands, Norway, Poland, Roumania, Russia, Slovakia, Sweden, Switzeland. *A. graeca* and *B. chalybata*, known as European species, are new records for the Turkish Fauna. In this study, the female of *A. graeca* and the female of *B. chalybata* are presented and photographs of two species are accompanied. Distribution of these species treated is briefly discussed.

Key words: *Alliocera graeca, beris chalybata*, entomology, new record, soldier flies (Diptera: Stratiomyidae), Turkey.

1. Introduction

According to the present knowledge, the genus *Alliocera* described by Saunders was discovered in Greece in 1845 [1-3]. The genus *Alliocera* has one of the smallest distributions of the European genera. This genus, belonging to the subfamily Stratiomyinae (Stratiomyidae), is southeast European in the Palaearctic Region. The only species described in this genus is *Alliocera graeca* Saunders, 1845. This species is extremely rare and only a few records are published [1-3]. This species is distributed in Albania, Croatia and Greece and limited in the Adriatic and Ionian Sea Subregion [1-3]. Until now this species, a typical ponto mediterranean element has not been recorded for Turkey.

So far, the genus *Beris* Latreille (1802), includes 33 Palaearctic species but only 14 of them are distributed in the western part [3-10]. The West Palaearctic species belonged to *Beris* are *B. chalybata* (Foster, 1771), *B. clavipes Linnaeus*, 1767, *B. cypria James*, 1970, *B. fuscipes Meigen*, 1820, *B. geniculata Curtis*, 1830, *B. hauseri Stuke* 2004, *B. kovalevi Rozkošný & Nartshuk*, 1980, *B. morrisii Dale*, 1841, *B. rex* (Poda, 1761), *B. rozkosnyi Kassebeer*, 1996, *B. schaposchnikowi Pleske*, 1926, *B. similis* (Foster, 1771), *B. strobli Dušek & Rozkošný*, *B. vallata* (Foster, 1771) [3, 4, 7, 8].

Beris chalybata (Forster, 1771) belonging to the genus *Beris* is distributed the greater part of Europe. The distribution areas of *B. chalybata* are located in Austria, Belgium, Bulgaria, Czech Republic, Denmark, England, Finland, France, Germany, Hungary, Ireland, Italy, Netherlands, Norway, Poland, Roumania, Russia, Slovakia, Sweden, Switzeland, and so on [2, 3]. So far, one species (*B. clavipes*) of *Beris* in Beridinae has been recorded in Turkey [9].

2. Materials and Methods

The materials, 1 female specimens belonging to *A. graeca* and 1 female specimens belonging to *B. chalybata*, deposited at the Zoological Museum, Gazi Üniversity (ZMGU) are examined in the present work.

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2.1 Alliocera Graeca Saunders, 1845

Material Examined: Turkey: Izmit, Gebze, Tavsanli Kö yü , Ballikayalar National Park, 40°50'575? N, 29°31'158? E, elev.150 m, 24 May 2004, 1 female, A. Hasbenli leg (Fig.1a, b, c; Fig. 2).

Female: Head nearly semicircular in dorsal view and in lateral view, with a distinct tubercule at middle of head in profil. Frons about 1/3 of head-width, frontal index about 0.7 (16: 20), mainly black, with a pair of transverse yellow spots above antennae at lower half of the frons. Face mainly yellow, with a pair of yellow facial pattern at the edge of eyes and a fairly narrow median black band in the middle (6:4:6). Yellow transverse spots above antennae are attached to yellow lateral parts of face. Upper half of frons covered with sparsely semi erect black hairs. Yellow facial patterns covered with as long as pedicel, densely semi erect whitish hairs from antennal tubercule to below corner of the each eyes in postocular area. Postocular area as long as pedicel is wide in lateral view (4:4), completely yellow, even here with densely adpressed whitish hairs, interrupted on vertex. Yellow postocular band reached on occipital margin. Cerebrale with two large yellow patchs that separated with black slim stripe. Antennae inserted on a distinct tubercule at middle of head, completely black, antennal index about 1.3 (16:12). Scape 3 times as long as pedicel (9:3); pedicel covered

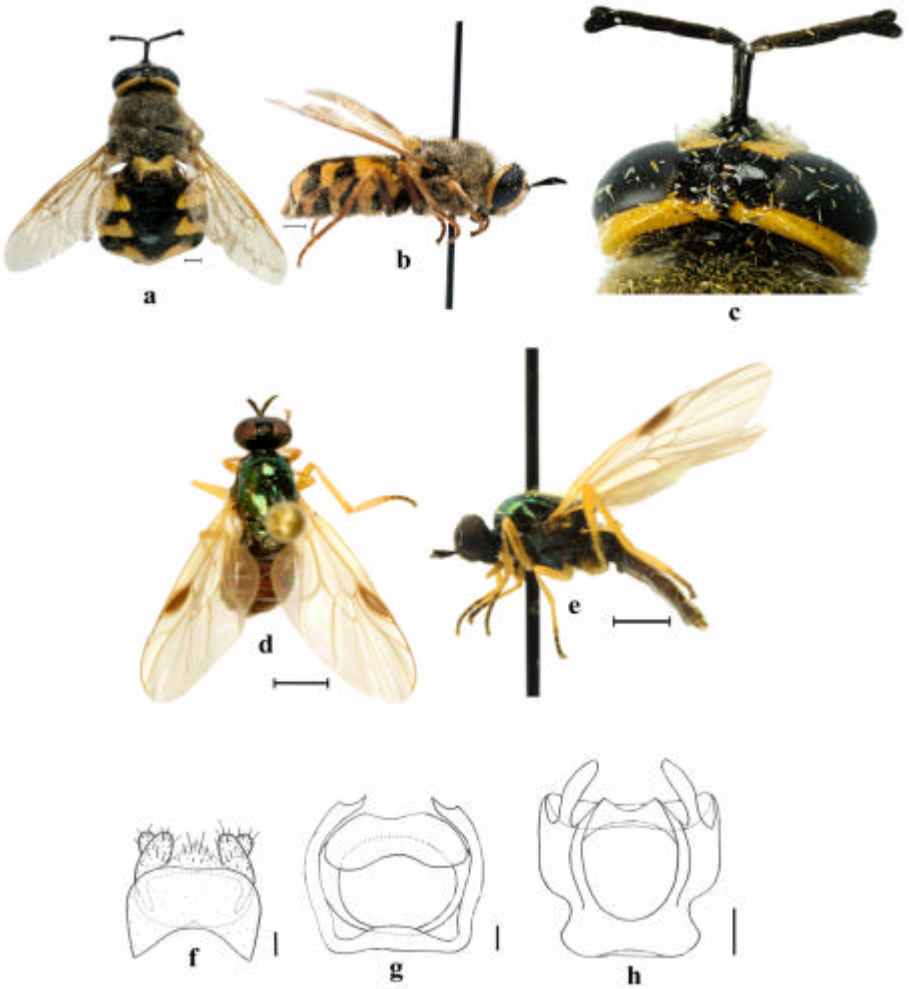

Fig. 1 a-c *Alliocera graeca* : a-female in dorsal view, b- female in lateral view, c-femele head and antennae in dorsal view (Scala 1 mm.); d-e *Beris chalybata* : d-female in dorsal view, e-female in lateral view (Scala 1mm.); f-g *Alliocera graeca* : f-female terminalia in dorsal wiew, g-female genital furca (Scala 0.1 mm.); h-*Beris chalybata*: female genital furca (Scala 0.1 mm.).

Fig. 2 Disrtibution of · *Alliocera graeca*, ¨ *Beris chalybata in Turkey* (The map was obtained from http://www.hgk.mil.tr/CografiUrunKatalogu/tematik/default.asp).

with densely semi adpressed black hairs; flagellum consisting of 5 flagellomeres, the last two flagellomeres forming a bilobed and dilated apex. One of them is much bigger than the other, Proboscis black.

Thorax is completely black and covered with long and densely yellow hairs. Scutellum and the strong spines yellow. Basal black spot on scutellum narrow and triangular, pointed medially. Legs are brown and yellowish. All coxae black, trochanters brownish. All femora mainly brown, tips of the femora yellowish. Fore tibia yellowish, but distal 1/3 of the tibia brownish. Middle and hind tibia yellowish. All tarsi are yellowish but paler inner part of the tarsi. All femora covered with semi-erect white sparsely hairs. All tibia and tarsi covered with adpressed white desely hairs. Wings tinged with yellow, stronger veins and pterostima yellowish. Halters yellow with darkened stalk.

Abdomen black dorsally with remarkable yellow side-markings is on terga 2-4 and apical spot on tergum 5. Lateral markings on terga 3 and 4 distinctly enlarged towards inner side. Apical spot on tergum 5 about 2/3 is as long as height of tergum 5 and narrow triangular on apex. Venter is mainly yellow with a variable black pattern. Sternum 2 mainly yellow with two black transverse bands, Sterna 3-5 mainly black with tarnsverse yellow band on posterior-side. Female terminalia is illustrated in Fig. 1f, 1g.

2.2. Beris chalybata (Forster, 1771)

Material Examined: Turkey: Izmit, Yuvacik Town, Beskayalar Natural Park, elev.729 m, 16 June 2004, 1 female, A. Hasbenli leg (Fig. 1d, e; Fig. 2).

Female: Head black, semiglobular in lateral view. Frons relatively wide, about 1/3 of head-wide, frontal index is 1.37. Eyes are very short, sparse, almost inconspicuous, whitish hairs. Frons and face shining black, densely punctate, covered with white hairs. Frons with shallow median groove is in lower half of frons. Face slightly prominent lateral view. BlackPostocular area as wide as 1/2 of pedicel-length, somewhat swollen, about as wide as pedicel-length. The postocolar area covered with adpressed silverish white hairs in upper half, with long pale yellowish hairs at the corner of eyes in lover half. Antennae moderately long, about 1.3 times as long as lenght of head, mainly black, but brownish distal side of pedicel. Basal antennal segments almost equal, flagellum about twice as long as the two basal segments combined, antennal index about

2.2. The basal antennal segments covered with long, thick, black hairs. The flagellum covered with very short addpressed densely silverish white hairs. Proboscis mainly yellow, with sparsely brown hairs.

Thorax with shining metalic green on mesonotum and scutellum, but darkened on pleura and sternum; covered with as long as scapus, erect, yellowish white hairs. These hairs are especially more long on side of mesonotum and distal side of scutellum. Scutellum with 6 spine like processes. Legs are mainly yellow, fore and hind coxae partly black. Fore about 1/3 of basitarsi at apex and 2-5. Tarsal segments darkened, middle and hind 2-5. Tarsal segments darkened. Wing slightly tinged with Brown, pterostigma contrasting dark brown. Wing weins brownish. Halters pale yellow.

Abdomen shining brown, but tergum 1 is dark brown or blackish. Venter shining brown, but sternum 1 black. Abdominal pile semi-addpressed, is densely yellowish, elongated on side. Female genital furca is illustrated in Fig. 1h.

3. Results and Discussion

Two species are recorded for the first time for the fauna of Turkey. It seems that these two species prefer the wetter climate between Black Sea and Sea of Marmara in our country. *Alliocera graeca* is a typical north-eastern mediterranean species that ranges from Croatia and Albania to Greece [2, 3]. Its occurence seemed to be limited to the Adriatic and Ionian Sea subregion on the western part of the Balkan Peninsula. Our record gives the first evidence of a member of the genus *Alliocera* in Turkey (in the western part of Asia) and represents thus a new eastern-most point in its range. *Beris chalybata is* a typical European species that ranges from Ireland and Finland to Roumania and Russia [2, 3]. As regards the southern boundary of its distribution area, its occurence seemed to be limited on the Balkan Peninsula and some southernmost records have been known in Roumania. Our record in Turkey represents thus a new southernmost point in its range and the first evidence of its occurrence in the western part of Asia.

4. Conclusions

This study consists of new record of two species belonging to Stratiomyidae from Turkey, summarising information on their morphology, distribution. The distribution of both species is shown in a distribution map. *A. graeca* is presented the first evidence of a member of the genus *Alliocera*. The two species belonging to photographs are shown distinctive features of this species in dorsal and lateral view in Fig. 1.

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Journal of Agricultural Science and Technology A 1 (2011) 235-243
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Evaluation of Neem-based Compound Fertilizer for Crop Production in Samaru, Moist Savanna of Nigeria

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Received: March 29, 2010 / Published: June 20, 2011.

Abstract: Nutrient mining is one of the major threats facing crop production in Africa and inputs of nutrients are required to overcome this constraint. Greenhouse and field trials were conducted to investigate the effect of sole and combined applications of neem-based and conventional compound (NPK 15-15-15) fertilizers on five crops. In another set of treatments, a factorial combination of the neem fertilizer and urea at 30, 60 and 90 kg N ha^{-1} was applied to maize, millet and sorghum in the field in a randomized complete block design. The results obtained on cowpea and soybean showed that application of 20 kg N ha^{-1} from the neem-based compound fertilizer produced both grain and haulm yields that were comparable to what was obtained with the application of 30 kg N ha^{-1} from the conventional fertilizer. Based on maize performance, combined application of neem-based and NPK in ratio 0.25: 0.75 respectively gave significantly ($P < 0.01$) higher plant height, stem girth, shoot and dry weight compared to other treatment combinations. The causes of the interactions between the two fertilizer sources resulting in added benefits from their mixed rather than sole application could be attributed to improvement in phosphorus availability and other soil conditions.

Key words: Neem, crop production, fertilizer, moist savanna.

1. Introduction

The moist savanna zone of Nigeria has a high potential for crop production but yield levels obtained are usually low. This is mainly attributed to low use of external sources of nutrients and breakdown of the traditional system of soil fertility maintenance. The traditional systems of shifting cultivation and bush fallow have been replaced by continuous cultivation due to burgeoning population. Although sufficient evidence exists in literature that animal manure could sustain crop yield, livestock densities in the moist savanna are generally low, therefore applicability of animal manure for crop production is limited [1]. Therefore, farmers have to rely on the application of mineral fertilizers for increased crop productivity and soil fertility maintenance. However, efficient use of mineral fertilizer is limited by high prices, lack of knowledge on correct method and time of application, inappropriate formulation and low yielding crop cultivars. Similarly, in an earlier work on soil fertility in the tropics, it was demonstrated that applying inorganic N fertilizers tended to deteriorate the soil physicochemical conditions of the often physically and chemically fragile moist savanna soils in the long term, especially in the case of ammonium sulfate [2, 3]. The soil micro flora and fauna community is also drastically affected. The beneficial microbes are destroyed resulting in the proliferation of microbes which take up fertilizer nitrogen as an energy source, for cell growth

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and development. The nitrogen which was supposed to be taken up by the plant is instead taken up by soil microbes resulting in the further loss of nitrogen through immobilization.

These observations led to testing different options of soil fertility maintenance and improved crop productivity that would be affordable, environmentally benign and sustainable. These include development of farming systems where inputs of mineral fertilizers were minimized or avoided and also led to increased research effort on the biological components of soil fertility dynamics in the tropics [4, 5]. However, it was soon realized that for optimum production and greater sustainability of farming systems in the tropics, organic and inorganic fertilizers should be applied together [6-8]. Organic inputs are needed to maintain the physical and biochemical health of the often shallow, sandy to sandy loam topsoils in the moist savanna zone while fertilizers are needed to supply a sufficient amount of nutrients to the crop.

Farmyard manure (FYM) is the most widely used organic input in the moist savanna of Nigeria and previous studies have concentrated on its impact on crop yield [9, 10]. Its use is often limited by large quantities required to satisfy crop need. For example, 100 kg of 10-0-10 mineral fertilizer contains about the same amount of N-P-K as 2,000 kg of FYM. Alternative sources of organic inputs produced from by-products of agro-allied industries, such as neem cake from bio-pesticide plant and sawdust from saw mill could also be considered for use in cropping systems in the moist savanna zone. These materials are of low quality leading to low C and N mineralization rates but their quality can be improved by combining them with other nutrient-rich inputs, such as poultry manure or inorganic fertilizers.

The objective of this study was to assess the role of neem-based fertilizer in improving crop yield in the treatments receiving only the fertilizer or in combination with the conventional NPK 15-15-15 fertilizer.

2. Materials and Methods

2.1 Experimental Site Characteristics

Potted experiment was set up in the greenhouse of the Institute for Agricultural Research (IAR), Samaru (11°11′ N and 7°38′ E) within the northern Guinea savanna (NGS) zone in 2008. Samaru soil was classified as an Alfisol using the USDA soil classification system [11]. A field trial was also set up in the same location on a relatively flat field. Common weeds observed on the field are *Setaria viridis* and *Vernonia galamensis*. The field has been left fallow for four years prior to the commencement of the trial. For characterization of the experimental soil, twenty core soil samples (0-15 cm depth) were taken at random along four transects. The soil samples were bulked to form four composite samples. A sub-sample of each composite was air-dried, sieved through a 2 mm screen and subjected to routine analysis following standard procedures [12]. The samples were analyzed for particle size distribution, pH (H_2O) and pH (0.01 M $CaCl_2$), organic carbon, total N and Bray 1-P. Exchangeable bases and acidity were also determined while the effective cation exchange capacity (ECEC) was calculated by summing the exchangeable bases and exchangeable acidity. Selected topsoil characteristics are shown in Table 1.

2.2 Analysis of Neem-Based Fertilizer

Neem, *Azadirachta indica* is an evergreen tree, usually tall and grown for its bark, resin and seed oil, which have medicinal and insecticidal properties. In Nigeria, it is found in the wild or planted on the streets and used for wind break especially in dry areas where there is potential for desert encroachment.

The neem-based fertilizer is a blend of neem cake and other organic and inorganic products, henceforth referred to as organo-mineral fertilizer (OMF). The neem cake was obtained after extraction of oil from the neem seeds. The oil contains azadirachtin, an active toxic ingredient used in the production of pesticide.

Table 1 Physico-chemical properties of the soil used.

Soil parameter	Mean
Physical properties	
Sand content (g kg^{-1})	500
Silt content (g kg^{-1})	320
Clay content (g kg^{-1})	180
Textural class	Sandy Loam
pH (Water) 1:2.5	5.60
pH (0.01 M $CaCl_2$) 1:2.5	5.00
Organic carbon (g kg^{-1})	8.6
Total N (g kg^{-1})	0.35
Available P (mg kg^{-1})	5.80
Exchangeable bases ($cmol_c$ kg^{-1})	
Ca^{2+}	2.50
Mg^{2+}	0.54
K^+	0.35
Na^+	0.15
ECEC	0.20
Exchangeable acidity ($H^+ + Al^{3+}$)	4.02

Other materials used in the manufacture of the fertilizer include: manure, limestone, phosphate rock, trace amounts of urea, muriate of potash and diammonium phosphate and binder. The end product was analyzed in the analytical laboratory of the Soil Science Department, IAR and was declared a compound fertilizer containing macro and micronutrients and organic matter and suitable for use as a source of plant nutrients source. The detailed result of the analysis is presented in Table 2.

Table 2 Results of physical and chemical analysis of the neem-based fertilizer.

Physical analysis	
Colour	Light grayish brown
Granular integrity	Good
Dustiness	Nil
Flow	Free-flowing
Caking	Non-caking
Chemical analysis	
Nitrogen (%)	7.26
Total phosphorus (%)	7.41
Potassium (%)	6.00
Calcium (%)	10.00
Magnesium (%)	0.54
Iron (%)	1.10
Copper (mg kg^{-1})	10.08
Zinc (mg kg^{-1})	61.68
Manganese (mg kg^{-1})	533.10
Boron (mg kg^{-1})	415.00
Sulphate (mg kg^{-1})	2,500.00
Organic matter (%)	12.73

2.3 Treatments and Experimental Layout

The test crops in the greenhouse were maize (variety, Across 97), millet (variety, SOSAT 26) and sorghum (variety, SK 5912). In addition to these, soybean (variety, TGx 1448-2E) and cowpea (variety, SAMPEA-7) were included in the field trials. Three sets of treatments were used in the whole experiment.

2.3.1 Greenhouse Protocol

In the greenhouse, neem fertilizer and conventional NPK 15-15-15 mineral fertilizer (MF) were applied in the following ratios: 0: 0, 0: 1.0, 0.25: 0.75, 0.5: 0.5, 0.75: 0.25 and 1.0: 0 respectively. 0:0 was the control i.e. no fertilizer material was applied while 1.0: 0 was neem fertilizer only was applied to meet the nutrient requirements of each of the crops based on their fertilizer recommendation (e.g. 120:60:60 for maize). The treatments were laid down in completely randomized design with three repetitions. 5 seeds were planted into pots containing 10 kg of soil each and thinned to two plants per pot a week after planting (WAP). The plants were watered regularly as soon as the soil starts drying up. Plastic covers were placed under each pot so that the drain were collected and returned to the pots. Plant heights, stem girth, shoot and root dry weights were measured at harvest, 8 WAP.

2.3.2 Field Protocol

In the field, the legumes received 10, 20 and 30 kg N ha^{-1} of both fertilizer materials while the cereals received these fertilizers at 30, 60 and 90 kg N ha^{-1} in separate plots, each comprising six ridges of 5m long, separated at 0.75m. Control plots were included in this set of treatments for each crop. The treatments were replicated three times in a randomized complete block design. Soybean seeds were planted by hand-drilling on the ridges. Seedlings were thinned to one plant $stand^{-1}$ at a spacing of 5 cm between plants two weeks after planting (WAP) to give a plant population of 266,667 plants ha^{-1}. Cowpea seeds were planted at 25

cm intra-row spacing. The seedlings were thinned to two plants per stand at 2 WAP giving a plant population of 106,666 plants ha^{-1}.

The third set of treatments was allocated to the cereal crops only. OMF and urea (46% N) were factorially combined at 30, 60 and 90 kg N ha^{-1} and applied to maize while millet and sorghum received the same fertilizer types at 15, 30 and 45 kg N ha^{-1}. Similar ridge number, length and spacing were maintained per plot. Two seeds of maize were hands sown per hill at 75 cm × 25 cm inter- and intra- row spacing and later thinned to one plant per stand to give approximately 53,333 plants ha^{-1}. About five seeds of sorghum were planted at the same spacing but thinned to two plants $stand^{-1}$. Approximately, ten seeds of millet were also planted at 75 cm × 50 cm inter- and intra- row spacing and thinned to two plants $stand^{-1}$. The plots were regularly weeded to minimize any impact of weed pressure on the crops' performance. At harvest, ear/head/pod and stover/haulm fresh weight were determined from the net plot (two inner rows of 4-m length). Sub samples of the plant parts collected in the greenhouse (shoot and root) and field (ears, heads, pods, haulms and stover) were taken, weighed and oven-dried to constant weight at 65 °C. After drying, the ears were separated into grains and cob, pods and heads were separated into grains and chaff.

2.4 Statistical Analysis

All agronomic parameters collected were subjected to analysis of variance using the Statistical Analysis System Institute Inc. [13] package. Where significant differences were observed between treatment means, experimentwise comparison of treatment levels was done using the least significant difference (LSD) at 5% level of probability.

3. Results

3.1 Greenhouse Trials

Maize: The control treatment produced significantly lower plant height, stem girth, shoot and root dry weights than the other treatments (Table 3). Plants that received combined application of OMF and MF in ratios 0.25: 0.75 and 0.50: 0.50 gave significantly higher ($P < 0.05$) values for all the parameters measured compared to other treatments. These were statistically followed by plants that received MF only. Except in the stem girth, plants that received OMF and MF in ratio 0.75: 0.25 produced similar results to plants treated with OMF only.

Millet: There were no significant differences in all the parameters measured between all the treatments set (Table 4). However, the trend observed showed that combined application of OMF and MF in ratios 0.25: 0.75 and 0.50: 0.50 gave relatively higher values than other treatments. This was followed by the application of MF only while OMF and MF in ratio 0.25: 0.75 produced similar result to sole application of OMF. The lowest values were obtained in the control treatment.

Sorghum: Unlike millet, the effect of the fertilizer treatment was significant on plant height and shoot

Table 3 Maize growth parameters as affected by the application of OMF, MF or the combination of both.

Treatments (OMF: MF)	Plant height (cm)	Stem girth (cm)	Shoot weight (g)	Root weight (g)
0: 0	31.1b	4.4c	20.0b	12.9c
0: 1.0	38.9a	5.2ab	26.8ab	20.3ab
0.25: 0.75	38.1a	5.6a	30.2a	22.6a
0.5: 0.5	38.5a	5.5a	31.5a	23.0a
0.75: 0.25	33.1ab	5.0abc	26.2ab	16.0bc
1.0: 0	33.7ab	4.8bc	25.1ab	16.5bc
SEM	1.85	0.19	1.12	1.40

Means within the same column followed by same letter(s) are statistically similar at 5% level of probability.

Table 4 Millet growth parameters as affected by the application of OMF, MF or the combination of both.

Treatments (OMF: MF)	Plant height (cm)	Stem girth (cm)	Shoot weight (g)	Root weight (g)
0: 0	21.0a	4.4a	9.8a	12.0a
0: 1.0	22.9a	4.8a	13.0a	14.2a
0.25: 0.75	23.6a	4.7a	12.8a	14.0a
0.5: 0.5	21.0a	4.9a	12.3a	17.4a
0.75: 0.25	21.1a	4.6a	11.2a	13.8a
1.0: 0	20.9a	4.5a	11.2a	12.8a
SEM	2.07	0.18	0.80	1.86

Means within the same column followed by same letter(s) are statistically similar at 5% level of probability.

weight (Table 5). Differences in stem girth and root dry weight were not significant. The control treatment produced significantly lower plant height than the other treatments. Plants that received combined application of OMF and MF in ratios 0.25: 0.75 and 0.50: 0.50 gave significantly higher ($P < 0.05$) values of shoot weight compared to other treatments. These were statistically followed by plants that received MF only while the control treatment had the lowest value. There was no significant difference between combined application of OMF and MF at ratio 0.75: 0.25 and sole application of OMF.

3.2 Field Trials (Comparison between OMF and MF)

Soybean: The results obtained showed that application of 20 kg N ha^{-1} from the OMF produced significantly ($P < 0.05$) higher grain yield than the control while no significant difference was observed between all fertilized plots (Table 6). However, application of 20 kg N ha^{-1} from the OMF produced relatively higher grain yield compared to other rates. Haulm yield was significantly ($P < 0.01$) higher with application of 20 kg N ha^{-1} from the MF compared to the control (Table 6). No significant difference was observed between all fertilized plots. The results obtained showed that application of 20 kg N ha^{-1} from OMF produced both grain and haulm yields that were comparable to what was obtained with the application of 30 kg N ha^{-1} from MF.

Cowpea: The results obtained showed that application of 30 kg N ha^{-1} from the conventional MF produced significantly ($P < 0.05$) higher grain yield than the control while no significant difference was observed between all fertilized plots (Table 7). Of the fertilized plots, application of 10 kg N ha^{-1} from both fertilizer products produced less than one tonne of grain yield while other rates produced grain yield well above one tonne per hectare. Similarly, haulm yield was significantly ($P < 0.01$) higher with application of 30 kg N ha^{-1} from MF compared to the control (Table 7). No significant difference was observed between all fertilized plots. Application of 20 kg N ha^{-1} from the OMF produced relatively higher grain and haulm yields compared to other rates. The results obtained at this rate were comparable to what was obtained with the application of 20 and 30 kg N ha^{-1} from MF.

Maize: The results obtained showed that the control treatment had significantly lower grain yield than other treatments (Table 8). Of the plots treated with MF

Table 5 Sorghum growth parameters as affected by the application of OMF, MF or the combination of both.

Treatments (OMF: MF)	Plant height (cm)	Stem girth (cm)	Shoot weight (g)	Root weight (g)
0: 0	25.1b	4.2a	8.1c	10.0a
0: 1.0	31.3a	4.9a	12.4abc	13.9a
0.25: 0.75	32.8a	5.3a	18.2a	14.6a
0.5: 0.5	31.7a	4.6a	14.8ab	13.3a
0.75: 0.25	29.6a	4.3a	11.9bc	12.1a
1.0: 0	29.2a	4.3a	11.9bc	11.5a
SEM	1.13	0.39	1.77	1.89

Means within the same column followed by same letter(s) are statistically similar at 5% level of probability.

Table 6 Effect of OMF and MF on the grain and haulm yields of soybean.

Treatments	Grain (kg ha^{-1})	Haulm (kg ha^{-1})
Control	1,055 b	1,161 b
10 kg N ha^{-1} (MF)	1,196 ab	1,455 ab
20 kg N ha^{-1} (MF)	1,305 ab	1,779 a
30 kg N ha^{-1} (MF)	1,306 ab	1,455 ab
10 kg N ha^{-1} (OMF)	1,161 ab	1,299 ab
20 kg N ha^{-1} (OMF)	1,448 a	1,636 ab
30 kg N ha^{-1} (OMF)	1,188 ab	1,381 ab
SEM	120.1	167.0

Means within the same column followed by same letter(s) are statistically similar at 5% level of probability.

Table 7 Effect of OMF and MF on the grain and haulm yields of soybean.

Treatments	Grain (kg ha^{-1})	Haulm (kg ha^{-1})
Control	620 b	238 b
10 kg N ha^{-1} (MF)	878 ab	613 ab
20 kg N ha^{-1} (MF)	1,311 ab	998 ab
30 kg N ha^{-1} (MF)	1,420 a	1,219 a
10 kg N ha^{-1} (OMF)	905 ab	590 ab
20 kg N ha^{-1} (OMF)	1,310 ab	613 ab
30 kg N ha^{-1} (OMF)	1,071 ab	608 ab
SEM	260.3	256.1

Means within the same column followed by same letter(s) are statistically similar at the 5% level of probability.

Table 8 Effect of OMF and MF on the grain and stover yield of maize, millet and sorghum.

Treatment	Maize yield (kg ha^{-1})		Millet yield (kg ha^{-1})		Sorghum yield (kg ha^{-1})	
	Grain	Stover	Grain	Stover	Grain	Stover
0	973 c	2,647 b	649 c	3,629 c	300 b	966 c
30 MF†	1,682 ab	4,771 ab	887 bc	4,171 bc	629 b	2,012 bc
60 MF†	1,972 a	4,944 ab	1,407 a	5,559 b	1,230 a	4,736 ab
90 MF†	2,089 a	6,098 a	1,490 a	7,682 a	1,444 a	5,829 a
30 OMF†	1,230 bc	3,575 ab	816 bc	3,851 bc	1,230 a	2,740 bc
60 OMF†	1,678 ab	4,430 ab	1,049 abc	4,250 bc	1,201 a	3,508 bc
90 OMF†	2,097 a	5,869 a	1,206 ab	5,670 b	1,426 a	3,571 bc
SEM	154.8	776.8	142.5	545.3	112.3	159.6

† Millet and sorghum received half of each rate. Means within the same column followed by same letter (s) are statistically similar at 5% level of probability.

only, application of 60 and 90 kg N ha^{-1} gave no significant difference in terms of grain yield but were significantly higher than plots treated with 30 kg N ha^{-1}. Similar result was obtained by the application of OMF with 90 kg N ha^{-1} performing significantly higher than other treatments. Analysis of all treatments showed that application of 60 kg N ha^{-1} from MF is more promising since it gave statistically similar grain yield to 90 kg N ha^{-1} from MF or OMF. On the contrary, 90 kg N ha^{-1} from MF or OMF produced significantly higher stover yield than other treatments. Except the control treatment, the remaining levels of MF and OMF gave statistically similar results (Table 8).

Millet: The results obtained showed that application of 30 and 45 kg N ha^{-1} from the MF produced significantly ($P < 0.05$) higher grain yield than other treatments (Table 8). This was followed by the application of 45 and 30 kg N ha^{-1} from OMF in that order. There was no significant difference in grain yield with application of 15 kg N ha^{-1} from either MF or OMF but these were higher than the control. Results obtained on the stover yield followed similar pattern with 45 kg N ha^{-1} from MF having significantly higher values followed by 30 and 45 kg N ha^{-1} from MF and OMF, which were at par. Other treatments followed similar pattern as enumerated for the grain yield.

Sorghum: The results on grain yield were statistically similar for all treatments except the control and 15 kg N ha^{-1} from MF, which were significantly, lower (Table 8). This was largely attributed to smut attack on the heads before harvest. However, results on stover yield showed that 45 kg N ha^{-1} from MF gave significantly higher value than other treatments. This was followed by the application of 30 kg N ha^{-1}. Except the control treatment, all other treatments were statistically similar.

3.3 Field Trials (Combined Application of OMF and Urea)

Grain and stover yield of all cereal crops did not respond to applied urea N while maize grain and millet stover yields responded significantly to OMF-N application (Table 9). Maize grain and millet stover yields increased with increasing rates of OMF-N. The interaction between urea N and OMF-N was not significant for all the parameters measured.

4. Discussion

4.1 Greenhouse Trials

The results on maize suggest slight additional benefit of combined application of OMF with MF. Application of MF could have directly improved availability of nutrients in OMF by enhancing the mineralization process through the supply of readily available nutrients to the soil microbial community. Because small addition of MF to OMF did not cause significant difference in the results obtained compared to sole application of OMF, it is not likely that this process is responsible for the additional benefit. Instead, it could be due to improved MF nutrients utilization by

Table 9 Effect of OMF and urea on the grain and stover yield of maize, millet and sorghum.

Treatment	Maize yield (kg ha^{-1})		Millet yield (kg ha^{-1})		Sorghum yield (kg ha^{-1})	
	Grain	Stover	Grain	Stover	Grain	Stover
Urea N						
30 MF†	1,970 a	5,528 a	1,215 a	4,924 a	1,543 a	5,671 a
60 MF†	2,062 a	5,692 a	1,331 a	4,997 a	1,708 a	6,589 a
90 MF†	2,160 a	6,100 a	1,438 a	5,870 a	1,803 a	6,866 a
Mean	2,064	5,733	1,328	5,264	1,685	6,375
SEM	94.3	516.8	139.9	521.9	103.0	132.9
OMF						
30 MF†	1,705 c	5,076 a	1,503 a	3912 b	1,556 a	3,869 a
60 MF†	2,053 b	5,888 a	1,240 a	5304 ab	1,680 a	4,437 a
90 MF†	2,494 a	6,356 a	1,241 a	6575 a	1,818 a	4,820 a
Mean	2,084	5,773	1,328	5264	1,685	4,375
SEM	94.3	516.8	139.9	521.9	103.0	132.9
Urea x OMF						
SEM	163.4	895.2	242.4	903.9	178.4	962.3
Significance	NS	NS	NS	NS	NS	NS

† Millet and sorghum received half of each rate. Means within the same column followed by same letter(s) are statistically similar at 5% level of probability. NS Not significant at 5% level of probability.

small application of OMF. Several authors have demonstrated significant maize response to MF in the moist savanna of Nigeria [5, 14, 15].

The lack of significant difference in plant height and stem girth could be due to large number of tillers produced by millet which makes it difficult to identify representative reference plant stand during data collection especially at the vegetative growth phase. Lack of significant difference in shoot and root weight suggests that the duration of the greenhouse trial may be insufficient to capture differences due to the treatment effect. Therefore, millet may have to be grown to maturity to be able to capture differences due to fertilizer effects. In this situation, grain and stover yields may be more useful indicator of treatment effects.

4.2 Field Trials

The results obtained on soybean and cowpea corroborates previous findings which recommend 20 kg N ha^{-1} for most grain legumes in the Nigerian savanna [16]. This recommendation was based on the premise that the soils are inherently low in N [17]. Other plausible reasons could be low population or ineffectiveness of the indigenous rhizobia to fix atmospheric N in symbiosis with the legumes. Higher grain and haulm yields obtained with the application of OMF compared to MF may be due to addition of other growth promoting substances in the organic material which are absent in the mineral fertilizer [15].

Generally, MF and OMF showed similar potential in improving cereal grain and stover yield. However, the lack of significant response to urea N application could be attributed to phosphorus and water limitations in the soil. Response of cereals to N application is usually more effective in the presence of applied P [18]. This was demonstrated in the crops performance where inorganic N was applied together with P in the MF (NPK 15-15-15). The only external P source in the combined OMF and urea study was the P derived from OMF which may not be sufficient to meet the crops' requirements due to competition by the decomposing microbial community and high sorption capacity of some tropical soils [19]. Moreover, the experimental soil is inherently low in available P (5.8 mg kg^{-1}), which implies that basal application of soluble P as chemical fertilizer would be necessary to obtain response to applied N. The sandy nature of the soil

texture may have also contributed to poor response to urea applied N. Urea N is easily brought into soil solution and losses can be facilitated in sandy soil if not utilized by crop.

Response to OMF-N could be attributed to the presence of P in the fertilizer material and slow release of N, which might have improved the N use efficiency. Moreover, application of OMF may alter the soil-related pest spectrum in the short term. M. Akhtar [20], for example, found that application of *Azadirachta indica* based organic products, sole or in combination with urea, significantly reduced the total number of plant-parasitic nematodes. In the longer term, continuous application of OMF may improve soil physical and chemical characteristics such as soil structure, bulk density, porosity, and nutrient retention among others, consequently lead to better crop growth.

5. Conclusion

The results obtained from the greenhouse study on the measured parameters showed that combined application of OMF and MF in ratio 0.25: 0.75 was more promising than other combinations or sole application of either fertilizer sources. This was due to improved MF nutrients utilization by small application of OMF and potentially higher availability of soil water in the sandy loam soil. In the field, OMF appear to have the potential to alleviate constraints to crop production other than N deficiency. The contribution of the applied OMF-N in combination with urea N was slightly higher compared with the contribution made by urea N. The low N contribution of urea is attributed to other growth-limiting factors especially phosphorus. Both greenhouse and field trials suggest that combined use of neem-based fertilizer and mineral fertilizer would alleviate constraints to soil productivity and boost crop production through efficient utilization of plant nutrients. However, future studies should consider the addition of soluble P in order to optimize benefits from both nutrient sources. Similarly, studies on the long term effect on soil properties would be useful in understanding the sustainability of the soil fertility management strategy.

Acknowledgments

This research was sponsored by grants from the Ahmadu Bello University Board of Research (UBR) and the National Research Institute for Chemical Technology (NARICT), Zaria, Nigeria. The neem based fertilizer was supplied by NARICT. The authors are grateful to Messrs U.O. Bello, A. Jibrin and I. Ibrahim of the Department of Soil Science, Ahmadu Bello University, Zaria for their technical assistance in both the field and the laboratory.

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Journal of Agricultural Science and Technology A 1 (2011) 244-248
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Measurement of Biological Oxygen Demand (BOD) in Sewage Wastewater Using Modified Inoculums

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Received: August 31, 2010 / Published: June 20, 2011.

Abstract: The objective of this studying was to accurate determination of Biological Oxygen Demand (BOD) through the use of two types of prepared inoculums, the natural activated sludge supplied from conventional wastewater treatment plant and the modified activated sludge prepared from activated sludge of wastewater treatment plant of refinery factory. Analytical method was used to measurement of BOD by preparing the standard curve of BOD in basal medium. The results showed to the large differences in BOD values in basal medium (30-300 mg/L) and conventional wastewater (80-320 mg/L) when they were inoculated with natural and modified activated sludge respectively. It was also found an ability of modified sludge to remove high concentrations of oil and greases.

Key words: BOD measurement, sludge, wastewaters, environmental biotechnology.

1. Introduction

Biochemical oxygen demand or biological oxygen demand is a chemical procedure for determining is the amount of dissolved oxygen needed by aerobic biological organisms in a body of water to break down organic material present in a given water sample at certain temperature over a specific time period. It is not a precise quantitative test, although it is widely used as an indication of the quality of water [1]. The Royal Commission on River Pollution, which was established in 1865 and the formation of the Royal Commission on Sewage Disposal in 1898 led to the selection in 1908 of BOD_5 as the definitive test for organic pollution of rivers. Five days was chosen as an appropriate test period because this is supposedly the longest time that river water takes to travel from source to estuary in the U.K. In 1912, the commission also set a standard of 20 ppm BOD_5 as the maximum concentration permitted in sewage works discharging to rivers, provided that there was at least an 8:1 dilution available at dry weather low. This was contained in the famous 20:30 (BOD: Suspended Solids) plus full nitrification standard which was used as a yardstick in the U.K. up to the 1970s for sewage works effluent quality. The United States includes BOD effluent limitations in its secondary treatment regulations. Secondary sewage treatment is generally expected to remove 85 percent of the BOD measured in sewage and produce effluent BOD concentrations with a 30-day average of less than 30 mg/L and a 7-day average of less than 45 mg/L. The regulations also describe “treatment equivalent to secondary treatment” as removing 65 percent of the BOD and producing effluent BOD concentrations with a 30-day average less than 45 mg/L and a 7-day average less than 65 mg/L [2]. The biochemical oxygen demand (BOD) determination is an empirical test in which standardized laboratory procedures are used to determine the relative oxygen requirements of wastewaters, effluent, and polluted waters. The test has its widest application in measuring waste loadings to treatment plants and in evaluating the BOD-removal efficiency of such treatment systems. Measurements of oxygen consumed in a 5-days test period (5210B),

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oxygen consumed after 60 to 90 days of incubation (5210C), and continuous oxygen uptake (5210D) are used [3].

There are two commonly recognized techniques for the measurement of BOD, the dilution method and Manometric method. In the dilution method, the BOD test is carried out by diluting the sample with oxygen saturated de-ionized water, inoculating it with a fixed aliquot of seed, measuring the dissolved oxygen (DO) and then sealing the sample to prevent further oxygen dissolving in. The sample is kept at 20 °C in the dark to prevent photosynthesis (and thereby the addition of oxygen) for five days, and the dissolved oxygen is measured again. The difference between the final DO and initial DO is the BOD. A nitrification inhibitor is added after the dilution water has been added to the sample. The inhibitor hinders the oxidation of nitrogen. Manometric method is limited to the measurement of the oxygen consumption due only to carbonaceous oxidation. Ammonia oxidation is inhibited. The main advantages of this method compared to the dilution method are simplicity and continuous display of BOD value at the current incubation time [4]. Most pristine rivers will have a 5-day carbonaceous BOD below 1 mg/L. Moderately polluted rivers may have a BOD value in the range of 2 to 8 mg/L. Municipal sewage that is efficiently treated by a three-stage process would have a value of about 20 mg/L or less. Untreated sewage varies, but averages around 600 mg/L in Europe and as low as 200 mg/L in the U.S., or where there is severe groundwater or surface water infiltration. The generally lower values in the U.S. derive from the much greater water use per capita than in other parts of the world. The test method involves variables limiting reproducibility. Tests normally show observations varying plus or minus ten to twenty percent around the mean. Some wastes contain chemicals capable of suppressing microbiological growth or activity. Potential sources include industrial wastes, antibiotics in pharmaceutical or medical wastes, sanitizers in food processing or commercial cleaning facilities, chlorination disinfection used following conventional sewage treatment, and odor-control formulations used in sanitary waste holding tanks in passenger vehicles or portable toilets. Suppression of the microbial community oxidizing the waste will lower the test result [5].

2. Materials and Methods

2.1 Types of Water

(a) Water, prepared according to the specification attached with a BOD (OXi-Top IS 12) procedure supplied by WTW company. It was used for the preparation of the standard curve for BOD as well as for calibration the device; (b) Wastewater after preparation treatment from the meddle petroleum company, (Al-dhora refinery). The water sample was centrifuged at 4,000 rpm for 15 minute to precipitate the microorganisms and other solids. The filtrate was withdrawn and transferred to a sterile container, heating at 60 °C and used in part to prepare the solid medium by adding (2%) of sterilized molting agar. It was poured in sterile Petri dishes and used in screening tests and maintained the isolates. The liquid medium was used in the experiences of screening and the subsequent selection of isolates and for the purpose of reactivation the isolates; (c) Wastewater from Alrsutamyia plant (in southern of Baghdad city) was prepared in the same way as above.

2.2 Preparation the Natural Inoculums

It was prepared from the activated sludge of the oxidation tank in the southern station of conventional wastewater treatment plant called Alrustamiya. The sludge was prepared by washing the precipitated sludge in standard phosphate solution (pH = 7. 3) of the same original volume. The inoculums was preserved in freezing by adding 20% glycerol. The sludge was activated in the recommended medium of BOD test according to the WTW instructions and after washing the sludge to remove the glycerol. BOD bottles were inoculated with 3% of the natural culture.

2.3 Preparation of Modified Inoculums

Water samples were taken from the different units of biological treatment station of the meddle petroleum company (Al- dhora refinery) as: (a) floating tank, (b) after floating tank, (c) oxidation tank.

Modified Inoculum was prepared from samples above by washing the precipitated biomass from three sources of cultures in standard phosphate solution (pH = 7.3) of the same original volume. These cultures were undergoing to re-isolation processes by culturing under aerobic and anaerobic conditions in solid medium consists of 1:1 (Aldhora-prearation wastewater: Al-Rustamyia-Prearation wastewater after centrifugation and discharged the deposit then addition the agar and sterilization). The growth colonies were taken and inoculated the broth medium of 1:1 (Aldhora: Al-Rustamyia-Prearation wastewaters after centrifugation and discharged the deposit and sterilization). Total Suspended Solids (TSS) were determined for modified sludge according to (APHA 1985) [6].

The inoculums was preserved in freezing by adding 20% glycerol. The Activated sludge was activated in the recommended medium of BOD test according to the WTW instructions and after washing the sludge to remove the glycerol. BOD bottles were inoculated with 3% of the modified inoculums.

2.4 Examination the Efficiency of the Cultures

The natural and modified sludge were examined in many formulas of Aldhora and Alrustamyia wastewaters as follows: (1) Alrustamyia (After prearation T1) 80% + Aldhora (after floatation) 20%; (2) Alrustamyia (After primary settling tank T2) 80% + Aldhora (after floatation) 20%; (3) Alrustamyia (After prearation T1) + Aldhora (after floatation) in ratio of (2:1); (4) Alrustamyia (After prearation T1) + Aldhora (after floatation) in ratio of (1:1). The formulas of wastewaters have been inoculated with modified inoculums to give the final TSS (2,500 mg/L). The cultures were incubated at mixing 175 rpm/min and 30 °C for 48 hours.

2.5 Determination the BOD

(1) Prepare the standard solution for the BOD (2000 mg/L) in demonized water. BOD standard powder was supplied from WTW company. The solution was used for preparation the media of serial BOD concentrations.

(2) Add the BOD solution to the recommended BOD medium (Basal medium prepared according to WTW instructions attached with BOD instrument) at concentrations of 0.0-1000 mg/L using the bellow equation:

C1 V1 = C2 V2

2,000 mg/L X V1 = 50 mg/L × 164 mL

V1 = 4.1 mL from standard solution

(C1) and complete to 164 mL of of basal medium in BOD bottle then inoculated with natural or modified sludge (Inoculums). All bottles are recoverd with OXiTop S12 digital electronic detectors and place it in rack shaker in the incubator for 5 days at 20 °C.

3. Results and Discussion

3.1 Examination of Aldhora Samples

Table 1 shows the results of analysis of wastewater samples from post-flotation and after oxidation treatment in aldhora refineries which are characterized by high oils up to 965 mg/L and BOD values of less than 200 mg/L. It found highly efficient of sludge in oxidative basin to remove high concentrations of oils up to 300 mg/L during the continuous treatments. The reason actually returns to the genetics adaptations of the cells of sludge in the high concentrations of oil.

Table 1 Wastewater analysis of some stages in the meddle petroleum company in Baghdad.

No.	Test	After floatation	After oxidation
1	BOD (mg/L)	230	95
2	Oil &Greases (mg/L)	965	620
3	pH	6.5	7.4
4	TDS(mg/L)	410	45

Seen from the table a good dip in the BOD reached 95 mg/L during the treatment. It was also observed low values of pH in the wastewater before oxidative tank.

3.2 Isolation and Production the Modified Sludge

The growth was appeared after 48 hours in the solid water medium of Aldhora. The colonies growth on the surface and bottom of the plates was creamy, rounded and flat. The cells are G-ve and filamentous. The growth was heavily in the liquid medium (1:1 Aldhora: Al-Rustamyia-Prearation wastewaters after centrifugation and discharged the deposit and sterilization). The growth was proliferated by scaling up to reach the TSS more then 3,000 mg/L to be sued in the experiment (Fig. 1).

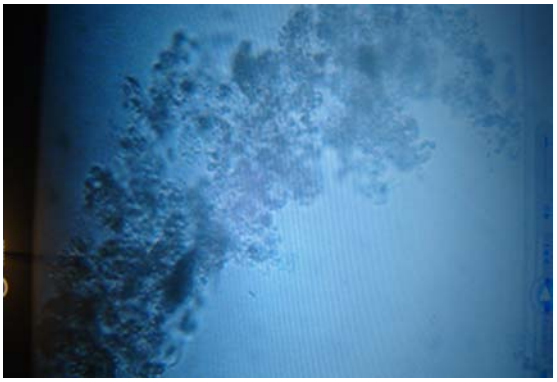

Fig. 1 The modified sludge.

3.3 Efficiency of the Modified Sludge

Table 2 shows the results of the efficiency of the modified sludge in the consumption and carrying high concentrations of the BOD and Oils. This means that the efficiency of these isolates on the consumption of carbon sources of degradable and non degradable of carbon sources and hydrocarbons. The water by mixing 1:1 increased the concentration of oil to 463 mg/L and reduced to 245 mg/L by the activity of modified sludge. These results indicate the tolerance and ability the modified sludge to consume high concentration of oils in the wastewater with high concentration of oils. It is also found in Table 2 the ability of modified sludge to utilize high concentrations of BOD in wastewater in addition to the oil especially in the treatment (1:1).

Table 2 Comparison the efficiency ofmodified sludge in different combinedmedia.

Water mixture	Oil & Grease (mg/L)*	Remaining oil (mg/L)**	Remaining BOD (mg/L)**	Growth density (550 nm)***
80% + 20% (alrustamyia after prearation + Aldhora after floation)	222	22	46	1.6
2:1 (alrustamyia after prearation + Aldhora after floation)	350	125	78	1.9
2:1 Alrustamyia (After prearation T1) + Aldhora (after floatation).	129	8	46	1.7
1:1 Alrustamyia (After prearation T1) + Aldhora (after floatation).	463	254	22	1.7

*The final concentration of oil the media.

** BOD concentration = 220 mg/L.

*** Mixing 175 rpm/min and 30 °C incubation for 48 h.

Table 3 The values of BOD in the standard media using different inoculums.

BOD required (mg/L)	Volume required (mL)	Weight of BOD powder required (mg)	BOD (mg/L)	
			Natural sludge	Modified sludge
0	0	0	2	4
50	4.1	8.1	23	48
100	8.2	16.4	56	96
150	12.3	24.3	77	144
200	16.4	32.4	110	195
300	24.6	48.6	167	292
400	32.8	64.8	217	394
600	49.8	97.2	298	596
800	65.6	129.6	388	790
1000	82	162	412	985

Table 4 BOD consumption by modified and natural sludge.

Test	Range of values / source of sample				
BOD_5 (mg/L)	After Praeration	After primary Settling	Oxidation tank	After Oxidation	After secondary tank
Natural sludge	180-300	120-170	80-120	70-100	70-100
Modified sludge	500-600	300-400	200-250	150-200	150-180

3.4 BOD Determination

Table 3 shows the comparison the values of BOD of the natural and modified sludge in a basal medium contains the standard BOD concentrations. The results reflect the situation of the real differences in the measurement of the BOD in the station or treatment plants. The modified sludge consumed about 98% of standard organic solution to give the nearest values of BOD standard and the difference ranged between 80 -300 mg/L. So we should select and imply the standard or suitable sludge inoculums. In Table 4, the application of modified and natural sludge in the determination of BOD in wastewaters taken from many stages of Alrustamyia plant.

The results reflect the important of using the modified sludge to obtain the real concentrations of BOD values compared with natural sludge.

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Journal of Agricultural Science and Technology A 1 (2011) 249-254
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Degradation of Soils in the Lowland of Kur-Araz

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Received: April 27, 2010 / Published: June 20, 2011.

Abstracts: The research works were carried out for determination of the washout, types, sorts and level of the development reasons of degradation with the purpose of preparation of the measures of fight against degradation in the soils in 2005-2009 in the lowland of Kur-Araz. The investigations were fulfilled by using of geographical and stasionar methods. It is revealed that 333.6 thous. ha of the soils of the lowland of Kur-Araz have been exposed to erosion. 97.4 thousand ha of them have been exposed to rainstorm erosion, 127.1 thous. have been exposed to irrigative and 122.1 thous. ha to wind erosion. The most dangerous of them are considered irrigative erosion. Under the influence of erosion process in deposited over the length of furrow, deposits the changes happened in maintenance of humus and nutritions elements. In dependence on slope and water expenditure on 1 ha 2.23-14.86 t of soil are leached. As a result of out wash from 1 ha of soil 55.88-304.59 kg of humus, 4.06-20.80 kg of nitrogen, 4.57-26.55 of phosphorus and 57.40-372.99 of potassium are lost. The soil is exposed to degradation and as a result a process of desertification begins. On the basis of the quantitative an intensity of soil outwash the possible losses of dry matter including humus and main elements of nutrition of plants as a result of erosion, their deposit with the water way, are calculated, too.

Key words: Irrigative erosion, erosion degradation, wind erosion, defination, capacity, removal of humus.

1. Introduction

Wind erosion in the eastern regions of the lowland brings irreplaceable damage which is not only defined by reduction of the collection of production of the agriculture with eroded soils, and decrease of potential fertility of soils.

Degradation brings a great damage to agriculture of the world including Azerbaijan. The German scientist Yustus Libikh noted in 1840 that “degradation of soils threatens all the humanity, sometimes it is called slow death of planet”. In this connection with the purpose of in assumption of irreversible degradation it is necessary to organize biological potential, to neutralize able completeness and to stop this process. That’s why the main problem of the rational use of the irrigative soils, of dry subtropics in Azerbaijan is an elaboration of the methods of soil stability to degradation, reproduction of the biological activity, soil fertility. It presents theoretical interest and possesses a great practical importance.

Degradation of soils-it is a totality of physic-geographical and antropogenic processes, subjecting to destruction of arid zones, degradation of all the forms of organic life and to reduction of natural economic potential of these territories.

An area of the soils of the exposing degradation in the Republic of Azerbaijan highly forms an important quantity: about 40% from a general area of the Republic. The cause of degradation of the soils, conveying global character, in particular it is erosion of soils [1-3].

2. Objects and Methods

Irrigative and wind erosion (defilation) spread in Kur-Araz lowland. Irrigative erosion is characteristic for the entire semi-desert zones and foothill parts where the slope forms 0.003, the wind erosion

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(defilation) for territory alond the river of Kur (southern-east Shirvan and Salyan steppe), for western regions of the lowland (Table 1). More than 55% of irrigative lands of the lowland are situated on the areas with the great slopes (0.003 and more).

The analysis of Table 1 shows that there are many erodible soils in the massive of Mugan-Salyan. Especially deflation and water (rain) have got an area where erosion is observed to a considerable extent in the plain of south-East Shirvan of the massif.

Its reason connects with the area which is inclined and broken. It connects with the strong winds on the seaside of the Caspean Sea, weak development of the vegetation on the surface.

Its reason also connects with the soils which are sandy. Irrigation erosion manly developed in the inclined areas of the plain of Mugan-Salyan.

The areas for investigations were selected in serozem, meadow-serozem and light-grey-brown soils, forming a base of the land fund here. A quantity of physical day (< 0.01 mm) in an arable layer vibrates in limits 32.40-56.84% a quantity of water stable aggregates (> 0.25 mm) 22.95-32.60%. Density of soils changes in limits of 1.18-1.59 g/sm^3, but specific weight forms 2.59-2.78 g/sm^3. Porosity of inter aggregates of the arable horizon is equal to 49.6-55.8%, a maintenance of humus vibrates from 1.61 to 1.80%.

Flows and out washes of the soils have been studied on natural conditions in all the versions of the experimental areas by means of selection of the samples of the irrigative waters every intervals: 5, 15, 60, 120, 180, 240 minutes after the beginning of flow and last hours of irrigation in each registration furrow in three repetition by M. S. Kuznetsov [4] and a method of dimension of the hollow.

Under carried out of irrigation they observed a quantity of irrigative stream at the beginning and at the end of furrows with the help of the spillway by Thomson [4, 5]; a rate of water flow in the furrows has been defined with the help of dye-liquid and second – measure, but regulation of the weight of irrigative stream is used of polyetilen napkins.

3. Results and Discussion

It was established over a result of the investigations that the display of irrigative erosion on character of the display in the given territory was divided into three groups:

(1) The surface outwash of the upper horizons of soils of the irrigative areas is under an influence of irrigation at rainfall.

(2) Flow erosion (including deep and side outwash of irrigative net) with the following formation of the ravines.

(3) Destruction of water economic net, outwash of soil layer, bringing of the soils by the earth flows and flood waters.

In connection with the peculiarities of hydrological regime on the irrigative areas from the development of irrigative erosion three zones are formed: erosion, stabilizative, and accumulative. The inequality of surface of the irrigative areas promotes intensive development of erosion-accumulative process.

Outwash of soils under irrigations on furrows is observed, first of all, in those places where irrigative furrows have got an important slope. In essence erosion processes spread in temporary and permanent inside economic irrigative net everywhere.

The consequences of our investigations on revealing of the influence of the quantity of slope, of the water expenditure on intensity of irrigative erosion are presented on Table 2.

It is obvious from Table 2 that the washout soil mass increases intensively while rising a quantity of water expense and inclination. The consequences of some investigations approve it, too [5-7].

From the getting data it is clearly visible that out wash of soil depends on slope, water expenditure and antierosion firmness of soils. Thus, under slope of the irrigative furrows it is 0.006, water expenditure is 0.6 and 1.2 L/s, length of furrow is 200 m in heavy loamy bright serozem soils, the turbidity of abandoned water accordingly forms 8.5 and 13.67 g/L, removal of soils is accordingly 2.26 and 4.68 t/h.

Table 1 Parameters of exposing to erosion over natural territories of Kur-Araz Lowland (thous. ha).

Natural territories	Total area	Eroded from them	Including eroded by types and kinds				
			From outflow of cloud-burst water			From irrigation	From defilation
Mugan-Salyan massiv	871.1	131.2	21.7	11.7	–	36.2	61.6
Shirvan steppe	508.0	116.9	16.0	8.3	8.6	41.5	42.5
Mil steppe	477.9	29.7	1.9	0.6	–	24.5	2.7
Garabagh steppe	241.8	55.8	18.1	3.8	6.7	24.9	16.3
In all	2098.8	333.6	57.7	24.4	15.3	127.1	122.1

Table 2 Irrigative outwash of soil in dependence of the slope of the field and water expenditure.

The slope of the soil	The length of the furrows (m)	Water expenditure in the furrow (L/s)	Turbidity (g/L)	Enleaching of the soil for one irrigation (t/h)
0.006	200	0.6	8.25	2.26
Heavyloamy bright serozem		0.8	13.67	4.68
		1.0	16.82	6.41
		1.2	21.54	8.84
0.018	200	0.2	9.44	2.78
Heavyloamy bright serozem		0.4	15.28	6.42
		0.6	20.65	10.74
		0.8	26.76	14.86
0.012	200	0.4	13.80	2.23
Middle loamy typical serozem		0.6	19.63	3.48
		0.8	23.84	5.20
		1.0	26.47	7.12
0.003	250	0.8	4.56	2.44
Mid loamy meadow-serozem		1.0	5.12	2.98
		1.2	7.24	3.45
		1.4	9.10	4.16
0.020	150	0.2	9.13	2.54
Mid loamy bright grey-brown		0.4	15.56	5.82
(chestnut)		0.6	19.34	9.16
		0.8	24.78	13.40

Under the slope it is 0.018 and on equal other conditions, the turbidity of water accordingly formed 19.63 and 23.84 g/L, enleaching is accordingly 10.74 and 14.86 t/ha.

From point of view of conducting of irrigations on furrows in the secure dimensions of outwash of soils the slope can be considered optimally < 0.003.

Granulometric composition in upper zone becomes lighter at expense of outwash of silty fraction, that is, decolmatation of genetic horizon, in low zone it grows heavier in consequence of enleaching of those fractions from year to year. Thus, granulometric composition of the irrigative soils in dependence on intensity of their usage changes to some and other degree.

The aggravation of the structural situation is unfavourably reflected on other soil regimes and peculiarities in its turn.

Destruction of structures and concentrating of irrigative soils can develop into sterilization which can be considered high display of structurelessness and concentrating. Mostly of stylization the upper, arable horizons are exposed, but there is information about this process in the underlying horizons, too.

Unevenness of granulometric composition on the fields complicates carrying out agricultural works, creates additional difficulty in growing of agricultural plants. For example, if the soil crustes in the irrigative furrows is formed only on surface of soil and reaches a little thickness, washed-over accumulation forms hard layer of a great thickness. These irrigative deposits under drying turn into cement plates with a high density, preventing from development of the roots.

Under an influence of irrigative erosion density and specific mass, also general porosity of soil change. The density of arable horizon in the erosion zone, an upper part of the field is slightly less and is connected with going out of the least concentrating horizon.

It is known that under erosion of soil finally the soil exhausts, as inevitable consequence, its fertility decreases, productivity of agricultural plants falls and water is polluted.

The getting data about maintenance of humus and nutritious elements in hard flow under irrigation testify that a quantity of their enleaching is significantly higher than their maintenance in upper horizons of the soils. So, a quantity of humus, gross nitrogen, and phosphorus in the hard flow is 20-30% higher than in entering water (Table 3).

It is known from Table 3 that humus, nitrogen, phosphorus and potassium in the float are higher than soil and it is completed by washing out of substances very much [5, 8].

The calculations of the loss of humus and nutritious elements showed that their enleaching was in a straight dependence of water expenditure. So, in the field with the slope of 0.20 (grey-brown bright soils) and length of the furrow of 150 m under water expenditure of 0.2 L/sec of enleaching of humus formed 55.88 kg/ha, nitrogen 40.6 kg/h, phosphorus 4.57 kg/h, potassium 67.82 kg/h, but under water expenditure of 0.8 L/sec is accordingly 297.98, 17.42, 21.44, 352.42 (Table 4).

As obvious that it depends on a quantity of water expense and inclination. The dependence of washout quantity of humus and biogen elements on inclination and water expense corresponds to a quantity of the washout soils. According to this Kh. Kh. Khamdamov [5], D. L. Carter, B. D. Berg, B. Z. Sanderes [6], R. Wallech, W. A. Jury, W. F. Speker [8], and others have got consequences.

The process of the degradation is characteristic only for a zone of irrigative agriculture and pasture zone. In degradation of the pacture lands of the semi-disserts defilation, causing irrational usage of the vegetation plays a great role in pasture stockbreeding. Defilation on the territory of Kur-Araz lowland appears both in the winter pastures and in the ploughing lands. It intensifies most under excessive pasture of cattle and in absence of elementary soil preserved agrotechnical methods. Its development promote over droughtness of the climate under wind, salinity and carbonatization of the soils, thinning of vegetation and effect of antropogen factors.

The defilation changes physicochemical peculiarities mainly in serozem and grey-brown soils. As compared with nonerodible on the eroded differences a quantity of sandy fraction increases 10-15%, a quantity of dusty fraction decreases 30-50% and silty 15-40%. On the eroded differences of the soils of the light granulometric composition maintenance of humus decreases 20-40%, gross nitrogen 8-45%, assimilating phosphorus 10-30%, exchangeable potassium 10-40%. Humus is 40-70% less, gross nitrogen 20-70%, assimilating phosphorus 20-60%, exchangeable potassium 5-35% in under bush Aeolian hillocks than in nonerodible differences.

As a matter of fact, erosion processes spread everywhere in temporary and constant inside economy irrigative net. As a result of annual outwash of canals with the slopes of 0.002-0.005 happens intensive sealing by products of erosin of temporary ditches, irrigative fields, pollution of the ponds under technical wastes, the canals themselves are washed out and collapsed. The valleys are formed in the places of the temporary and constant irrigative net.

The analysis of the erosion situation in the arable soils of the lowland of Kur-Araz, grounded on contemporary erosion condition of the soil cover and quantitative value of intensity of soil out wash, we allow to prognosticate development of the erosion processes in dependence on prolongation of the land usage, character of agrotechnics and composition of agrocenoses.

One of the main measures of fight against irrigative erosion is a correct selection of technology of irrigation, an establishment of the optimal length of the irrigative

Table 3 Maintenance of humus and nutritious elements in hard flow.

The slope of the soil	Water expenditure, L/s	Percentage maintenance in hard flow			
		Humus	Nitrogen	Phosphorus	Potassium
0.006 Heavy loamy bright serozem	0.6	3.00	0.20	0.23	2.54
	0.8	2.92	0.19	0.23	2.50
	1.0	2.80	0.19	0.22	2.51
	1.2	2.80	0.19	0.22	2.50
0.018 Heavy loamy bright serozem	0.2	2.11	0.16	0.19	2.49
	0.4	2.05	0.16	0.18	2.52
	0.6	2.05	0.15	0.18	2.50
	0.8	1.98	0.15	0.17	2.51
0.012 Mid loamy typical-serozem	0.4	3.208	0.22	0.27	2.68
	0.6	3.00	0.21	0.25	2.64
	0.8	3.00	0.19	0.25	2.66
	1.0	2.90	0.19	0.25	2.64
0.020 Mid loamy bright grey-brown (chestnut)	0.2	2.84	0.16	0.18	2.67
	0.4	2.72	0.14	0.17	2.69
	0.6	2.53	0.13	0.17	2.65
	0.8	2.50	0.13	0.16	2.68

Table 4 Enleaching of humus and main nutritious matters under irrigative erosion.

The slope of the soil	Water expenditure (L/s)	Enleaching with the flow (s. 1h, in kvt)			
		Humus	Gross		Total analysis
			Nitrogen	Phosphorus	
0.006 Heavy loamy bright serozem	0.6	67.80	4.52	5.20	57.40
	0.8	133.66	8.89	10.76	117.00
	1.0	179.48	12.18	14.10	160.89
	1.2	239.56	15.91	18.56	221.00
0.018 Heavy loamy bright serozem	0.2	58.66	4.45	5.28	69.22
	0.4	131.61	10.27	11.56	161.78
	0.6	212.65	16.11	19.33	258.50
	0.8	304.59	20.80	26.55	372.99
0.012 Mid loamy typical-serozem	0.4	68.68	4.91	5.99	59.76
	0.6	104.40	7.31	8.70	91.87
	0.8	154.96	9.88	13.00	138.32
	1.0	206.48	14.24	17.08	187.97
0.020 Mid loamy bright grey-brown (chestnut)	0.2	55.88	4.06	4.57	67.82
	0.4	115.40	8.15	9.89	156.56
	0.6	170.04	11.91	15.57	242.74
	0.8	247.90	17.42	21.44	352.42

furrows and water expenditure in them in dependence on slope of the area and type of the soil.

Permissible quantity of water expenditure in the furrow depends on degree of water permeability and granulometric composition of the soil. The main information must be paid attention to warning of outwash by means of the establishment of the permissible expenditure of water (Table). The length of the furrow should be established in dependence on slope of locality, waterpermeability of soils, a quantity of water stream, depth of humid layer and eveness of the fields. The insignificant out wash of the soil under investigative lengths of the furrows and irrigative streams has been observed (2.0-2.5 t/h for a year) at the beginning of furrows and accumulation of outwash soil at the end of the furrow in our experiments.

The systemless pasture age changes structural condition of the soil, in dependence on humidity to formation of the large beo-formed crumblers and lumps, to soil dispertion under significant decrease of

wind durability of aggregates and increase defilation.

For a struggle of defilation the optimization of the pasture capacity is carried out by rationing of the quantity of the grazing small and big horned cattle on one of the area.

4. Consequences

It was determined during the carried out investigations that the main reasons of degradation in the soils of the lowland of Kur-Araz were rain erosion, irrigation erosion, deflation, irrigation and intensive use of the pastures.

The rain erosion happens on bare and inclined slopes and it is a cause for degradation of the pasture soils. At first complex measures must be fulfilled against it. A development of the irrigation erosion exposes the soils to the most intensive degradation in the soils where the irrigation inclination is 0.003-0.005 and higher than it.

Degradation happens by increase of the quantities of inclination and water expenditure, an expense of humus, nitrogen and other biogen elements which belong to washout soil and its structure. Running the danger of degradation of these areas was studied very exactly.

Graing turned into the main reason of degradation in the pastures. The intensive grazing intensifies erosion and deflation and degradation in the soils.

It was known that a level of the development of degradation in the lowland of Kur-Araz required fulfillment of the fight against it.

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Journal of Agricultural Science and Technology A 1 (2011) 255-261
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Assessment of Variabilities in Wheat Genotypes to Yellow and or Strip Rust, *Puccinia striiformis* in Glass House Conditions

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Received: February 22, 2010 / Published: June 20, 2011.

Abstract: Yellow/stripe rust caused by *Puccinia striiformis* is a major problem in the Middle East specially Iran. Virulence survey of cereal rust fungi have traditionally used differential host genotype and virulent physiologic race 134 E 134 AT was received to evaluate the segregating lines. A set of 35 segregating lines and three additional susceptible cultivars were added, making a total of 38 cultivars-lines. Spreader plants served as a source of inoculums. The plants were evaluated after two months based on infection percentage and the disease scoring scales. The results indicated that, there are significant changes in susceptibility assessments of the segregating lines in contrast to cultivated varieties to the yellow rust *P. striiformis* as far as the disease incidence and rust development is concerned in these experiments. The selected differential genotypes associated with various levels of resistance. The infection percentages were varied on the lines and the varieties, whereas the severity percentages were entirely different. The lowest severity is of line 38 with 34.70% and the highest in line 1 with 93.49% indicating 2.7 folds reduction in severity. For discrimination among the lines, the results on scoring scales gave distinctions of five classes of resistance, in which, the highest score is 29.93 and the lowest one 11.46. The cluster analysis showed almost similar results and confirmed, the analysis by Duncan's Multiple-lest rang as the above five distinctive groups.

Key words: Genotype, Isfahan, race, resistance, severity, varieties.

1. Introduction

Yellow/stripe rust caused by *Puccinia striiformis* West, is an important disease of wheat in countries, where wheat cultivars are grown in cool environment. The disease is a major problem in the Middle East specially Iran [1] and has been known as an endemic disease in Central and Western Asia. The apparent increasing frequency and severity of yellow rust epidemic in recent years, has resulted from the occurrence of new physiological races which were able to overcome widely utilized sources of resistance in wheat [2].

Screening for the resistant sources against yellow rust in advanced bread and durum wheat (*Triticum aestivum* and *T. turgidum* var. *durum*) in Iran, has indicated that the bread wheat is more susceptible than the durum wheat. The results showed that 88.45% of the advanced generations of the bread wheat were with high susceptibility whereas, 80% of the durum were highly resistant to the yellow rust disease. Also, in a similar studies [3], the field reactions of 19 bread wheat lines and three durum wheat lines to *Puccinia triticina* showed that the bread wheat lines were susceptible to moderately susceptible while that of three durum wheat lines were resistant, and moderately resistant. Breeding for resistance in two wheat cultivars, Azadi cross and Morocco caused the reduction of pustule size, infection percentage and increased the latent period of the disease incidence. Also, susceptibility assessments and

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grading of the yellow rust on wild gramine species in various areas in Iran showed four scoring scales, i.e. susceptible moderately susceptible, moderately resistance and resistance. Screening of 48 genotypes of wheat to yellow rust indicated the moderately resistant in two lines, i.e. M-73-4 and M-73-7. Out of a hundred advance wheat lines 62 percent were susceptible [4]. A total of 98 Chinese lines were inoculated with 26 pathotypes of *Puccinia striiformis* f. sp. *tritici* for postulation of stripe rust resistance genes effective at the seedling stage. A total of 135 wheat lines were planted at two locations to characterize their slow rusting responses to stripe. Thirty-three lines showed slow stripe rusting resistance at two locations in two seasons [5].

Pathogenic changes have been a significant factor in recurrent *P. striiforms* epidemic in the Middle East. In Syria, yellow rust has been observed annually since 1987, and the disease has spread to all wheat-growing areas [2]. The severity of infection was variable based on the different climatic condition in South and North of France, reflecting the genetically variability of physiological races of the pathogen [6]. Also, the physiological races are tremendously variable in Australia and New Zealand, where the mutations are taken places stepwise [7]. The serious outbreak of the yellow rust also happened in South Africa leading to screening for resistance and out of the Kriga cultivars [8]. In England, the two resistant cultivars Claire and Brigudier became susceptible [9, 10]. In Hungary, also, the few varieties were found to be resistant [11]. However, some resistance genes have been found against this disease. The *Lr34/Yr18* resistance gene provides durable, adult-plant, slow rusting resistance to leaf rust, yellow rust, and several other diseases of wheat. Expression was often higher in resistant plants, suggesting a possible role for *Lr34/Yr18* in priming of defense responses [12].

Hence, the objective of this study was to access the susceptibility of 35 segregating lines against 3 susceptible cultivars, i.e. Bollani, Ghods and Omid to yellow rust in glass-house conditions in Isfahan, Iran.

2. Material and Methods

Virulence survey of cereal rust fungi has traditionally used differential host genotype that express resistance in the primary leaves of seedlings plants which is assessed every year by pathologist in Cereal Research Institute, Karaj, Iran, where the virulent physiologic race 134 E 134 AT was received to evaluate the segregating lines [1].

In the study, a set of 35 segregating lines and three additional susceptible cultivars were added, making a total of 38 cultivars-lines (Table 1). The dry frozen inoculum was lyophilized in laboratory conditions and spread on the plants under glass-house at 10 °C and above 80 percent humidity. Spreader plants served as a source of inoculum. Primary infection by spreading the rust spores developed rapidly, then the subsequent spread of urediospores occurred on the surrounding pots of the different lines and cultivars, which was repeated four times for the insurance of the infectors on the leaves. The plants were evaluated after two months based on infection percentage and the disease scoring scales [1, 2].

Infections were assessed based on infection percentage (0-100) and the severity on the seven scoring scales, i.e. 0, 1, 2, 5, 10, 25 and 50 percent (Fig. 1). For the quantitative assessment of the disease severity, the detentions of rust development and/or scoring scales were determined with the disease severity index scale. For each line the number of plant of the severity classes was multiplied by 0, 1, 2, 4, 8, 16 and 32 respectively. The product added, and the sum divided by the total number of plants. A mean score 0-32 scale was calculated for each line [7, 13, 14].

When the rust severity was rated on a scale of 0-32, scores were ARC/Sine transformed prior to statistical analysis of variance with PROC ANOVA (complete block design) (DMRT). The Pearson's correlation coefficient was calculated. The data were also subjected to cluster analysis according to Ward’s

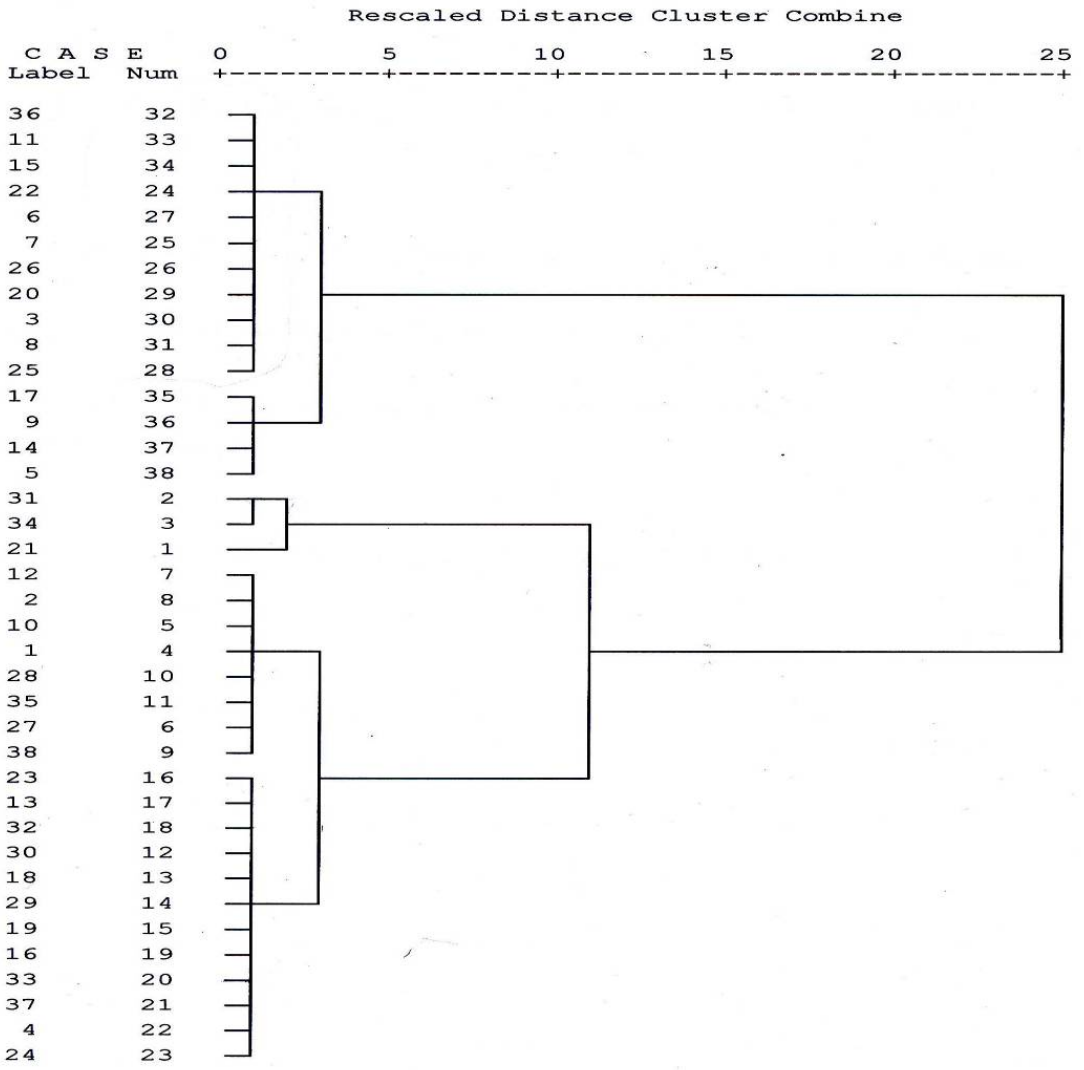

Fig. 1 Dendrogram of susceptibility assessment of bread wheat lines to yellow and/or stripe rust.

minimum variance method, using the cluster procedure of SAS computer software [15].

3. Results and Discussions

The results indicated that, there are significant changes in susceptibility assessments of the segregating lines in contrast to cultivated varieties to the yellow rust *P. striiformis* as far as the disease incidence and rust development is concerned in these experiments. The parents of segregating lines were shown in Table 2. The consequence of the crossing of these related parents is the generation of segregating populations which are used in this study.

This shows that, the selected differential genotypes associated with various levels of resistance, which agrees with other statement [7, 13]. The wheat-rye lines Lankao 1, 3, 4, and 5 were reported as resistant lines to a wide spectrum of wheat powdery mildew and also were resistant to a mixture of wheat stem rust (*Puccinia graminis* f. sp. *tritici* and wheat stripe rust (*P. striiformis* f. sp. *tritici*) [16]. The wheat cultivars Luke and Aquileja have been reported to possess resistance to stripe rust. Aquileja displayed less number of stripes per unit leaf area than Luke, while Luke showed lower infection type than Aquileja [17].

The infection percentages varied on the lines and the varieties, 76.33-100, whereas the severity percentages were entirely different, 34.70-93.43 indicating that

there is a reserve condition with a negative direction (r = -0.45). The lowest severity is of line 38 with 34.70% and the highest in line 1 with 93.49% indicating 2.7 folds reduction in severity (Table 1). These variations in severity of the rust the plant leaves of screened lines have been observed by others on various bread/durum wheat in other countries [4, 9, 18, 19].

For discrimination among the lines, the results on scoring scales gave distinctions of five classes, i.e. resistant (36-38), moderately resistant (32-35), a clear tolerance, moderately susceptible (2-3) and susceptible (1) in which, the highest score is 29.93 and the lowest one 11.46, indicating a various ranges of resistance with a significant effect (P = 0.01). And, also there is direct and high correlation between the severity and scoring scale (0.99) (Table 1).

The cluster analysis showed almost a similar results and confirmed, the analysis by Duncan's Multiple-lest rang as the above five distinctive groups (Fig. 2). Singh et al. [20] investigated the genetic basis of resistance in seedlings and adult plants of spring wheat 'Brambling', which has a high level of adult-plant resistance (APR) to leaf rust caused by *Puccinia triticina*, and the magnitude of genotype × environment effects on the expression of APR. Brambling was crossed with spring wheat 'Jupateco 73S' that is highly susceptible to current predominant *P. triticina* races in Mexico and the United States. Expression of APR was influenced by the environment in the recombinant inbred lines (RILs), even though Brambling displayed a consistent response, indicating that stability of APR can be achieved by combinations of slow-rusting resistance genes.

4. Conclusions

In this study, we can conclude that, the diverse virulence phenotypes of *P. storiiformis* can exist and eventually develop and spread over larger areas in the region. So, the wheat crop can be protected from rust and/or occurrence of epidemics could be reduced by emphasizing the regional cooperation in monitoring the

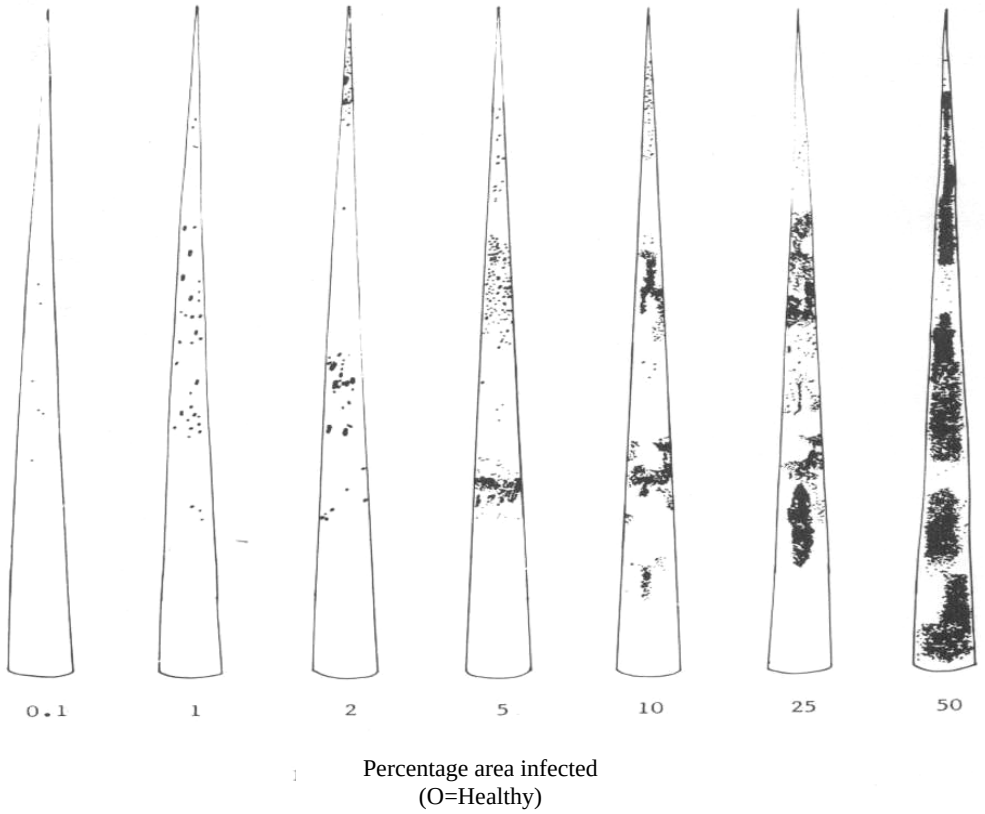

Fig. 2 Scoring scale of rust infections on various ranges.

Table 1 The mean of infection, severity and scoring scale of yellow rust on segregating lines in glass-house condition.

Serial number	Lines	Infection	Severity	Scoring scale	Standard deviation[1]	Duncan's group[2]	CV
1	21	100	93.46	29.93	± 2.06	A	11.95
2	31	100	74.33	26.4	± 3.62	A-C	23.81
3	34	76.33	74.00	23.73	± 5.39	A-C	39.35
4	1	100	68.00	22	± 2.03	A-D	20.82
5	10	100	66.78	21.66	± 4.14	A-D	33.15
6	27	100	65.26	21.20	± 3.93	A-D	32.18
7	12	100	65.26	21.06	± 2.53	A-D	20.82
8	2	100	64.94	21.06	± 3.34	A-D	27.47
9	38	100	60.76	20.86	± 1.24	A-D	11.04
10	18	100	60.93	20.40	± 3.20	A-D	27.24
11	35	100	61.85	20.33	± 5.02	A-D	42.83
12	20	100	60.73	19.66	± 3.94	A-D	32.40
13	18	100	60.70	19.60	± 1.84	A-D	16.32
14	29	100	59.61	19.53	± 3.94	A-D	34.70
15	19	90	57.92	19.30	± 2.35	A-D	21.10
16	23	100	58.72	19.13	± 2.99	A-D	27.14
17	13	100	58.38	19	± 0.57	A-D	5.26
18	32	100	58.06	18.86	± 3.92	A-D	36.01
19	16	100	56.60	18.64	± 2.80	A-D	26.29
20	33	100	55.54	18.16	± 4.03	A-D	28.43
21	37	96.33	54.4	17.8	± 4.11	A-D	40.08
22	24	93.33	53.88	17.6	± 2.02	A-D	19.91
23	24	100	52.53	17.2	± 1.79	B-D	18.05
24	22	100	50.40	16.66	± 1.48	B-D	15.62
25	7	100	50.30	16.50	± 3.17	B-D	33.36
26	26	100	50.26	16.40	± 2.07	B-D	21.95
27	6	100	49.00	16.06	± 0.92	B-D	9.92
28	25	100	48.87	16.00	± 4.04	B-D	43.80
29	30	93.33	47.64	15.66	± 0.67	B-D	7.48
30	3	100	47.69	15.53	± 1.86	B-D	20.81
31	8	100	45.92	15.36	± 3.39	B-D	38.28
32	36	100	44.60	14.80	± 0.52	B-D	6.19
33	11	100	44.73	14.80	± 1.66	B-D	19.48
34	15	100	44.26	14.30	± 1.32	D-C	15.99
35	17	93.33	41.20	13.53	± 1.36	D-C	17.50
36	9	100	36.86	12.90	± 1.56	D	21.70
37	14	93.33	36.72	12.23	± 4.35	D	61.64
38	5	93.33	34.70	11.46	± 0.86	D	13.12

[1] It is defined as the root-mean-square (RMS) deviation of the values from their mean, or as the square root of the variance.

[2] Means followed by the same letter are not significantly different at $P = 0.05$ level in Duncan's multiple range test.

evolution and migration of new races of rust fungi, enhanced information on the genetics; basic of resistance in important wheat cultivars and shift towards breeding and deploying wheat cultivars with durable resistance. Therefore, this is suggested that by referring to the Tables 1 and 2 segregating line can be

Table 2 The parents of segregating lines in these studies.

Plot. No.		Cross. No.	Parentage
2004	2003		
01	601	1-16940	Rsh/5/kvz/cgn/4/Hys//Drc*2/7/3/2*Rsh
02	602	1-16941	Rash/3/vee"s"/Nac//1-66-22
03	650	1-16989	Bloudna/3/Bb/7c*2//Y50E/Kal*3/4/kavir
04	651	1-16990	Bloudan/3/Bb/7c*2//Y50E/Kal*3/4/Arvand
05	652	1-16991	Alvand//Aldan"s"/lAS 58/3/Hmd
06	662	1-17001	Vee"s"/Nac//1-66-22/5/Kvz/Cgn/4/Hys//Drc*2/7C/3/2*Rsh
07	663	1-17002	Vee"s"/Nac//1-66-22/4/1-63-31/3/12300/Tob//Cno/Sx
08	664	1-17003	Vee"s"/Nac//1-66-22/3/Shi#4414/Crow"s"//1-66-22
09	665	1-17004	Vee"s"/Nac//1-66-22/3/Bank"s"/Vee"s"
10	666	1-17005	Vee"s"/Nac//2*1-66-22
11	667	1-17006	V82 187/1-66-22/5/Kvz/Cgn/4/Hys//Drc*2/7c/3/2*Rsh
12	668	1-17007	Shi#4414/Crow"s"//1-66-22/3/Rsh
13	669	1-17008	V82 187/1-66-22//Rsh
14	670	1-17009	Anza/3/Pi/Nar//Hys/4/Alborz/5/1-66-75/6/Hmd
15	671	1-17010	Anza/3/Pi/Nar//Hys/4/Alborz/5/1-66-75/6/Mat/2*Skauz
16	672	1-17011	Anza/3/Pi/Nar//Hys/4/Alborz/5/1-66-75/6/Alvand//Aldan"s"/las 58
17	673	1-17012	Anza/3/Pi/Nar//Hys/4/Alborz/5/1-66-75/6/Gv/D630//Ald"s"/Azd
18	677	1-17016	477 1//Fkn/Gb/3/Vee "s"/ Vee "s"/4/Buc "s"/5/1-66-44/6/Alvand //Alvand "s"/las 58
19	698	1-17037	Mrn/Catbird/4/Alvand//Aldan "s"/las 58
20	705	1-17044	Catbird/Rsh/3/Alvand//Aldan "s"/las 58
21	706	1-17045	Thb/Kea//Skauz/3/Rsh/4/Alvand//Aldan"s"/las 58
22	707	1-17046	Thb/Kea//Skauz/3/Rsh/4/Bloudan/3/Bb/7c*2//Y50E/Kal*3
23	708	1-17047	Mat/2*Skauz//Rsh/4/Bloudan/3/Bb/7C*2//….Y50E/Kal*3
24	713	1-17052	Kal/Bb//Cj"s"/3/Hork"s"/4/2*Alvd//Aldan/las 58
25	718	1-17057	Alvd//Aldan/las58/3/Rsh/4/Kauz/Stm/3/Alvand//Aldan/las 58
26	719	1-17058	Alvd//Aldan/las 58/3/2*Rsh
27	720	1-17059	Alvd//Aldan/las 58/3/2*Warbler"s"
28	721	1-17060	Alvd//Aldan/las 58/3/2*Moncho"s"
29	722	1-17061	Alvd//Aldan/las 58/4/2*Ndd/WW//lee/Fn/3/N/4/Ti71/Resel
30	723	1-17062	Alvd//Aldan/las 58/3/Sakha 8/4/Alvand//Aldan"s"/las 58
31	724	1-17063	Alvd//Aldan/las 58/3/1-60-3/5/Kal/Bb//Cj"s"/3/Hork"s"/4/Alvd//Aldan/las58
32	725	1-17064	Kayson/Glenson//Attila/3/Kvr/4/Alvand//Aldan"s"/las 58
33	726	1-17063	Flt/Rsh/3/Alvd/Aldan/IAS 58/4/Chamran
34	727	1-17066	Flt/Rsh/3/Alvd/Aldan/IAS 58/4/Flt/Rsh/3/Kauz*2/Opata//Kauz
35	728	1-17067	Flt/Rsh/3/2*Alvd//Aldan/las 58

selected accordingly and get in used by any breeder and or kept as resistant genotypes for further programs.

Acknowledgments

Thanks go to Agricultural and Natural Resources Research Center, Isfahan, for financial supports and also to Dr. Zeinali for critical statistical analysis and Mr. H. Almassi for technical assistance.

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Journal of Agricultural Science and Technology A 1 (2011) 262-269
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

The Roles of Plant Secondary Metabolites from Cowpea Floral Structures in Resistance to the Flower Bud Thrips

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Received: September 10, 2010 / Published: June 20, 2011.

Abstract: Floral structures of six cowpea (*Vigna unguiculata*) cultivars were analysed for secondary plant metabolites (polyphenols, terpenoids and flavonoids) to study their relationships with resistance to *Megalurothrips sjostedti* (Trybom). Polyphenols varied significantly ($P < 0.001$) among the floral structures of the cultivars at the same growth stage. Significant negative correlations were obtained between polyphenols and damage indices (r = -0.57), mean adult counts (r = -0.56) and mean larval counts (r = -0.64) of resistant cowpea cultivars especially in the late season, indicating that polyphenols play a significant role in cowpea resistance to *M. sjostedti*. High levels of polyphenols obtained from Sanzibanili and Sewe cultivars, coupled with highly significant correlations between the polyphenols and thrips population on resistant cultivars, and their damage indices, suggests that these polyphenols could be inhibitors or deterrents in this case. Terpenoid extracts (10 mg/mL) of IT90K-277-2, Sewe, Sanzibanili, TVu 1509 and KV × 404-8-1 racemes; KV × 404-8-1 and TVu 1509 floral buds; IT90K-277-2, Sewe and Sanzibanili flowers caused significant ($P < 0.001$) larval mortalities, since mortality ranged between 56.7%-96.7%. Hence terpenoid extracts from floral structures of the cultivars are biologically active and confers antibiotic resistance to *M. sjostedti* larvae; this compound could be promising candidates for genetic transformation of cowpea cultivars.

Key words: *Vigna unguiculata*, terpenoids, flavonoids, polyphenols, *Megalurothrips sjostedti*.

1. Introduction

The flower bud thrips *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae) is one of the major pests of cowpea, *Vigna unguiculata* L. Walp. in tropical Africa [1] that attacks the reproductive structures of cowpea during plant development [2, 3]; which cause yield losses of up to 100% in Tanzania, Ghana, Cameroon and Nigeria [4]. Consequently there is the need to protect cowpea from such insect pests as *M. sjostedti* to obtain high pod production which will result into high grain yield. Recently the production of cowpea with natural pest resistance has assumed a major importance in recent years [3, 5-7]. Identification of compounds responsible for resistance can be of great value in developing resistant varieties [8]. Plant primary and secondary metabolites play a decisive role in determining host plant favourite by insects [7, 9] and they greatly influence the behaviour and physiology of insects thereby imparting resistance or susceptibility of plants. Although work has been done on the total phenolic content of cowpea floral buds in relation to resistance to *M. sjostedti* [10], little has been done on all the cowpea reproductive structures (racemes, floral buds and flowers) chemical composition in relation to thrips resistance since thrips damage the entire reproductive structures. The objective of this paper is to investigate the role(s) of polyphenols, terpenoids compounds, aglycones of flavones and flavonols of cowpea reproductive structures on resistance to *Megalurothrips sjostedti*.

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2. Materials and Methods

2.1 Field Screening of Cultivars

Six cowpea cultivars were screened for resistance to *Megalurothrips sjostedti* during the early and late seasons of 1998 at the International Institute of Tropical Agriculture (IITA), Ibadan. TVu1509 has been reported as resistant to thrips and Vita7 as susceptible [11, 12]. These two cultivars were used as control standards throughout the experiment. Each cultivar was planted in five row plots of 5m long with an inter-row spacing of 1m. Distance between plants was 0.2 m and a distance of 1.5 m was left between adjacent plots.

The test cultivars were planted to fit a randomised complete block design with four replicates. An increase in thrips population was achieved by planting a susceptible cultivar (Vita 7) as spreader rows in a checkerboard design two weeks prior to planting the experimental materials. At the raceme stage of the test plants, the spreader rows were uprooted and plants laid between rows of the test plants. This caused the thrips to move away from the drying plants to those of the test green rows.

2.2 Field Data Collection

Visual rating of thrips damage on cowpea plants from raceme stage to mid-poding stage within a 2m row staked area per plot was performed using a modification of the visual rating scale developed by Jackai and Singh [11]. Population densities of *M. sjostedti* were estimated by randomly picking 20 racemes and 20 flowers of cowpea per plot. The racemes or flowers were placed separately in labelled glass vials containing 30% ethanol solution. Later, larvae and adults of *M. sjostedti* were separated from the plant parts and counted under a macroprojector. The level of infestation was assessed for 2 weeks each time the visual rating was performed. Pod counts were collected within the 2 m row staked area and recorded. Treatments were analysed by using the SAS system and means were compared using the Student Newman-Keuls Test (SNK).

2.3 Plant Secondary Metabolites Determination

All solvents were analysed reagent grade. All analysis done to investigate the role of secondary plant metabolites in some cowpea cultivars were conducted at the IITA Ibadan Nigeria. Seeds of four cowpea cultivars IT90K-277-2, KV × 404-8-1, Sanzibanili and Sewe and the resistant, TVu 1509 and susceptible, Vita 7 controls were planted in 18 cm diameter pots in the screen house. No insecticide applications were made prior, during or after reproductive structures of culture were collected for polyphenol, terpenoids and aglycones of flavones and flavonols analyses and laboratory bioassays.

2.3.1 Polyphenols

Concentrations of polyphenols in reproductive structures of cowpea cultivars were obtained following the method described by Anderson and Ingram [13]. Samples of racemes, floral buds and flowers of the six cowpea cultivars were collected in labelled paper bags from growing plants in the screen house. The samples were dried in an oven at 65 °C for 3 days and later ground with an electric grinder (Janke and Kunkel, D-6075 Dreieich, West Germany). Ground samples (0.75 ± 0.001 g) were weighed into a 50 mL beaker. 20 mL of 50% methanol was added to the ground sample, the beaker was covered with Parafilm M® and afterwards placed in a water bath at 77 °C-80 °C for 1 hr. Whatman No 1 filter paper was used to quantitatively filter the extract into a 50 mL volumetric flask and made up to the mark with 50% aqueous methanol. 1 mL of the extract or standard, 0.1 mg/mL tannic acid (0, 1, 2 and 4 mL) was pipetted into a 50 mL volumetric flask. 20 mL distilled water, 2.5 mL Folin-Denis reagent and 10 ml sodium 17% carbonate was added in sequence to the extract. The mixture was made up to 50 mL mark and mixed together. After 20 min Spectrophotometric estimation was read at 760 nm VIS. The method detected polyphenols by the

formation of the bluish colour. Extraction and determination was conducted in triplicate.

2.3.2 Concentrations of Flavonoids

Racemes, floral buds and flowers of the cowpea cultivars were collected from potted plants in the screen house and freeze dried within 10 min of cutting for 3 days. The samples were later ground with an electric grinder. 100 mg of freeze-dried tissues of each cultivar was hydrolysed with 2N HCl in a water bath at 100 °C for 45 min and then filtered. After filtering the solution was extracted twice with ethyl acetate using a separatory funnel and the combined extract taken to dryness under the fume cupboard.

2.3.3 Concentrations of Terpenoids

Racemes, floral buds and flowers of the cowpea cultivars were collected from potted plants in the screen house. The reproductive structures were dried at 40 °C for 3 days, and ground with an electric grinder. Samples (3 g) were individually extracted three times with 30 mL ethyl acetate for 15 min at 40 °C. After filtration the acidic compounds were extracted three times with 10 mL aqueous 5% Potassium hydroxide this was followed by the extraction of basic compounds with 10 mL aqueous 5% HCl. The organic fraction which contained the neutral compounds, was washed with distilled water (10 mL, three times) and concentrated in a rotary evaporator to a volume of approx. 20 mL, after which the extracts were centrifuged for 10 min at 6,000 rpm to remove suspended particles. The solvent was evaporated to dryness under the fume cupboard in each of the extracts to give terpenoid compounds.

2.3.4 Megalurothrips Sjostedti Bioassay

Second larval stages of *Megalurothrips sjostedti* were obtained from a laboratory culture maintained on Ife-Brown cowpea pods at the Insect rearing unit of the IITA. Bioassays were initiated by placing 10 larvae in a 4 cm diameter polyvinyl chloride (PVC) tubes lined with folded paper towels and Vita 7 floral buds coated with 10 mg/mL terpenoid extracts from the reproductive structures of the six tested cultivars. The PVC tubes were placed in the laboratory at 25 ± 2 °C and 60%-70% relative humidity with a 12:12 (L/D) photoperiod for 48 hr in four replications per treatment. Percent larval mortalities after 48 hr were recorded. Vita 7 floral buds coated with terpenoid extracts from reproductive structures of TVu 1509 served as the resistant controls while uncoated Vita 7 floral buds, floral buds coated with acetone and floral buds coated with terpenoid extracts from reproductive structures of Vita 7 served as susceptible controls. Bioassay data were subjected to an analysis of variance and means were separated using Student Newman Keuls Test.

3. Results and Discussion

3.1 Thrips Populations and Damage

In the early season IT90K-277-2 cultivar had the highest mean larval counts (54.2) but it was not different from mean larval counts on Vita7 and KV × 404-8-1. Sewe recorded the least mean larval counts of 7.2. However in the late season, there were no significant differences between cultivars in the number of larvae, although Vita 7 had the highest while IT90K-277-2 had the least (Table 1). IT90K-277-2 had the highest mean adult counts in the early season (15.5) but it was not significantly different from mean adult counts on TVu1509 and Vita 7 while Sewe had the least mean adult counts (1.5) which was not significantly ($P < 0.01$) different from Sanzibanili (2.7). In the late season when thrips infestation was high on the field Vita 7 had the highest mean adult counts (185.3) and it was not different from IT90K-277-2, KV × 404-8-1 and TVu1509. The lowest mean adult count was recorded on Sewe.

In the two seasons, Vita 7 was badly damaged by thrips compared with all the other cultivars. Sewe suffered the least damage in the early season but it was not different from damage observed on Sanzibanili (2.3) and the resistant cultivar TVu 1509 (2.3). Damage observed on IT90K-277-2 and KV × 404-8-1 was significantly higher ($P < 0.01$) than those observed on Sanzibanili and Sewe in the early season. However

Table 1 Damage indices and thrips population on six cultivars grown under field conditions.

Cultivar	Number of larvae		Number of adults		Damage indices	
	ES	LS	ES	LS	ES	LS
IT90K-277-2	54.2 ± 5.9 a	111.3 ± 39.0 a	15.5 ± 3.8 a	80.3 ± 41.7 ab	4.9 ± 0.4 b	2.3 ± 0.3 bc
KV × 404-8-1	46.3 ± 6.5 a	114.0 ± 37.4 a	8.5 ± 2.6 b	160.0 ± 77.7 a	5.0 ± 0.3 b	3.3 ± 0.3 b
Sanzibanili	16.0 ± 3.2 bc	123.2 ± 52.2 a	2.7 ± 0.5 c	67.8 ± 30.4 c	2.3 ± 0.2 c	1.3 ± 0.2 c
Sewe	7.2 ± 1.6 c	139.7 ± 58.7 a	1.5 ± 0.6 c	57.2 ± 28.4 c	2.0 ± 0.2 c	2.5 ± 0.3 b
TVu1509 (RC)	28.2 ± 3.8 b	119.2 ± 47.4 a	10.0 ± 1.2 ab	113.0 ± 51.2 ab	2.3 ± 0.2 c	2.3 ± 0.2 bc
Vita 7 (SC)	53.7 ± 8.4 a	191.3 ± 51.1 a	10.8 ± 2.2 ab	185.3 ± 76.1 a	7.8 ± 0.2 a	7.6 ± 0.7 a

Means in the same column followed by the same letter(s) are not significantly different using Student-Newman-Keuls at $P < 0.01$. Values are means of 4 replicates.

RC = Resistant control; SC = Susceptible control; ES = Early season; LS = Late season.

Vita 7 was significantly ($P < 0.01$) damaged compared with all the other cultivars that harboured similar number of thrips.

Variations in the levels of damage among resistant cultivars harboring similar number of thrips as vividly exemplified by Sanzibanili and Sewe in the late season, suggests that there are inherent underlying plant factors that are responsible for such differences. Plant defensive secondary compounds such as phenolics, tannin, alkaloids, terpenoids and flavonoids have been shown to increase resistance to thrips [14, 15]. Ananthakrishnan [16] reported that plant phenolics reduce feeding; alter fecundity and the duration of post-embryonic development of thrips.

3.2 Secondary Metabolites Composition in Relation to Thrips Populations and Damage

Percentage polyphenols extracted from racemes, floral buds and flowers ($P < 0.001$) varied considerably among all the cultivars (Table 2). Vita 7 floral structures consistently had significantly ($P < 0.001$) higher polyphenol content compared with TVu1509 (Table 2). Floral structures of Sanzibanili consistently had significantly higher polyphenol level compared with Sewe except in the racemes where there was no significant difference between the two cultivars. Polyphenol content in floral structures of KV × 404-8-1 and IT90K-277-2 were not significantly different from each other. Across all the cultivars tested, polyphenols were lowest in racemes (9.76%), highest in floral buds (17.0%) and declined in the flowers (14.4%) (Table 2) Oghiakhe et al. [17] and Woodhead [18] reported a similar trend in variation of phenolic acid concentration in *Vigna unguiculata* and *Sorghum bicolor* respectively.

Table 2 Polyphenol content (percentage) in cowpea racemes, floral buds and flowers.

Cultivar	Racemes	Floral buds	Flowers
IT90K-277-2	9.29 ± 0.15 b	15.55 ± 0.16 d	11.29 ± 0.05 d
KV × 404-8-1	9.36 ± 0.10 b	15.21 ± 0.02 d	10.00 ± 0.11 d
Sanzibanili	11.22 ± 0.11a	21.08 ± 0.11 a	20.87 ± 0.36 a
Sewe	11.34 ± 0.12 a	18.88 ± 0.16 b	12.85 ± 0.13 c
TVu 1509 (RC)	7.95 ± 0.06 c	15.42 ± 0.07 d	10.73 ± 0.17 d
Vita 7 (SC)	11.53 ± 0.04 a	16.75 ± 0.13 c	14.29 ± 0.18 b
Means ± S.E.	9.76 ± 0.34	17.0 ± 0.46	14.4 ± 0.97

Means in the same column followed by the same letter(s) are not significantly different using Student-Newman-Keuls at $P < 0.001$. Values are means of 3 replicates.

RC=Resistant control; SC=Susceptible control.

Correlations between percentage polyphenol content extracted from reproductive structures of resistant cowpea cultivars excluding Vita 7 (susceptible cultivar) and damage indices, number of larvae and number of adults across the two seasons are presented in Table 3. Negative and highly significant correlations were obtained between all the parameters and polyphenols of resistant cultivars in the two seasons. The inverse and highly significant ($P < 0.001$) relationships between polyphenol content and damage indices; number of larvae, number of adults, of resistant cultivars especially in the late season suggests that polyphenols in resistant cowpea cultivars play some

role in reducing population of thrips and consequently damage to floral structures of cowpea. Salifu [10] also obtained a highly significant ($P < 0.01$) negative correlation between thrips populations and phenolic content of cowpea floral buds. He concluded that perhaps phenolics in cowpea serve as repellents or phagodeterrents to thrips. Also condensed tannins have been reported in the resistance of cowpea to the cowpea curculio, *Chalcodermus aenueus* [19]. Although it has been established from literature that high polyphenols in plant tissues confer resistance to plant from thrips attack [16, 20], however significantly ($P < 0.001$) higher levels of polyphenols were extracted from floral structures of Vita 7, (susceptible check), compared with TVu 1509, the (resistant check). Nevertheless this result should not be discarded because Bi, et al. [21] had earlier stated that phenolics are a structurally diverse class of phytochemicals and one should not draw inference that all phenolics act similarly on these insects. Also, Leszczynski et al. [22] reported that the phenolic compounds naturally occurring in wheat cultivars had an effect on the preference of cereal aphids on the wheat. They further concluded from their studies that the quality of the phenols is as important as their quantity in host-plant selection by the aphids. Therefore there is the need to further investigate to determine the compositions (qualities) of these polyphenols in the reproductive structures of the various cowpea cultivars by gas chromatography-mass spectrometry to unravel these findings. Furthermore this study provides a precautionary note that reliance on presence of high polyphenols in plants as indicators of plant resistance to insect attack probably requires re-evaluation. Also resistance of the cowpea cultivar, TVu 1509 to *M. sjostedti* may not be attributed solely to the low level of polyphenols (quantity) but perhaps to some other factors such as other biochemical and structural defences of cowpea.

Table 3 Correlation between percentage polyphenol content extracted from reproductive structures of resistant cowpea cultivars and resistance parameters during the early and late planting seasons.

Resistance parameters	Percentage polyphenol content	
	Early season	Late season
Damage indices	-0.47**	-0.57***
Number of larvae	-0.41*	-0.64***
Number of adults	-0.38*	-0.56***

$P < 0.05$ = *; $P < 0.01$ = **; $P < 0.001$ = ***.

In an attempt to identify specific biochemical compounds that could be responsible for cowpea resistance to *Megalurothrips* sjostedti; terpenoid compounds and aglycones of flavones and flavonols (flavonoids) were extracted from reproductive structures of the tested cowpea cultivars (Table 4). Flavonoid content in floral buds and flowers of the cowpea cultivars were not significant. They were only significant ($P < 0.01$) for the racemes of the different cowpea cultivars. The flavonoid content of IT90K-277-2 racemes, only was the highest, differed significantly ($P < 0.01$) from those of other cultivars.

Table 4 Flavonoid and terpenoid compositions of cowpea floral structures.

Floral structures	Cultivar	Flavonoids (µg/100mg)	Terpenoid composition (%)
Racemes	IT90K-277-2	4.00 ± 0.20 a	2.40
	KV × 404-8-1	2.00 ± 0.35 b	2.40
	Sanzibanili	2.90 ± 0.21 b	2.32
	Sewe	2.97 ± 0.07 b	2.59
	TVu 1509 (RC)	2.40 ± 0.46 b	2.02
	Vita 7 (SC)	1.80 ± 0.12 b	2.59
	Mean ± S.E.	2.678 ± 0.235	2.39 ± 0.09
Floral buds	IT90K-277-2	3.03 ± 0.59a	1.75
	KV × 404-8-1	2.63 ± 0.96a	1.98
	Sanzibanili	2.87 ± 0.18a	2.11
	Sewe	3.07 ± 0.38a	2.29
	TVu 1509 (RC)	1.76 ± 0.15a	1.60
	Vita 7 (SC)	2.57 ± 0.52a	2.57
	Mean ± S.E.	2.655 ± 0.463	2.05 ± 0.14
Flowers	IT90K-277-2	2.10 ± 0.23a	1.91
	KV × 404-8-1	3.50 ± 1.10a	1.35
	Sanzibanili	3.27 ±0.24a	2.02
	Sewe	3.07 ± 0.09a	1.33
	TVu 1509 (RC)	4.63 ± 1.71a	4.00
	Vita 7 (SC)	2.00 ± 0.58a	1.18
	Mean ± S.E.	3.095 ± 0.658	1.97 ± 0.43

Means in a column within each floral structure followed by the same letter are not significantly different at $P < 0.01$ using Student-Newman-Keuls. Values are means of 3 replicates.
RC = Resistant control; SC = Susceptible control.

Highest percentage terpenoids was found in racemes of all the cowpea cultivars (2.39%). It is expected that the resistant cultivar (RC), should contain higher levels of terpenoids while the susceptible cultivar (SC) should contain lower concentration. However, surprisingly Sewe (2.59%), a resistant cultivar and Vita 7 (SC) (2.59%) racemes had the highest percentage terpenoids while TVu 1509 (RC) had the lowest (2.02%). Among the floral buds of the cowpea cultivars, Vita 7 (SC) (2.57%) had the highest percentage terpenoids while TVu 1509 (RC) had the lowest (1.60%). However, TVu 1509 (RC) flowers had the highest percentage terpenoids (4.00%) and Vita 7 (SC) had the lowest 1.18%. There was a decrease in the percentage terpenoids from racemes to floral buds to flowers in all the cultivars except in IT90K-277-2 flowers where a slight increase occurred while in TVu 1509 (RC) flowers a markedly high increase (4%) was observed (Table 4).

3.3 Thrips Response to Terpenoid Extracts

The effects of terpenoid extracts from racemes, floral buds and flowers of the different cowpea cultivars on mortality of *M. sjostedti* larvae are shown in (Table 5). Highest larval mortality was recorded on larvae fed on terpenoid extracts from racemes of IT90K-277-2 (93.33%) and Sewe (93.33%). However the larval mortalities were not significantly different ($P < 0.001$) from mortalities recorded on racemes extracts of all the other cultivars except acetone (20.00%), control (13.33%) and extracts from Vita 7 racemes (16.67%). Although the highest mortality was recorded on larvae fed with extracts from TVu 1509 floral bud (90.00%), this was not significantly different from the mortalities recorded with extracts of KV × 404-8-1 (86.67%). Low mortalities were observed in larvae fed with Sewe (20.00%) and Sanzibanili (23.33%) floral bud extracts. Terpenoid extracts from flowers of the different cowpea cultivars bioassayed against larvae of thrips shows that Sanzibanili (96.67%), IT90K-277-2 (93.33%), and Sewe (83.33%) extracts caused significant ($P < 0.001$) mortalities on the larvae that fed on them. TVu 1509 flower extracts caused a significant low mortality (30.00 ± 15.28%) on larvae fed with the extract. Low mortalities were recorded for larvae fed on extracts of Vita 7 racemes (16.67%), floral buds (26.67%) and flowers (26.67%) (Table 5). Flavonoids and carotenoids impart particular floral hue to plants and these may also affect the attractiveness as well as the susceptibility of the host to thrips [15]. In this study, high larval mortality was recorded from larvae fed with terpenoid extracts from TVu 1509 racemes and floral buds compared with low larval mortality observed when larvae fed on TVu 1509 flower extracts. This suggests that each reproductive structure has a different mechanism by which they resist insect attack. Oghiakhe and Odulaja [23] reported that there were differences in the mechanisms and levels of resistance in floral buds, flowers and sliced pods of cowpea cultivars to *Maruca testulalis* (Geyer), which was also evident in varying mortality rates obtained from *Megalurothrips sjostedti* larvae fed with terpenoid extracts of the cowpea cultivars. Although a high percentage terpenoid compositions was obtained from TVu 1509 flowers, when this terpenoid extracts was bioassayed on larvae for 48 hr, mortality was low (30.00%). This proves that the quantity of an

Table 5 Percentage mortality (Means ± S. E.) of *M. sjostedti* larvae fed on floral buds of Vita 7 coated with 10 mg/mL terpenoids extracted from racemes, floral buds and flowers of cowpea cultivars.

Treatments	Racemes (%)	Floral buds (%)	Flowers (%)
IT90K-277-2	93.33 ± 6.67 a	30.00 ± 15.28 b	93.33 ± 3.33 a
KV × 404-8-1	56.67 ± 17.64 a	86.67 ± 8.82 a	46.67 ± 17.64 b
Sanzibanili	66.67 ± 13.33 a	23.33 ± 6.67 b	96.67 ± 3.33 a
Sewe	93.33 ± 3.33 a	20.00 ± 15.28 b	83.33 ± 8.82 a
TVu 1509 (RC)	90.00 ± 10.00 a	90.00 ± 10.00 a	30.00 ± 15.28 b
Vita 7 (SC)	16.67 ± 8.82 b	26.67 ± 6.67 b	26.67 ± 8.82 b
No treatment (Vita 7 buds)	13.33 ± 3.33 b	13.33 ± 3.33 b	13.33 ± 3.33 b
Acetone	20.00 11.55 b	20.00 11.55 b	20.00 11.55 b

Means in the same column followed by the same letter are not significantly different using Student-Newman-Keuls at $P = 0.001$. Values are means ± SE of 3 replicates. RC = Resistant control; SC = Susceptible control.

antinutritional factor/allelochemical alone is not responsible for resistance or susceptibility of cowpea cultivars to thrips but the concentrations of the other various compounds that may make up the antinutritional factor. Also, Khan and Saxena [24] have demonstrated that resistance or susceptibility to feeding of rice leafhopper, *Nephotettix virescens* was not necessarily due to the presence or absence of feeding deterrents, but was governed by the sum total of allomones or kairomones present in rice plant volatiles. The same result was obtained for rice planthopper, *Nilaparvata lugens* feeding on rice cultivars [25].

Furthermore, terpenoid extracts from TVu 1509 flowers probably have other functions other than feeding deterrence or toxicity. However if larval populations on TVu 1509 had fed on the terpenoid extracts from racemes and floral buds, a significant reduction in their population would have occurred before the flowers emerged. Nevertheless it can be inferred from this study that terpenoid extracts from IT90K-277-2 racemes and flowers, Sanzibanili racemes and flowers, Sewe racemes and flowers, KV×404-8-1 racemes and floral buds, and TVu 1509 racemes and floral buds, play a significant role in resistance of these cultivars to *M. sjostedti* larvae.

4. Conclusions

Cowpea plant secondary metabolites investigated in this study play significant roles in conferring resistance to *Megalurothrips sjostedti.* It is noteworthy that terpenoid extracts from racemes of resistant cowpea cultivars caused significant mortality to second instar larvae. Presence of resistant factors in cowpea racemes is highly desirable in screening and breeding for resistance to thrips. At the raceme stage, adult thrips infest cowpea racemes on which they oviposit and feed. Consequently if the emergent larvae face the problem of unpalatable food, because of unsuitable inherent biochemical factors, larval survival will be impaired and this will lead to subsequent reduction of adult thrips populations to perpetuate the next generation. Thus reduction in thrips populations will lead to production of more flowers and increase in cowpea yields. Therefore the present study highlights the potentials of secondary plant metabolites in the management of *Megalurothrips sjostedti* on cowpea.

Acknowledgments

The authors acknowledge the support of International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria, for providing facilities such as, field plots, laboratory space, equipments and reagents to carry out the research. O. Y. Alabi was a research fellow at the IITA when the studies were carried out.

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Journal of Agricultural Science and Technology A 1 (2011) 270-284
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Between and Within-Farm Variability in Soil Fertility Management and Status in the Central Highlands of Kenya

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Received: September 10, 2010 / Published: June 20, 2011.

Abstract: The processes of nutrient depletion and soil degradation within smallholder farms of central Kenya are spatially heterogeneous, determined by both biophysical and socio-economic factors. A monitoring study involving nutrient stocks, flows and balances was conducted in central Kenya to explore between and within-farm variability in soil fertility management and identify spatial niches for targeting soil fertility management strategies. Focus group discussions were conducted and farms grouped into 3 farm types (rich, medium and poor). Nine case-study farms - three from each of the farm types - were randomly selected from the 50 farms studied, for detailed resource flow mapping. The farms were visited to record movement of nutrients inputs using a monitoring protocol covering soil, crops, livestock, and socio-economic aspects of the farm. Soil in different plots were sampled at a depth of 0-20 cm and analyzed for texture, pH, C, N, available P, exchangeable K^+, Mg^{2+} and Ca^{2+}. Results revealed that wealthy farmers added an average of 51.3 kg/ha N, 37 kg/ha P, and 244 kg/ha K, compared to 25.9 kg/ha N, 14.5 kg/ha P and 50.7 kg/ha K for the poor farmers. In all farm types, home fields received more nutrient inputs compared to the outfields. Consequently, maize grain yields, partial nutrient balances and soil nutrient stocks were significantly higher in wealthy farms and home fields compared to poor farms and outfields, respectively. These results imply that different soil management strategies are required to achieve similar yields on the different field and farm types and avert soil degradation.

Key words: Soil fertility, home fields, outfields, soil degradation, soil nutrient budgets.

1. Introduction

Soil fertility depletion has been described as one of the fundamental constraints to crop productivity on smallholder farms in sub-Saharan Africa [1]. Aggregated nutrient balance estimates at national and regional levels have shown overall large negative balances [2, 3]. An average of 660 kg N ha^{-1}, 75 kg P ha^{-1}, and 450 kg K ha^{-1} has been lost during the last 30 years from about 200 million ha of cultivated land in 37 African countries excluding South Africa [4, 5].

At farm level, soil fertility status of different plots on smallholder farms in sub-Saharan Africa may vary considerably due to both inherent factors and different resource management strategies [6-9]. Smallholder farms consist of multiple plots managed differently in terms of crops grown, fertilizers and labour resources [10]. In most cases, both organic inputs and fertilizer are preferentially allocated to plots close to the homestead whilst plots further away are neglected. Even where small quantities of manure and fertilizers are available, farmers still apply these at high rates by concentrating them on small areas [9]. The underlying reasons for targeting of nutrient resources to few fields vary from farmer to farmer, but important factors include farm size, distance of different plots from the homestead, restricted availability of fertilizers and manures, availability and efficiency of labour use, farmers' own experiences on

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fields that give higher yields, type of crop (more nutrients are applied to the high value crop), risk of theft and the need to reduce risk associated with erratic rainfall [11, 12].

To overcome inherent low soil quality, smallholder farmers use a wide range of nutrient sources in their farms such as fertilizers, manure, compost, leaf litter, green manures, cereal-legume intercropping and rotations, and rock phosphates [13-16]. However, the choice of the sources and amounts used is mainly based on the availability of the resources (nutrient inputs, labour and cash) at the farm rather than a systematic and intentional way to increase soil fertility [17]. Availability of these resources is not uniform among smallholder farmers and is dependent on their resource endowment/wealth status. In fact, fertilizers are unaffordable to the majority of farmers due to the high costs of purchase and transport [18] and production of manure and other organic nutrient resources are generally limited and restricted to those who have greater access to livestock, cash, labour and land [17, 19]. Arising from these factors, smallholder farmers can be classified into different wealth categories (farm types) that reflect the level of household's resource endowment. These farm types represent different scenarios for soil fertility management within smallholder farming systems. This socio-economic heterogeneity in smallholder farms needs to be considered when developing soil fertility management interventions.

Availability of organic inputs and fertilizers and their management under spatially variable soil fertility conditions has consequences on the soil resource base, cropping patterns and crop yields on smallholder farms. Understanding the spatial and dynamic resource use strategies is necessary as a foundation for designing relevant and sustainable interventions to improve resource use efficiency at the farm level. Past studies have ignored variation in soil fertility associated with resource management despite evidence that this affects nutrient use efficiencies and productivity of both crops and livestock [10]. There are strong indications that nutrient balances differ widely between farms in different wealth categories and between plots at different distances from homesteads [7, 8, 20].

Nutrient budgets of agro-ecosystems can be used as a tool to increase the understanding of nutrient cycling, or as a performance indicator and awareness raiser in nutrient management and environmental policy [21]. In central Kenya, information on the dynamics of total nutrient stocks and balances in agro-ecosystems, i.e., budgets and flows between different production compartments are scarce and hence the need for this study. Here, we describe a monitoring study that was undertaken to understand within and between-farm variability in soil fertility management on smallholder farms in Kirege location, Central highlands of Kenya. This was seen as a necessary step in identifying spatial-temporal niches for targeting of soil fertility management strategies and technologies. The objectives were (1) to construct farm types that reflect potential access of households to resources for managing their soils, (2) to compare nutrient inputs between farm types and between field types within a farm and, (3) to determine the magnitude of the soil nutrient flows, balances and stocks at farm scale as affected by farm types (4) to assess the influence of resource availability on soil fertility management and nutrient balances.

2. Materials and Methods

2.1 Study Area

The study was conducted in Kirege location (00°20′0.56″ S, 037°37′58.6″ E), in Meru South District. It is in the upper midland agro-ecological zones two and three (UM2-UM3); tea-coffee-maize growing zones [22]. The area lies on the eastern slopes of Mt. Kenya at an altitude of approximately 1500 m above sea level with an annual mean temperature of 20 °C. It has an annual rainfall ranging from 1200 to 1,400 mm and is bimodal, falling in two distinct seasons. The long rains (LR) occur from March to June,

and the short rains (SR) from October to December. The soils are deep, well drained; weathered Humic Nitisols (locally known as red Kikuyu loams) with moderate to high inherent fertility [22]. However, soil fertility has been declining due to continuous cropping without adequate nutrient addition.

Smallholder mixed farming is the most predominant farming system in the area. Coffee (*Coffea arabica*) and tea (*Camellia sinensis*) are the major cash crops, while maize (*Zea mays*) and beans (*Phaselous vulgaris*), are the main food crops in the area. There are two cropping seasons for some crops such as maize, beans, potatoes and vegetables whilst other crops such as coffee, tea, bananas and Napier grass are perennial. The farmers also keep livestock under a zero grazing system. The study area typically represents a demographic, agro-ecological and cropping system scenario for other surrounding high potential areas in the central highlands of Kenya including parts of the greater Meru, Embu, Kirinyaga and Nyeri districts. These areas are faced with intense soil degradation arising out of continuous cultivation without addition of adequate nutrient resources. Farmers in these areas also face similar challenges in soil fertility management key among them being limited availability of nutrient and labour resources. This means that the results of this study could provide an insight into the status and trends in soil fertility management in the central highlands of Kenya. During the study period, the area received approximately 1,272.8 mm of rainfall, indicating near normal cropping year, though long rains were slightly diminished (Fig. 1).

2.2 Development of Farm Types and Selection of Case Study Farms

Focused group discussions were conducted to identify farmer criteria to be used for wealth ranking. Farmer-identified indicators of wealth status were ranked, and this formed a basis for grouping farmers into different wealth categories. A rapid household survey was conducted using a sample of 50 households randomly selected out of the list of households in Kirege obtained from the local chief's office to characterize and classify the farms into three different categories (rich, medium and poor). During the survey and transect walks, data on socio-economic characteristics of households (gender of household head, age, marital status, education level and average family size) were also collected. Having confirmed the

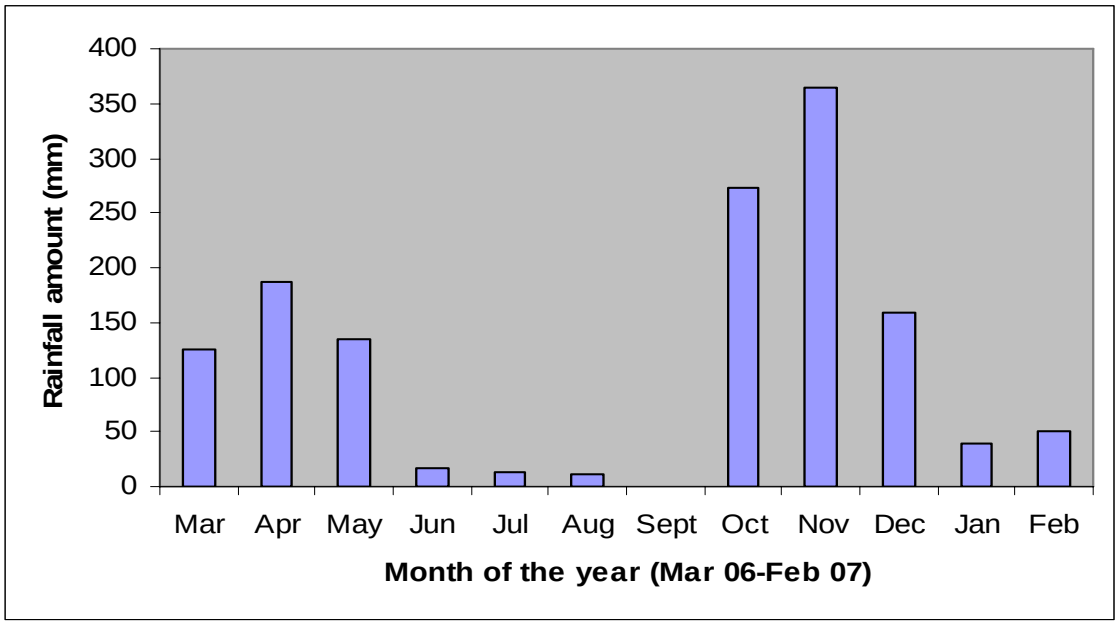

Fig. 1 Rainfall distribution in Kirege during the period March 2006 to February 2007.

resource endowments of the farms, nine case-study farms (three from each of the farm types) were randomly selected for detailed resource flow mapping.

2.3 Development of Field Types

The nine farms were visited to inform the farmers of the nutrient monitoring exercise. During the visit, the researcher together with the farmers drew sketch farm maps to indicate location of farm plots under various activities/enterprises. An inventory was conducted to identify the important features of the farm to be studied, such as fields, crops, animals, compost/manure pits, household composition, farm size, farm implements and facilities. Area of farm plots, their coordinates and distance from the homestead were obtained by use of a Global Positioning System (GPS) unit. Land use and distance from the homestead were the main criteria used to classify field types (home fields, mid-fields and outfields). These had been identified as the main indicators of within-farm soil fertility management strategies in similar studies in other regions [7, 8, 19, 23].

2.4 Resource Flow Mapping and Calculation of Partial Nutrient Balances

The farms were first visited in February 2006 and farmers asked to draw schematic maps and indicate all production units. Additionally, the type of crops grown, use or destination of the outputs, type and amount of inputs used, timing of crop and soil management activities and sequential order within the farm, sources of labour, off-farm income, average yields and general crop and livestock husbandry practices adopted were recorded. A seasonal time frame was used, considering the long rains (March to August) of 2006 and short rains (October 2006 to February 2007). In the long rains season, a monitoring approach was adopted where farms were regularly (monthly) visited from the period of land preparation to harvesting time to monitor the internal flow of nutrients. During the monthly visits, farmers were interviewed to provide information on crop and livestock husbandry practices between then and the previous visit. In the short rains season, farmers were asked to be recording information on various aspects of soil, crop and livestock management, and household consumption patterns. Farm visits by the researcher were limited to planting, weeding and harvesting periods when the information recorded by farmers was collected.

During resource flow mapping, farmers indicated quantities of inputs and outputs to the different production units in local units, such as *tins* (± 2 kg of grains), *debes* (± 16 kg of grains), *bags* (± 90 kg of grains), *bunches of bananas* (± 40 kg) *head loads* (± 40 kg of Napier grass or maize stover) and these were converted into SI units. Many of the values in kg given to local units were taken from previous work and farmers own experiences with the products [15, 24]. Six manure samples (2 from each of the farm types) were collected and analyzed for proportion of N, P, K, Mg and Ca and results used for the corresponding farm types in the IMPACT model to calculate the amount of nutrients available from manure.

2.5 Soil Sampling and Analysis

Soil in the plots identified in resource flow mapping at different distances from the homesteads were sampled at the end of 2006/2007 short rains cropping season (March). The sampling depth was 0-20 cm to capture the level of nutrient stocks in the topsoil. Soil sub-samples (number dependent on plot size) were taken randomly in each plot using an Alderman auger and then bulked to one sample to reduce variability. Soil samples were analyzed for pH, C, N, available P, exchangeable K, Ca^{2+} and Mg^{2+} [25].

2.6 Data Analysis and Presentation

The data obtained during the monitoring exercise were analyzed by use of IMPACT (Integrated Modeling Platform for mixed Animal-Cropping Systems) model version 2.0 [26]. IMPACT is modeling platform that integrates aspects of crop and animal

management and aggregates the nutrient inputs, outputs and balances from the different production systems. It enables the calculation of nutrient balances, economic performance, labour efficiency and nutrition status of households thereby providing an opportunity for improving labour and resource-use efficiencies [26]. Nutrient inputs and balances were calculated for both the farms as units and for field(s), separating nutrient sources into organic inputs and fertilizer. The data obtained from IMPACT model and soil analyses were subjected to Analysis of Variance (ANOVA) using Genstat Discovery edition 2 [27], using split plot design with "farm type" as the main plot and "field type" as the sub-plot. Comparisons were made between "farm types", and "field types" for nutrient inputs, balances, and soil nutrient stocks and their means separated using least square difference (LSD) at 5% level of significance. The nutrient inputs and balances were presented as summaries for the 2006/2007 cropping year (long and short rains cropping seasons combined as this was the capability of the model).

3. Results and Discussion

3.1. General Description of Households and Farm Types in Kirege

Results from the rapid household survey indicated that about 78% of the sampled households were male headed (Table 1). In many communities, gender influences access to resources, which are vital to farm management in general, and to soil fertility management specifically. The age of the household head and the family structure are important factors that influence farmers' investment in soil fertility management [28]. Young people work hard to improve their status, are receptive of new ideas and are therefore more likely to adopt new technologies for soil fertility replenishment. At least 96% of the household heads had basic primary education (Table 1). Education level of the household head also influences the kind of decisions made regarding general farm management.

The type of housing, type of livestock housing, livestock ownership, off-farm income, intensity of use of fertilizer and the frequency of hiring or selling labour were important indicators of wealth in the community (Table 2). This is in agreement with observations from a study in western Kenya, which reported these factors as important wealth indicators in the study area [7]. In contrast to the study in western Kenya, this study did not identify land size as an indicator of wealth. This can be attributed to the fact that the study area is close to Chuka town (about 3 km) and rich farmers were mainly immigrants who had bought small pieces of land in the area due to its proximity to town compared to the poor farmers who were mainly the original inhabitants of the area or their descendants who have inherited the land (Table 3). The distribution of household types (rich, medium, poor) in Kirege location revealed that out of the 50 farms studied during the rapid survey, 22% were rich (11 households), 52% medium (26 households) and 26% were poor (13 households) (Table 3). Rich farmers owned more cattle than any other group but the differences between the groups were not significant (Table 3).

Poor farmers generated most of their income from on-farm activities (Table 3), indicating limited opportunities for off-farm income amongst poor farmers compared to the wealthy farmers. Off-farm

Table 1 Demographic characteristics of households in Kirege (n = 50).

Characteristic	Type	Percentage
Gender of household head (HH)	Male	78
	Female	22
Age of HH (years)	25-40	52
	41-60	30
	> 60	18
Marital status of HH	Single	6
	Widow/er	12
	Married spouse present	70
	Married spouse absent	12
Education level of HH	None	4
	Primary	46
	Secondary	42
	Tertiary	8
Average family size	0-4	48
	5-8	52

HH-Refers to household head.

Table 2 Indicators of the wealth status of the farmers and characteristics of the different farm types at Kirege, central Kenya.

Indicator of wealth status	Rich	Medium	Poor
Type of housing	Concrete floor, stone wall, tiled roofing	Concrete floor, timber wall, iron roofing	Earth floor, timber/ mud wall, iron roofing
Livestock ownership	Own more than 2 cattle, sheep, goats and chicken	Own 1 cattle, sheep, goats and chicken	No cattle but own goats, sheep and chicken
Type of livestock housing	Roofed and with concrete floor	Roofed without concrete floor	No roofing and no concrete floor
Production orientation	Produce surplus for sale (approx. 1000 kg of maize)	Produce mainly for subsistence (approx. 400 kg of maize)	Produce mainly for subsistence (approx. 270 kg of maize)
Fertilizer use	Use in all seasons and in large amounts (> 150 kg ha^{-1})	Not used in all seasons and in smaller amounts (50-100 kg ha^{-1})	Not regularly and in small quantities (< 25 kg ha^{-1})
Off-farm income	High, mainly in permanent employment	Medium, mainly short-term employment and small businesses	Low, mainly selling casual in farms
Hire or sell labour	Afford to hire regularly (all seasons)	Do not afford to hire regularly (all seasons)	Sell labour locally

Table 3 Average resource endowment on the farms in Kirege location (n = 50) and in selected case study farms (n = 9) in the different farm types.

Level	Farm type	No. of farms	Estimated income (%)		No. of plots	Farm size (ha)	No. of cattle	No. of sheep & goats	No. of chicken
			On-farm	Off-farm					
Village	Rich	11 (22%)	40	60	6	0.5	3	6	24
	Medium	26 (52%)	50	50	6	0.8	2	3	12
	Poor	13 (26%)	70	30	5	1.0	0	2	6
		Mean	55	45	5.3	0.8	1.7	3.7	14
		SED	5.1	5.1	1.4	0.2	0.4	1.1	3.4
Case study farms									
	Rich	3	40	60	6	0.5	2	5	25
	Medium	3	55	45	6	0.5	1	3	14
	Poor	3	75	25	5	1.2	0	3	5
		Mean	60	40	5.3	0.7	1	3.7	14.7
		SED	5.8	5.8	1.4	0.3	0.8	1.4	6.7

income has a variety of effects on soil fertility; for instance, cash flows generated allow a higher rate of input use but may cause a shortage of labour within the farm. According to the magnitude of these cash flows, hiring labour may compensate its shortage especially in the wealthy farms. Similar observations were made in a study among smallholder farmers in Murewa, Zimbabwe, which identified farm size, livestock ownership, production orientation, use of fertilizers, off-farm income and labour supply as important wealth indicators [19].

3.2 Categorizing and Describing Field Types

Different field types were identified within a farm, varying in enterprises/ production activities, resource allocation and management practices, as revealed by the farm transects. These fields were distinct in terms of distance from the homestead, crop allocation and management strategies employed by farmers. Crop allocation was most diversified in the home fields, which had an average of 8 different crops, compared to outfields with 4 crops (Table 4). Most of the home fields were under high value crops such as fruits and vegetables while lower value crops were allocated to the outfields (Table 4). The home fields were normally managed by women and were often the first fields to be planted and weeded, receiving kitchen wastes and the sweepings from the house. The home fields also received spills of manure from animal shed or manure stored in heaps due to their proximity to these structures.

In the midfields an intermediate management situation was found (Table 4), where a mixture of high and low value crops were found. Intercrop of maize and beans was the most dominant crop in these fields followed by coffee, vegetables, Napier grass and tea. In terms of management, these fields received greater attention than the outfields though less than the home fields. In some cases, they were managed in a similar way to the home fields, though input use was less intense. The outfields were distant and/or difficult to access, and the crop produce more prone to theft, particularly in areas of steep slopes. In this type of field, associated with poor quality land, farmers planted woodlots or crops that are known to produce under poor soil fertility conditions, such as sweet potatoes, cassava or Napier grass. On some farms, outfields were located in the flood plain (river banks) and in such cases, farmers planted vegetables such as arrowroot and kale.

Table 4 Average area, distance from the homestead, and most frequently grown crops for the different field types, averaged over all farm types in Kirege, central Kenya (n = 9 farms).

	Home fields (n = 35)	Mid-distance fields (n = 15)	Outfields (n = 12)
Average area (ha)	0.07 (0.01-0.1)[a]	0.18 (0.08-0.21)	0.23 (0.15-0.25)
Average distance (m)	25 (5-40)	84 (45-80)	158 (85-180)
Most frequently grown crops (frequency %)			
Maize	25 (18-32)	7 (3-16)	18 (12-26)
Beans	12 (5-16)	0	7 (3-9)
Maize/beans	35 (20-45)	83 (53-90)	0
Bananas	19 (15-24)	0	0
Coffee	2 (1-4)	20 (5-28)	0
Tea	0	7 (6-13)	0
Vegetables[b]	23 (12-17)	13 (2-14)	6 (2-7)
Potatoes	18 (8-26)	9 (1-12)	0
Sweet potatoes	8 (3-11)	4 (3-8)	11 (3-20)
Cassava	6 (4-8)	0	14 (8-22)
Woodlot	0	8 (5-11)	24 (12-35)
Napier grass	9 (1-15)	13 (7-24)	41(20-64)

[a] Ranges in parentheses; [b] Include kales, cabbages, tomatoes and onions.

3.3 Variability in Resource Allocation within Farms

Resource allocation studies revealed that farmers in Kirege location allocated most of the nutrients (organic inputs and fertilizers) to the home fields with outfields receiving very little inputs across farm types (Table 5). The use of organic resources varied clearly for different field and farm types, and was strongly influenced by distance from the homestead and farmers' level of resource endowment. Wealthy farmers used large amounts of organic resources, which provided 33.9-105.2 kg/ha N, 19.4-64.8 kg/ha P and 69.4-208.5 kg/ha K compared to the poor farmers who used about 29.2-39.5 kg/ha N, 14.7-22.3 kg/ha P and 50.3-77.0 kg/ha K from organic resources (Table 5). Vegetable crops grown in the home fields received most of the organic resources, followed by the cash and grain crops grown in the mid-distance fields. Very little organic resources were applied to the outfields, due to their limited availability and the extra effort required in transporting coarse, heavy and bulky materials to distant parts of the farm (Table 4). Similar results have been reported in a study in Uganda, where crop residues from the 'outfields' were harvested, fed to livestock and manures applied to crops intended for the market, resulting in nutrient mining of 'outfield' soils and the creation of characteristic patches, plots and fields of nutrient-deficient crops [29]. A similar study in western Kenya also reported widespread variations in allocation of organic resources to different field types in Kakamega District, western Kenya [7, 8]. Fields close to the homesteads received more manure compared to remote fields.

Fertilizers were used with varying intensities in the different field types (Table 5). The wealthy farmers applied fertilizers to all field types, and relatively high rates were used in the outfields. Poor farmers did not apply fertilizers to outfields (Table 5) because the resources were not enough to be used in all the fields. With regard to farm type, there was a large difference in amounts of fertilizers used by the wealthiest farmers (18.6-28.5 kg/ha N and 13.8-20.7 kg/ha P) and the poorest farmers (0.4-4.6 kg/ha N and 0.4-4.6 kg/ha P).

Table 5 Allocation of organic (mainly farmyard manure) inputs and fertilizer to different field types by different farm types in Kirege, central Kenya.

Resource group	Field type	Organic inputs (kg/ha/yr)			Fertilizer inputs (kg/ha/yr)		
		N	P	K	N	P	K
Rich	Home fields	75.0	61.3	379.0	12.5	7.0	0.0
	Mid-fields	60.0	40.7	298.0	23.0	11.6	0.0
	Outfields	19.0	9.1	55.0	34.1	23.0	0.0
	Mean/farm	73.3	40.9	244.0	21.6	16.3	0.0
Medium	Home fields	65.0	42.7	145.0	25.6	24.6	0.0
	Mid-fields	57.0	36.2	119.0	1.8	6.3	0.0
	Outfields	15.5	16.1	33.0	5.6	2.0	0.0
	Mean/farm	61.6	36.5	99.0	14.6	11.2	0.0
Poor	Home fields	54.2	30.5	106	2.6	2.6	0.0
	Mid-fields	15.5	8.5	30.0	2.3	2.3	0.0
	Outfields	8.1	4.6	16.0	0.0	0.0	0.0
	Mean/farm	32.4	18.2	50.7	1.5	1.6	0.0
LSD (field)		17.8	12.9	84.7	8.7	6.1	0.0
LSD (farm)		25.4	14.4	116.1	11.4	7.4	0.0

LSD (field) was used to compare means across fields while LSD (farm) was used to compare means across farms at $P = 0.05$.

The wealthy farmers distributed fertilizers evenly across their farms, but preferentially targeted organic resources (manure) to the plots closest to the homesteads (Table 5). As previously observed in other similar studies [7, 11, 19, 30, 31], this study also found that distance from the homestead tended to affect the allocation of production activities and resources (Table 5).

3.4 Variations in Manure Quality between Farms

The results from analysis of manure revealed significant differences in the proportion of N, P, K, Mg and Ca between rich and poor farms but not between rich and medium resource farms (Table 6). Manure from the rich farms was of higher quality compared to that from the poor farms. The variations in manure quality could be related to several factors among them type of animal, quality and utilization of materials fed to the animals, animal housing, manure collection, storage and processing method, and addition of organic materials to the animal stalls as beddings. The high quality of manure from the rich farms may be as a result of feeding the animals with high quality fodder and concentrate feeds, reduced nutrient losses from leaching and volatilization because the livestock housing had concrete floor and roofing as well as storage of manure in pits. Conversely, the low quality of manure from poor farms can be associated with low quality animal feeds and poor livestock housing and manure storage methods, which led to increased nutrient losses through leaching and volatilization.

Table 6 Quality of manure from different farm types (rich, medium, poor) in Kirege, central Kenya.

Farm type	Manure quality (%)				
	N	P	K	Mg	Ca
Rich	1.62	0.62	4.45	0.90	1.24
Medium	1.24	0.36	2.39	0.40	1.02
Poor	0.86	0.15	0.95	0.15	0.05
Mean	1.12	0.32	2.62	0.48	0.77
SED	0.25	0.16	1.72	0.13	0.21

Several studies have shown that manure stored in pits until application has substantially greater contents of N, P, K, Ca and Mg compared to when stored in heaps [32-34]. The studies further reported that animal housing and floor type influenced N, P and Ca concentration in manure. Results on improvement of manure quality through provision of high quality feed

(e.g. calliandra, leucaena) to the animal and through management of manure are available [34, 35].

3.5 Total Nutrient Inputs, Outputs and Partial Nutrient Balances

The total nutrient inputs calculated for different field and farm types indicated that wealthy farms received more nutrients inputs compared to the poor farms (Table 7). It was further observed that more nutrients were applied to the home fields compared to the outfields. Although the average rates of nutrients inputs at farm level for wealthy and medium groups were close to the recommended rates for this region (60 kg N and 60 kg P per ha), calculations at field level revealed that little nutrients were applied to the outfields and especially in the medium and poor farms. Total N and P inputs differed insignificantly in the home fields of rich and medium farms. Large amounts of K additions were observed as a result of high levels of K in materials used e.g. up to 3.31% for manure, up to 4.1% in banana residues, and up to 2% for beans residues.

Partial nutrient balances at field scale revealed an existence of N 'accumulation' areas within the wealthy farms and home fields of medium farms (Table 7). However, these were found to be small patches and the accumulation was not reflected in the soil total N, indicating the need to link soil nutrient inputs, output and balances to the actual nutrient stocks. The partial balances on the wealthy farms were large and positive on the home fields, but decreased in the midfields and outfields. The partial N balances were negative for outfields in medium farms and all fields of the poor farms, illustrating that the amount of N added from both organic inputs and fertilizer was less than the amount of N harvested with the biomass removed (Table 7). The results indicate that while the major obstacle to soil fertility management among the resource-poor farmers could be unavailability of nutrient resources, the wealthy farmers could be having access to enough nutrient resources but have labour limitation hence do not distribute the resources evenly within the farms. Phosphorus balances were positive in all the farms and field types although in some fields the P balances were close to zero (equilibrium) (Table 7). The areas being depleted were much larger than the areas of "accumulation", leading to an overall negative

Table 7 Total nutrient inputs, outputs and partial nutrient balances for different field types of the three different farm types in Kirege, central Kenya.

Farm type	Field type	Total nutrient inputs (kg/ha/yr)			Total nutrient outputs (kg/ha/yr)			Partial nutrient balances (kg/ha/yr)		
		N	P	K	N	P	K	N	P	K
Rich	Home fields	87.5	68.3	379.0	46.5	21.5	350.0	41.0	46.8	29.0
	Mid-fields	83.0	52.3	298.0	75.0	18.5	223.5	8.0	33.8	-20.0
	Outfields	53.1	32.1	55.0	51.1	10.9	70.0	2.0	21.2	-15.0
	Mean/farm	84.4	57.2	244.0	58.4	16.6	246.0	26.0	40.6	-2.0
Medium	Home fields	90.6	67.3	145.0	74.5	28.3	98.0	16.1	39.0	47.0
	Mid-fields	58.8	42.5	119.0	56.7	8.5	115.0	2.1	34.0	4.0
	Outfields	21.1	18.1	33.0	64.3	7.1	117.0	-43.2	11.0	-84.0
	Mean/farm	64.8	47.8	99.0	71.8	15.1	110.0	-7.0	32.7	-11.0
Poor	Home fields	56.8	30.5	106	85.8	2.6	163.0	-29.0	27.9	-57.0
	Mid-fields	17.8	10.8	30.0	94.8	4.8	201.0	-77.0	6.0	-171.0
	Outfields	8.1	4.6	16.0	177.1	2.0	204.0	-169.0	2.6	-188.0
	Mean/farm	33.9	19.7	50.7	103.9	4.0	189.4	-70.0	15.1	-138.7
LSD (field)		24.0	16.0	84.7	19.4	7.4	34.3	26.0	6.0	65.0
LSD (farm)		28.0	22.0	116.1	33.7	9.1	42.7	66.0	33.0	88.0

LSD (field) was used for comparing means across fields while LSD (farm) was used for comparing means across farms at $P = 0.05$.

nutrient balance at farm scale for medium and poor farms. This agrees with observations that larger areas of smallholder farms in Zimbabwe were highly depleted while nutrient accumulations were observed in home fields [17]. Large amounts of K off-take were observed especially in the home fields. This could be partly explained by high levels of K in materials removed e.g. up to 4.1% in banana residues, and up to 2% for beans residues, which were the main crops.

3.6 Production Orientation and Soil Nutrient Balances

The study revealed that cash crop fields in wealthy farms had significantly higher soil nutrient balances compared to food crop fields but this was not the case with other farm types (Table 8). However, there were significant differences in soil nutrient balances between cash crop and pasture fields across all farm types. As a result of high inputs in fields planted with cash crops (mainly coffee, tea, bananas and vegetables) in terms of fertilizers and manure, nutrient balances were found to be highly positive in the cash crop fields compared to those for pasture fields (Table 8). In particular, most of the nutrients applied to tea fields were mainly from fertilizers available to farmers on credit from farmers' co-operatives. For coffee and bananas, the nutrients were mainly from manure. However, farmers combined fertilizers and manure in the vegetables fields largely because these fields were the smallest and therefore required only small amounts of manure and fertilizers.

Food crops received limited amounts of fertilizer and manure. Maize and beans were grown at subsistence level. Despite low outputs, the balances for maize and beans fields were negative because very little inputs were used for these crops. Similarly, nutrient balance studies in Kisii, Kenya revealed that most of the nutrients were allocated to cash crops while food crops received little inputs resulting in large negative balances in food crop fields compared to cash crop fields [5]. Hardly any fertilizer was used in Napier grass fields, whereas high outflows of N and K were recorded resulting in negative balances. The severe nutrient mining under Napier grass is explained by high fodder removal, which was not offset by manure application though considerable amounts were applied. Discussions with farmers revealed that on average about 20% of the available manure was used for fertilization of Napier grass fields. It was also observed that farmers usually applied manure on a bare plot where Napier grass was to be planted or had just been harvested and left uncovered. Such a mode of application induces high losses by volatilization and leaching. Low P circulation (though positive balance) was recorded and this could be as a result of low P values in Napier grass and manure.

Table 8 Influence of farm type and production orientation on soil nutrient balances in smallholder farms of Kirege, Central Kenya.

Farm type	Production orientation	Nutrient balances (kg ha^{-1} yr^{-1})		
		N	P	K
Rich	Cash	45	36.8	47.0
	Food	14.2	22.8	-20.0
	Pasture	2.3	10.2	-15.0
	Mean	20.5	23.3	4.0
Medium	Cash	19.1	34.7	29.0
	Food	2.1	18.6	4.0
	Pasture	-43.2	11.0	-84.0
	Mean	-7.3	21.4	-17.0
Poor	Cash	7.5	17.9	-17.0
	Food	-19.6	14.6	-56.0
	Pasture	-92.0	2.6	-118.0
	Mean	-34.7	11.7	-63.7
LSD (production)		15.3	3.5	19.3
LSD (farm)		26.2	6.2	63.0

LSD (production) was used to compare means across production orientation while LSD (farm) was used to compare means across farm types at $P = 0.05$.

3.7 Implications of Nutrient Allocation Strategies on Crop Production

Maize was the most predominant crop among the farmers studied and therefore provided an opportunity for comparing production in different fields and farm

types (Table 5). The results revealed that maize grain yields decreased with increasing distance from the homestead across all farm types, although the decline in yields was less steep in wealthy farm types (Table 9). For instance, field measurements in the long rains season indicated within and between-farm differences in average maize grain yields of 18% (2.7 vs. 1.9 t ha^{-1}) in rich farms and of 58% (1.4 vs. 0.3 t ha^{-1}) in poor farms, between the fields that were close and far from the homestead, respectively. The decrease in yields with increasing distance from the homestead can be explained by the preferential allocation of nutrient materials to the home fields compared to the outfields. This kind of management over a long period of time may have resulted to soil fertility gradients with increasing distance from the homestead. These findings concur with Tittonell et al. (2007) in western Kenya, who reported within-farm differences in average maize grain yields of 48% (2.7 vs. 1.4 t ha^{-1}) in Vihiga District and of 60% (1.5 vs. 0.6 t ha^{-1}) in Teso District, between close fields and remote fields, respectively.

Table 9 Mean maize grain yields for different field types of the three different farm types in Kirege, central Kenya for the 2006 long rains (LR) and short rains (SR) seasons.

Farm type	Field type	Maize grain yields (t/ha)	
		2006 LR	2006 SR
Rich	Home fields	2.7	4.2
	Mid-fields	2.4	3.8
	Outfields	1.9	2.9
	Mean/farm	2.4	3.6
Medium	Home fields	2.3	3.9
	Mid-fields	1.8	3.2
	Outfields	1.1	2.1
	Mean/farm	1.9	3.1
Poor	Home fields	1.4	2.2
	Mid-fields	0.7	1.7
	Outfields	0.3	1.1
	Mean/farm	0.8	2.3
LSD (field)		0.3	0.4
LSD (farm)		0.4	0.6

LSD (field) was used for comparing means across fields while LSD (farm) was used for comparing means across farms at $P = 0.05$.

Soil fertility gradient could also be as a result of inherent differences in soil texture which is known to influence other soil characteristics such as soil carbon and exchangeable bases. This would mean that different fields within a farm would require different amounts of nutrients to achieve similar crop responses and therefore yields. The higher yields in wealthy farms compared to the poor farms can be attributed the fact that wealthy farmers could afford relatively more nutrient and labour resources. A related study in western Kenya equally observed that fields close to the homesteads produced substantially larger amounts of maize than fields farther away [9]. It is worth noting that the yields for the 2006 long rains were lower compared to the short rains. This can be attributed to the diminished rains in the long rains season compared to the short rains season, which reflected the average amount for the area.

3.8 Soil Fertility Status

The analytical results of soil collected from the different farms and fields in Kirege are summarized in Table 10. The results show that the soil pH ranged from acidic (4.83) to near neutral (6.01). The optimum pH range for most agronomic crops is between 6.0 and 7.0 [37]. There were significant differences between farm and field types in terms of available P. However, available P levels in all the fields and farms were found to be above critical level of 10 mg/kg [25], which could be related to the relatively high additions through manure and P fertilizer, and low removals through harvested products.

Soil organic carbon, total N, available P and exchangeable K^+, Ca^{2+} and Mg^{2+} decreased with distance from the homestead on most farms. For most of the soil nutrients, there were significant differences between home fields and outfields in the different farm types, with home fields having the highest nutrient stocks compared to the outfields. In rich farms, there were significant differences between soil pH in home fields, midfields and outfields. For SOC, total N, P,

exchangeable K^+ Ca^{2+} and Mg^{2+}, there were no significant differences between home fields and midfields. Strong differences were, however, observed between home fields and outfields for those soil properties on all farms types (Table 10). This cannot be solely linked to the differential management observed between the different fields but could also be related to the inherent soil characteristics such as clay content (Table 10). At farm level, home fields and midfields were located close to one another, meaning they shared almost similar soil characteristics as opposed to the outfield, which were mostly located far away from the home fields and mostly in steep slopes. This could mean that their inherent clay content was different from the home fields. The differences in texture were also reflected by the trends in soil carbon and exchangeable cations across farm types. Almost similar values were observed in two case study farms of poor type that were next-door neighbours indicating that texture could have played a role. Soils richer in clay and silt content have a greater capacity for physicochemical carbon stabilization and will therefore contain higher levels of carbon [38].

At farm level, significant differences were observed between farms of different resource endowment levels. There were significant differences between rich and poor farms for soil pH, P, exchangeable K^+, Ca^{2+} and Mg^{2+}, but no clear differences between rich and poor farms for total N and SOC. Rich farmers tried to distribute nutrient resources to all fields compared to poor farmers who accumulated the resources in the home fields. This could be as a result of differences in availability and affordability of nutrient and labour resources especially considering that manure, which is the major source of soil organic matter, is bulky and therefore high amounts of labour are required to enable even distribution within the farms. Similar results were reported in a study on smallholder farms in Emuhaya, Shinyalu and Aludeka divisions of western Kenya [7, 8]. An implication of these results is that different amounts of nutrients are required to achieve similar yields on the different filed and farm types. This also indicates that the response to applied inputs or their agronomic use efficiency is likely to decrease with increasing soil fertility status arising from previous management practices.

Table 10 Variability in soil nutrient stocks as influenced by resource endowment and distance from the homestead in Kirege, central Kenya.

Farm type	Field type	Average content							
		pH	SOC	Total N	Clay	Extractable P	Exchangeable cations ($cmol_c\ kg^{-1}$)		
		(in H_2O)	(%)	(%)	(%)	($mg\ kg^{-1}$)	K^+	Ca^{2+}	Mg^{2+}
Rich	Home fields	5.72	2.35	0.28	36.43	14.50	1.01	2.64	0.74
	Midfields	6.01	2.31	0.27	38.13	13.90	1.12	3.26	1.10
	Outfields	5.12	2.20	0.22	52.85	10.70	0.57	0.88	0.34
	Mean/farm	5.62	2.28	0.26	42.47	13.03	0.90	2.26	0.73
Medium	Home fields	5.90	2.28	0.26	31.07	13.63	0.92	3.05	1.21
	Midfields	5.29	2.26	0.25	44.50	13.07	0.76	1.39	0.58
	Outfields	5.04	2.26	0.24	43.70	12.06	0.50	0.56	0.24
	Mean/farm	5.41	2.27	0.25	39.76	11.92	0.73	1.67	0.68
Poor	Home fields	5.65	2.21	0.31	53.34	11.70	1.04	2.30	2.31
	Midfields	5.04	2.19	0.24	48.50	11.08	0.50	0.77	0.21
	Outfields	4.83	2.01	0.24	46.58	10.27	0.43	0.55	0.16
	Mean/farm	5.17	2.14	0.26	49.76	10.68	0.66	1.21	0.89
LSD (field)		0.26	0.08	0.01	4.17	0.96	0.14	0.6	0.12
LSD (farm)		0.28	0.14	0.02	6.56	1.38	0.18	0.74	0.46

LSD (field) was used for comparing means across field types while LSD (farm) was used for comparing means across farm types at $P = 0.05$.

4. Conclusions and Recommendations

The allocation of both organic inputs and fertilizers varied clearly for the different field types and was strongly influenced by distance from the homestead. Home fields received more nutrient inputs compared to the outfields. Wealthy farmers used large amounts of nutrient inputs (both organic inputs and fertilizers) compared to the poor farmers. N, P and K partial balances were higher on the wealthy farms and all home fields compared to poor farms and outfields, respectively. The same trend was observed with regard to soil nutrient stocks and maize grain yields, indicating the farm and field types are important factors in soil fertility management in this area. These findings highlight the need for site- and farm type-specific nutrient inputs recommendations, especially where these inputs are either relatively expensive and/or scarce. However, in order to provide these recommendations, it will be essential to base the diagnostic results to local soil assessment qualities as conventional soil analyses are beyond the financial reach of most smallholder farmers. Fortunately, farmers are aware of within-farm soil fertility gradients and will use local terms to describe them. What is required is extension advice on how these fertility niches can be managed with the limited inputs available to individual farmers. For the rich and medium farmers who have relatively more nutrient resources, it would be beneficial to distribute them more evenly in the farms. Of particular importance here is to increase the organic inputs to the outfields to enable them respond better to fertilizers. For poor farmers, what is critical is developing strategies to enable them access more nutrient resources and how to efficiently use the limited labour resources.

Acknowledgments

The authors acknowledge the Belgium government through Vlaamse Interuniversitaire Raad (VLIR), European Union through AfricaNUANCES project, Kenyatta University and Lewa Education Trust for providing financial support to carry out this study. Special thanks also go to the staff at World Agroforestry Centre (ICRAF) who assisted in many ways during soil testing and to Kirege farmers who participated in this study.

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Journal of Agricultural Science and Technology A 1 (2011) 285-291
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Some Studies on the Ecology and Host Range of Eriophyid Mites (Acarina: Eriophyoidea) in Sudan

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Received: January 20, 2010 / Published: June 20, 2011.

Abstract: In this study, some Eriophyid mite species showing different morphological characters and host-plant symptoms were obtained. Five mites species collected from different localities in Khartoum State (Sudan) during the period (2002-2003) which classified under family Eriophyidae from different hosts were selected and examined carefully. These were recognized as *Cisaberoptus kenyae* (K.) on Mango *Mangifera indica*, *Acalitus hassani* (K.) onArrak *Salvadora persica*, *Aceria balanites* (Massee) on Heglig *Balanites aegyptiaca*, *Colomerus vitis* on Grape *Vitis* sp. (for the first time in the Sudan) and *Eriophyes tulipae* (K.) on Onion *Allium cepa* and garlic *Allium sativum* respectively. The remaining six eriophyid mites were not completely described but designated as *Eriophyes* sp. 1, 2, 3, 4, 5 and 6 on Sidir *Ziziphus spina*, Aradeib *Tamarindus indica*, Harazz, *Acacia albida*, Talha *Acacia seyal* var. *seyal*, Seyal *Acacia tortilis* var. *raddiana* and Mormeet *Pergia capensis* respectively. The response of 12 different plant species infested artificially with *Cisaberoptus kenyae* (K.), *Acalitus hassani* (K.) and *Eriophyes tulipae* (K.) were studied after two months of infestation. The results showed that onion and garlic plants were severely infested with *Eriophyes tulipae* (K.) while tomato was found to be susceptible for *Cisaberoptus kenyae* (K.) and *Acalitus hassani* (K.) showing moderate infestations. On the other hand, sorghum and wheat infested with *Eriophyes tulipae* (K.) showed moderate and light infestations respectively. On the contrary, mite infestations were not detected on the rest of plant species. The degree of infestation of Eriophyid mites species on different test plants, measured by symptoms appeared on plant leaves.

Key words: Eriophyid mite, morphological characters, host-plant symptoms.

1. Introduction

Eriophyids rank relatively high in value among phytophagous mites of economic importance. Numbers of the eriophyid mites have caused considerable agricultural problems to cultivated plant. They are not well known in the Sudan, and apparently vast areas exist where no studies or collection have been made. Despite the fact that, plant parasitic mites attack several economic plants over the world, our knowledge on its distribution, and economic role is extremely meager. This state of deficiency is attributed to entirely little attention laid on investigations of identity of this important group of pests.

In view of the progressive studies carried out recently, its potential ability to limit production is gradually perceived. Information dealing with plant parasitic mites, their taxonomic status, economic importance, hosts range and control has long been very important concern for acarologists in many countries of the world including Sudan.

Recently, additional advanced knowledge is recorded, and eriophid mites have become important pests, and plant disease vectors on various field, and horticultural crops [1].

Some are highly specialized and feed on a single host species, whereas others extend their ranges to a wide array of hosts [2-7]. However, the preponderance of narrow host specificity has been often observed, and herbivores species tend to be specialized in their use of particular plant taxa [8-11].

In the Sudan, few reports dealing with taxonomy,

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economic importance and control measures were recorded. Hassan [12] gave a record of new species of eriophyid from Arrak trees in Sudan. It was described and named *Acalitus hassani* (K.). He also studied elaborately the infection of *Cisaberoptus kenyae* (K.) in mango leaves. Elhag [13] investigated phytophagous mite species in Khartoum State. He also reported a survey on host-range, determination of the efficacy of Dimethoate E. c-32%, and elucidation of Eriophyidae, especially on plants belonging to different families. Emphasis was made on eriophyid mites, with identification of different mites species.

Generally, eriophid mites gained comparatively less attention than other phytophagous groups probably because of their small sizes which make them difficult to handle. Eriophyid invades series of plants and constitutes considerable damage with varying degrees.

The present investigation was carried out on the phytophagous mite species in Khartoum State. Emphasis was made on eriophid mites attacking different plant families, with the following objectives:

(1) To study the susceptibility of different host plants to eriophyid mite infestation;

(2) To survey suspected infested host plants and identificate it's mites;

(3) To assessment of different plant damages affected by the direct or indirect mites infestation.

2. Materials and Methods

2.1 Survey of Eriophyid Mites in Khartoum North Province

In this work, trials have been made primarily to survey and collect eriophid mites from different areas in Khartoum North Province and around this province with the intention to study its distribution, host plants, and the degree of damage imposed upon them.

Plant species belonging to different families were collected and examined for the presence of eriophyid mite. About 15 leaves sample were collected randomly from each plant showing visual eriophyid mite symptoms. The samples were kept in labeled plastic bags and brought to the laboratory for further examination. For the practical work, some laboratory equipments and tools were use i.e. Dissecting needles, camel-hair's brush, metal hair-clips to hold the leaves flat, scalpel, 20-45 power compound microscope and a binocular.

For mounting of temporary and permanent eriophyid mite slides lactophenol and Hoyer's solution were used respectively. The method of examination and identification of these mites was performed in a similar way as described by Keifer [1] which was based on the morphological character of adult mite collected from their host-plants. Other mite specimens were identified according to method used by Elhag [13]. In an exact way as Elhag [13], the examination of Eriophyid mite on Sidir *Ziziphus spima*, Aradeib *Tamariudus indica*, Harazz *Acacia albida*, Talha *Acacia seyal* var. *seyal* and Seyal *Acacia tortilis* var. *raddiana* were designated 1, 2, 3, 4 and 5 respectively with the exception of the appearance of an abnormal eriophyid mite like symptoms on Marmeet *Pergia capensis* which was noted for the first time in Shambat area. The Hoyer's solution for permanent slides was prepared as follows:

(1) Distilled water 50 mL;

(2) Chloral-hydrate 200 gm;

(3) Gum-arabic 30 gm;

(4) Glycerin 20 mL;

(5) Potasium-lodide 1 gm.

The ingredients were mixed at room temperature, and it may be necessary to filter the mountant through bolting-silk before use.

2.2 Eriophyid Mites Host-Range Procedure

Three species of mites viz. *Cisaberoptus kenyae* (K.), *Acalitus hassani* (K.) and *Eriophyes tulipae* (K.) were used to study the response of various plant species to the infestation as measured by the symptoms produced by these mites. Twelve plants belonging to 7 families namely Malvaceae, Portulaceae, Gramineae, Tiliaceae, Solanaceae,

Fabaceae and liliaceae (Amaryllidaceae) were used.

For each plant species, 4 clay pots of 9 inches in diameter were prepared. Each pot contained soil consisted of thoroughly mixed three equal parts of loam manure, and one part of sand. Four seeds of the test plants were sown in a separated pot except in the case of tomato, onion and hot-pepper, where 2-week old, well grown as free seedlings were planted. The test plants were kept under cheese-cloth cages, and allowed to grow under glass-house conditions at 20-37 °C and 15% relative humidity for three weeks. Thereafter, only two well grown plants were left to develop in each pot, and half of these selected species were used for mites infestation. The rest of the group was treated as control. Infestation was achieved by collecting enough number from each mite species, then satisfactory number from each Eriophyes species were carefully placed on each test plant using camel-hairs brushes. The test plants were allowed to develop for two months. The experimental plants were examined once a week. Observations and description of symptoms produced by Eriophyes mites infestation were recorded.

3. Results

3.1 Survey of Eriophyid Mites in Khartoum North Province

From Table 1, it is clear that among the 48 examined plants belonging to different families, Eriophyes mites were encountered in only Heglig *Balanites aegyptiaca*, Arrak *Salvadora persica*, Garlic *Allium sativum*, Onion *Allium cepa*, Mango *Mangifera indica*, and Grape *Vitis sp*. were found infested with, *Aceria balanites* (Massee), *Acalitus hassani* (K.), *Eriophyes tulipae* (K.), *Cisaberoptus kenyae* (K.) and *Colomerus vitis*, respectively.

The symptoms produced by different eriophyid mite species on infested host-plants under field and store conditions were summarized as follows.

3.1.1 *Aceria balanites* (Massee) on Heglig *Balanities aegyptiaca*

Newly infested green leaves of balanites with *Aceria balanites* show pale green colour and at the sites of infestation the leaves tend to curl on the upper leaf-surface. Against this site at the lower leaf-surface, numerous plant hairs are produced. With the continuous feeding present mites population is increased and the leaves cell increased in number and size forming deformed galls of different shapes and sizes according to different mites population. The mites continue to feed and hide on this gall as long as plants cell sap is available in these galls. Each gall contains numbers of small opening (orifices) within the gall body structure, maintaining ventilation and mites movement. It has also been noted that the gall getting dry after four to six months. Examination of desiccated dry galls shows no living mites but big numbers of white mite moulting skin were found.

Table 1 Eriophyes mite species collected from different localities in Khartoum State during the period (2002-2003).

Plant type	Locality	Host plant		Eriophyes mite species
		Scientific name	Common name	
Field crops	Shambat	Hibiscus sabdarifa	Karkadae	
		Gossypium spp.	Cotton	
		Sorghum spp.	Dura	
		Nicotina tabacum	Tobacco	
		Ricinus communis	Caster	
Vegetable crops	Shambat	Allium sativum	Garlic	*Eriophyes tulipae*
		Allium cepa	Onion	*Eriophyes tulipae*
		Cucumis sativus	Cucumber	
		Hibiscus spp.	Okra	
		Solanum melongena	Egg-plant	
Vegetable crops	Shambat	*Chorochorus olitorius*	Jews mellows	

(to be continued)

Plant type	Locality	Host plant		Eriophyes mite species
		Scientific name	Common name	
		Vigna sinensis	Cow-pea	
		Cucumis melo	Sweet-Melon	
		Vicia faba	Broad-Bean	
		Phaseolus vulgaris	Snap-bean	
		Solanum tuberosum	Potato	
		Capsicum frutescens	Hot pepper	
		Portulaca oleracea	Purslane	
Weeds	Shambat	*Cyperus rotandus*	Sieda	
		Dicanthium annulatum	Lokh	
		Cynodon dactylon	Nagela	
		Dinebra retroflexa	Omginagra	
Weeds	Shambat	*Aristolochia bractedata*	Omgalagil	
		Calotropis procera	Oshar	
Weeds	Shambat	Cassia italica	Sanamaca	
		Cuscuta hyalina	Dodder	
		Pergia capensis	Marmeet	*Eriophyes* spp. (6)
Shade trees	Elfakihashim, Shambat and Omdwanban	Ziziphus spina christi	Sidir	*Eriophyes* spp. (1)
		Azadirachta indica	Neem	
		Salvadora persica	Arrak	*Acalitus hassani* (K.)
		Balanites aegyptiaca	Heglig	*Aceria balanites* (Massee)
		Terminalia brazilia	Brazilia	
		Tamarindus indica	Aradeib	*Eriphyes* spp. (2)
Shade trees	Elfakihashim, Shambat	*Acacia seyal* var. *fIstula*	Saffar abeyad	
	and Omdwanban	*Acacia albia*	Harazz	*Eriphyes* spp. (3)
		Acacia seyal var. *seyal*	Talha	*Eriphyes* spp. (4)
		Acacia gerrardii	Sangam	
		Acacia derpanolabium	Saffar azrag	
		Acacia tortilis var. *raddiana*	Seyal	*Eriphyes* spp. (5)
Fruit trees	Toti lsland, Shambat and Elgayli	Mangifera indica	Mango	*Cisberoptus Kenyae* (K.)
		Citrus sinensis	Orange	
		Citrus aurantium	Rough-lemon	
		Citrus paradisi	Group-fruit	
	Shambat	*Vitis* sp.	Grape	*Colomerus vitis*
Ornmentals	Shambat	Ficus nitida	Ficus	
		Rosa poinsettia	Rose	
		Nerium oleander	Nerium	

3.1.2 Eriophyes Species (1) on Sidir *Ziziphus spina christi*

Mites form galls varying from small to large size with different colours ranging from greenish-yellow to reddish-brown, lobed tuberculate and usually crowded on the branches. Microscopic examinations indicated that mites moved constantly into the crevices of tuberculated galls. Old branches have severe infestation, later when they dry, their colour change into a dark brown. They can be easily crushed.

3.1.3 *Acalitus hassani* (Keifer) on Arrak *Salvadora persica*

This mite attacked leaves, flower buds, fruits and growing tips of the plants. The pattern of infested plants generally shows erineum pockets on the lower leaves surfaces with hairy outgrowths within which mites concealing themselves. The appearances of heavily infested young leaves are marked by

longitudinal down-ward rolling. Behavioural observations showed that, mites preferred living on the young growths to old infested plant parts.

3.1.4 Eriophyes Species on Aradeib *Tamarindus indica*

The leaf-let showed pale and rolled up longitudinally from either side towards the mid-rib showing pod-like structure. On the newly examined infested leaves, swollen greenish yellow spongy hairy leaf-tissues were noted, and among these dense hairs mites commonly live. In severe infestations mite's, frass and cast skins appear as specks scattered among the leaf-hairs.

3.1.5 *Eriophyes tulipae* (K.), on Garlic Bulbs *Allium sativum*

In the stored garlic bulbs, mites are usually found under the surface layers, and due to the effect of feeding brownish sunken spots appeared. Severely infested cloves showing mite's cast skins on the infested area was observed. Also symptoms appear on garlic grown in the field. Due to the effect of mite feeding, the leaf rolled longitudinally and mites lived and protected themselves inside these rolled leaf. The infested foliage were stunted, twisted and curled showing mosaic colour similar to that produce by a virus. In severe infested plants, clumps of interloop leaves were produced showing broom symptoms.

3.1.6 *Eriophyes tulipae* (K.), on Onion Bulbs *Allium cepa*

Mites are principally found feeding on the green foliage, they live vagrant. In severe infestations, light yellow streaks appear on the leaf-surface and the plant's development slow-down. In store, the mites were found in dorsal and ventral sides of the leaf-base and surface. In heavy infestation, the leaf-surface appears light brown and leaves desiccated.

3.1.7 Eriophyes species 3, 4 and 5 on Harazz *Acacia albida*, Talha *Acacia seyal* var. *seyal*, and Seyal *Acacia tortilis* var. *Raddiana*

On infested Acacia trees, the axillary buds turn into lobed greenish grey irregular shaped galls with different sizes, and the mites live inside these galls.

3.1.8 *Cisaberoptus kenyae* (Keifer) on Mango Leaves *Mangifera indica*

The mites infested the upper leaf-surface mainly, the severe infested upper leaves-surface covered with silvery sheath. The latter make shelter to the mite colonies, while on the newly formed leaves symptoms were noted.

3.1.9 Eriophyes Species on Marmeet *Pergia capensis*

The young leaves are more infested than old ones. The infestations appeared as small galls advanced with the development of infestation. The abnormal leaves showed whitish-yellow to white colour. Spong hairy leaf-tissues with cast skin and frass were noted under a binocular microscope examination. In severe infestations, the plant leaves seemed to be yellow and fell down later.

3.1.10 *Colomerus vitis* on Grape Vine *Vitis* sp.

Feeding by mites on leaf surfaces resulted in a abnormal proliferation of the surface hairs, with the production of a felt-like mass called an erineum. The mites shelter within this growth. Leaves and tip of stems were desiccated, discoloured and fell down. In advanced infestation, the plant leaves showed a virus-like symptom.

3.2 Eriophyid Mites Host-Range

The results obtained are summarized in Table 2.

4. Discussion

In this study, more than 45 different vegetable, field crops, fruits, shade trees and weeds belonging to different families were examined for Eriophyes mites infestations. Five Eriophyes species, *Aceria balanites* (Massee), *Cisaberoptus kenyae* (K.), *Acalitus hassani* (K.), *Eriophyes tulipae* (K.) and *Colomerus vitis* were identified on *Balanites aegyptiaca* (heglig), *Mangifera indica* (mango), *Salvadora persica* (arrak), and *Allium sativum* (garlic), *Allium cepa* (onion) and Grape *Vitis* sp**.** respectively. The infestation on grape vine was recorded for the first time in the Sudan.

It is evident that Eriophyes species invade several

Table 2 The response of various plant species infested with *Cisaberoptus kenyae* (K.), *Acalitus hassani* (K.) and *Eriophyes tulipae* (K.), measured by infestations.

Plant family	Common name	Eriophyes mite species		
		Cisaberoptus kenyae (K.)	*Acalitus hassani* (K.)	*Eriophyes tulipae* (K.)
Malvaceae	Okra	0	0	0
Portulaceae	Purslane	0	0	0
	Lokh	+	0	0
Gramineae	Wheat	0	0	+
	Sorghum	0	0	++
Tiliaceae	Jew'smalow	0	0	0
Solanaceae	Tomato	++	++	0
	Hot-pepper	0	0	0
Fabaceae	Cow-pea	0	0	0
	Broad bean	0	0	0
Liliaceae	Onion	0	0	+++
	Garlic	0	0	+++

+ + + Severe infestation; + + Moderate infestation; + Light infestation; 0 no visible symptoms.

plants species such as *Pergia capensis* (marmeet), *Ziziphus spina christi* (sidir), *Tamarindus indica* (aradeib), *Acacia tortilis* var-*radiana* (seyal), *Acacia seyal* var. *seyal* (talha), and *Acacia albida* (harazz) (Table 1).

The species of Eriophyes mites mentioned above were not fully identified and described. Elhag [13] sent some specimens from *Tamarindus indica* (aradeib), *Ziziphus spina christi* (sidir), and *Acacia tortilis* var-*raddiana* (seyal), to stored product institute, Georgia U.S.A for description and identification. It is believed that they are newly recorded species of Eriophyid, but its description and nomenclature, till the end of this study, remain to be reported.

The close observations and examination of the symptoms produced by various Eriophyes mite species on their host-plants reflected the pattern and degree of severity of damage caused by this group of Phytophagous mites. In general, the result obtained in this study our findings are to a great extent in agreement with the findings of Refs. [1], [13] and [14], as far as the signs of damage and habits of mites are concerned. They stated that all eriophyids were essentially parasites of perennial plants.

Slykhuis [15] noticed that eriophyid mites had intimate and highly specific host relations. Some species parasitized only certain species of one plant genus, others preferred hosts in several genera. However, rare information was available to support this argument.

In the present investigation, *Eriophyes tulipae* (K.) was recorded on cultivated garlic in the glass house of the Faculty of Agriculture in Shambat. Infested plants foliage showed virus-like damage, stunting, twisting, curling and light yellow leaf streak. Those of Smalley [16] who pointed out that many fields of garlic were associated with infestation by Eriophyes tulipae (K.) support the above findings. Ahmed et al. [17] found that early symptoms appear on garlic plants included twisting, curling, stunting and dwarfing of the plant. Wahba [18] reported the infestation of stored garlic bulbs with *Eriophyes tulipae* (K.). He also noticed virus-like symptoms on the foliage.

The results obtained in this study indicated that the response of the representatives of different plant families to various mite species was different. The rate of response among susceptible plants as measured by the symptoms was also different. Tomato plants were found to be susceptible and moderately infested with *Cisaberoptus kenyae* (K.) and *Acalitus hassani* (K.). These two mites did not produce visible symptoms on the other plant species except *Cisaberoptus kenyae* (K.) on lokh where light symptoms appeared (Table 2). In this work, *Eriophyes tulipae* (K.) was found to infest onion, garlic, sorghum and wheat only, although with different rates. The degree of infestation on garlic and onion was severe, but for sorghum and wheat it was moderate and light.

These results are not different from Anderson [19] who found that the tomato russet mite *Vasates lycopersici* (Massee), developed on various solanaceous plants and some other hosts. It has been mentioned that *Eriophyes tulipae* (K.) infested a large number of plants belonging to the families Liliaceae

and Gramineae [20-22]. Connin [23] reported that *Aceria tulipae* (K.) infested wheat, maize, corn, sorghum and various kinds of grasses. He concluded that wheat and barley are the best hosts. Eriophyid mites of genus Eriophyes (K.) collected from sorghum, onion and garlic bulbs, reproduced and increased in numbers on leaves [13].

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Journal of Agricultural Science and Technology A 1 (2011) 292-302
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Risk Management Strategies of Vulnerable Rural Households in Southeast Asia: A Case Study from Vietnam

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Received: July 27, 2010 / Published: June 20, 2011.

Abstract: In the Uplands of Southeast Asia, poor and near-poor farm households endure considerable livelihood vulnerability. Access to formal insurance services is scarce. Rural farm households in mountainous Northern Vietnam have developed alternative risk management strategies. This article investigates the theoretical links between poverty, vulnerability and risk. The concept of vulnerability to poverty lays the analytical framework. Based on empirical evidence from more than 200 ethnic minority households, major risks and risk management strategies are presented and analyzed. Results suggest that households suffer from limited endowment with and access to capital assets and service institutions. Human and economic risks (e.g. illness of family members and loss of livestock) were identified as the main components affecting rural livelihoods. Constrained access to adequate risk management strategies increase household's vulnerability, drowning them more and more in poverty. Major policy implications are that anti-poverty programs should focus on a broader target group, the currently poor as well as the vulnerable households.

Key words: Risk management, vulnerability, Southeast Asia, Vietnam.

1. Introduction

Subsistence farmers in developing countries face many types of dangers in their everyday life. With low livelihood resilience at the best of times, unmitigated income and consumption shocks can have devastating consequences [1]. In mountainous Northern Vietnam, poor and near-poor farm households endure manifold risks and income shocks, which threaten their existence. Normally, insurance systems would step in to assist. In developing countries however, where access to formal insurance services is hardly available, rural farm households have developed alternative risk management strategies. The better-off households might have access to so-called (ex-ante) adaptive risk management strategies. The accumulation of savings in cash or kind counts as such an adaptive risk management strategy. Poorer households have to rely primarily on (ex-post) risk coping strategies, for instance the sale of livestock. In contrast to risk management strategies, risk coping strategies, however, enhance the long-term level of vulnerability.

According to Ligon and Schlechter [2], economists have long used poverty measures to describe the well-being of less fortunate households. Meanwhile, it is obvious that a household's well-being depends not just on its average income or expenditures, but that risk plays an important role, too. While the concept of risk has been extensively considered by the scholarship, it has often remained on the periphery in the design of anti-poverty policies. Hence, in order to better understand the highly diverse livelihood strategies of

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vulnerable rural farm households, this article investigates the theoretical links between poverty, vulnerability and risk. The concept of vulnerability to poverty serves as analytical framework. Focusing on the concept of vulnerability and its measurement, there are numerous dimensions, definitions and methods available since it's emerging in the course of the 1980s. However, there is no consensus yet on a universal theoretical framework. Up to date, the implementation of many theoretical suggestions and policy recommendations is largely impossible due to the absence of intertemporal data on shocks, risks, their determinants, and possible coping mechanisms at the household level. These issues have been incorporated in our empirical research. Major risks and shocks that rural ethnic minority households face in Northern Vietnam will be analyzed. In addition, the applied risk management strategies will be presented.

The article is organized as follows: The economic situation in Vietnam, focusing on the rural Northern Uplands will be summarized in Chapter 2. Thereafter, a brief conceptual overview will be given in Chapter 3, clarifying the links between the concepts of poverty and vulnerability. The risk management strategies of ethnic minority households will be analyzed and presented in Chapter 4. Chapter 5 concludes.

2. Economic Situation in Vietnam

Despite the well-known achievements of the *doi moi* reform process[1], which was launched in 1986, almost one third (28.9% in 2007) of Vietnam's total population (85 million in 2007) is still living below the national poverty line [4]. Especially the mountainous, rural areas of Northern Vietnam, which are mainly populated by ethnic minorities[2] (e.g. Black Thai, Tay, Hmong, etc.) are underdeveloped. Following Minot and Baulch [9] as well as Gaiha et al. [10], "poverty rates, which decreased in urban areas, remained much higher in rural areas. Especially in high mountain areas, poverty is a major problem of ethnic minorities (in 2002, 69% of ethnic minorities were poor, in contrast to 23% of the Kinh [Vietnamese] and Chinese)".

Vietnam has made good progress concerning the implementation of the United Nations' Millennium Development Goals (MDGs). Nevertheless, regional disparities are still apparent. According to United Nations Vietnam [11], poverty levels are already meeting the standards. Nevertheless, there are still many challenges, including the reduction of child mortality as well as the improvement of maternal healthcare and the quality of primary education. As "it is estimated that 20 million children (59% of all children) still lack access to proper sanitation", major improvements are necessary, particularly in remote and ethnic minority areas. Even though Vietnam is well on its way to achieving its goals to promote gender equality in primary and secondary education, "women continue to face serious obstacles-including poverty, limited access to higher education, and employment opportunities, as well as persistent discriminatory attitudes and behaviour" [11].

Focusing on the Northern Uplands[3], it may be summarized that farming remains the most important economic sector and the population in this region is increasing fast. To date, the natural resources have been depleting as well as the natural environment has been degrading seriously due to unsustainable expansion of agriculture on sloping lands. Once again, the success of the agricultural reforms in the Deltas had little impact on the Northern Uplands. Currently, the Northern Uplands remains the poorest regions in the country. With the recent enforcement of forest protection polices, poor ethnic minority farmers are in

[1] According to the World Bank [3], Vietnam's average annual real GDP growth was 7.3% and per capita income grew by 6.2% (1995-2005). The economy has proven resilient to shocks and negative impacts from SARS, avian influenza, poor weather, high commodity prices, inflation, and anti-dumping suits. In US dollar terms, income per capita rose from US$260 in 1995 to a 2007 level of US$835.

[2] For more detailed information concerning ethnic minorities in Mainland Southeast Asia please refer to ADB [5], Friederichsen and Neef [6], Michaud [7] as well as Rambo and Jamieson [8].

[3] The next paragraphs are based on World Bank [12].

a more difficult situation because (additional) incomes from harvesting natural forests are no longer available.

The governmental infrastructure programs (i.e. the Program 135) have increased accessibility but also increased migration to the regions and widened the gaps between the rich and the poor and among different ethnic groups. Infrastructure development alone is not sufficient to address the poverty and restoration of the degraded environment. If the current trends continue, there are high risks that poverty will persist while the increased population will further put pressure on the environment. In addition, the vulnerable situation of rural households in Northern Vietnam is intensified by the lack of a formal agricultural or more general rural insurance markets [13] and by a non-functioning or non existent social welfare system. For instance, health insurance[4] is limited to a few groups [16]. All other households have to rely on sub-optimal coping strategies in the event of a crisis, dependent on their endowment with and access to resources and capital assets.

Admittedly, state interventions are common during covariate shocks. For instance, in the event of a natural calamity such as a flood or a drought, the State Bank of Vietnam can postpone or terminate the term of payment of formal loans disbursed through government schemes or through the state owned banks [17]. However, in the incidence of idiosyncratic income shocks, rural households in Vietnam have to rely mainly on informal mutual aid schemes within their social networks to cope with shocks and to reduce their risk. Usually, immediate and extended kin provide material and financial help and/or inexpensive farm labour. This is in line with the findings of Dalton et al. [18], Rosenzweig and Binswanger [19] and others who showed that the family plays a vital role within the social risk-sharing networks as well as in daily social life.

3. Poverty and Vulnerability

According to van Dillen [20], "the term 'vulnerability' found its way into common use in the social sciences in the course of the 1980s". Chambers [21] defines that it "refers to exposure to contingencies and stress, which is defencelessness, meaning a lack of means to cope without damaging loss". Extensive literature review[5] on 'vulnerability' reveals that it "has diverse but related meanings in different academic disciplines. In the social sciences in general, and in economics in particular, vulnerability is perceived as the existence and the extent of a threat of poverty and destitution [23]. In the natural sciences, in general, and environmental sciences and geography in particular, vulnerability refers to the susceptibility of a household or community to the impact of natural hazards or climate change" [27].

Following Dercon [28], "economic vulnerability refers to risks faced by households and/or communities arising from exogenous shocks to systems of production, distribution and consumption. In the economics literature, however, this is referred to as "vulnerability to poverty". One of the most important components in the concept of vulnerability to poverty is 'risk'. The term 'risk' is defined as potentially dangerous event that is likely to cause a loss in individual and/or household welfare when it occurs (Chaudhuri, Jalan and Suryahadi [29], Dercon [30], Harrower and Hoddinott [31]). In the same vain,

[4] Even if people possess health insurance cards, they usually face additional expenses including costs for transport, special treatment and medication. People staying in a hospital must cover the expenses for a second family member accompanying the sick to provide for the basic needs of the sick and themselves. For more detailed information concerning health and access to social security in Vietnam, please refer to Fischer and Salehin [14] and Tran [15].

[5] Alwang, Siegel and Jorgensen [22] introduce different definitions, discuss various views and provide important references. More recently, Dercon [23] discuss on risk, vulnerability and its links with poverty. Gaiha, Katsushi and Imai [24] focus on vulnerability and poverty dynamics in Vietnam. Makoka [25] reviews theoretical and empirical literature on vulnerability to poverty, risk management strategies and consumption smoothing. Shakya [26] provides a detailed review of "Theoretical Approaches to Vulnerability in the Context of Development Research".

a 'shock' is defined as an actual occurrence of a risk." Major risks and shocks, which affect vulnerable livelihoods of ethnic minority farm households in Northern Vietnam, will be presented in the next chapter, after a brief conceptual overview of poverty, vulnerability and vulnerability to poverty.

Poverty and vulnerability are closely interlinked and while poverty is usually defined as economic deprivation (lack of income), vulnerability entails "the relationship between poverty, risk and efforts to manage risk" [32]. Households may not be poor at present. They may be, however, vulnerable-to-poverty in the future. Poor households without potential to escape poverty are also characterized as vulnerable [33]. Moreover, poverty is a static and vulnerability a dynamic concept. While the poor can be quantified relatively easily ex-post despite the many dimensions of poverty (absolute poverty with regard to food consumption, housing etc. and relative poverty with regard to income), quantification of the vulnerable is much more difficult due to the dynamic and ex-ante perspective. Nevertheless, a thorough understanding of the characteristics and priorities of the poor and vulnerable is crucial to formulate effective strategies for reducing the share of those who are currently poor and will remain in poverty and those of the vulnerable non-poor [29, 34].

Like poverty, vulnerability is a multi-dimensional concept, based on both, monetary (relative) and non-monetary (absolute) indicators[6]. The specification of the future over which shortfall in welfare could be, represents one of the major differences between poverty and vulnerability. The specification of the period over which to measure vulnerability affects the level and magnitude of vulnerability. The longer the period, the higher is the probability of a household falling under a certain poverty-line [38]. The concept of vulnerability is thus forward looking: Vulnerability is seen as a certain probability that a household would find itself poor in the future or that a household that is currently poor will remain in poverty in the future [29, 33]. Contrary to the ex-post concept of poverty, vulnerability is not directly observable as it is an ex-ante concept. The seminal review of poverty and vulnerability by Alwang, Siegel and Jorgensen [22] shed substantial light on the poverty-to-vulnerability issue.[7] By way of summary, poverty and vulnerability to poverty are two sides of a coin. The observed poverty status of a household (defined as whether or not the consumption expenditures are above or below a given poverty-line) is the ex-post realization of a state; the ex-ante probability of which can be taken to be the household's level of vulnerability [29].

According to Gaiha, Katsushi and Imai [39], "there has been a surge of interest in measuring vulnerability in developing countries (e.g. Chaudhuri, Jalan and Suryahadi [29], Dercon [23], Gaiha and Imai [40 and 23], Hoddinott and Quisumbing [41, 42], Ligon [43], Ligon and Schechter [2])." All of these studies suggest developing special anti-poverty policies to address vulnerability in remote rural areas, where risks are boosted by lack of formal insurance, credit market imperfections, and weak infrastructure. "Low income households and/or those living in remote areas are also subject to idiosyncratic risks arising from morbidity, dependence on a single adult male for much of household income and exclusion from community networks of support" [44].

4. Risk Management Strategies of Ethnic Minority Households

As mentioned above, Vietnam has experienced a remarkable reduction in poverty in recent years. However, it does not necessarily imply that the reduction is durable. In fact, there are fears that the pace

[6] Poverty and vulnerability can be assessed through monetary parameters (for instance income and consumption expenditures), or non-monetary dimensions, such as food consumption, education, anthropometry and mortality (Deaton [35], Sahn and Stiffel [36], Baulch and Masset [37]).

[7] They comprehensively reviewed the economics, sociology, and environmental literature, the so-called sustainable livelihood, food security and nutritional, and disaster management literature.

of poverty reduction is slowing down or even reverse [45]. According to Dercon [46], "despite the fact that households actively try to manage risk, shocks affect them, and at best, the evidence suggests only partial smoothing of welfare and nutrition. Assets, and more in general, households" livelihoods and their ability to generate future income is affected, in part due to the necessity to cope with shocks, so that assets are sold-off, or, more directly, the asset base is often instantly affected by the shocks–such as death of livestock or a loss of human capital due to illness or temporary poor nutrition." In rural livelihood systems, the household consumption is inseparable from the agricultural production activities. Literature review on risk and vulnerability indicates that rural households in developing countries are usually affected by multiple shocks (e.g. Christiaensen and Sarris [47], Dercon [48], Hoddinott and Quisumbing [41, 42], Makoka [25]).

5. Data Collection

Quantitative and qualitative field research took place in ten villages, in Ba Be and Pac Nam districts in Bac Kan province, as well as in Yen Chau district in Son La province (2004-05). Both provinces are located in the mountainous regions of Northern Vietnam and belong to the poorest provinces of the country. Data concerning vulnerability and risk management of ethnic minority farm households were collected at different administrative levels in both provinces. Key informant interviews with officials of so-called mass organizations (e.g., the farmers union and the women's union) and political cadre at the commune and district level provided general information and gave hints on common risks that rural households usually face. Special focus was laid on difficulties concerning livestock as well as information on access to public services (e.g., finance, extension, education, health care, etc.) and differences concerning the wealth strata[8] of rural livelihoods.

At the village level, general household interviews with a structured questionnaire[9] were conducted among 203 households with 670 adult[10] household members. Beside collecting basic data on demographic characteristics, asset endowment and livestock ownership, special emphasis was laid on financial transactions (effective demand and supply for savings, credit and insurance services) and social networks as well as on experienced and expected difficulties and applied risk management strategies of the interviewee's household[11]. Hence, the respondents were asked to name those shocks/difficulties, which severely influenced the livelihoods of their households during the last year and the last two to five years respectively. The majority of the surveyed households experienced multiple shocks in both periods. Only a few households did not have to endure any difficulties during the recall period. In addition, the respondents were asked to name future risks that they fear might affect their household, as well as risk management strategies they have previously used and those, which they intend to apply in future. Furthermore, 44 male and female respondents joined complementary, visualized participatory rural appraisal (PRA) sessions.

6. Results and Discussion

Own empirical evidence (Table 1) confirms existing data concerning major shocks and risks. As anticipated, death of livestock and sickness of household members (working and non-working) were named as last years' top two livelihood shocks. The surpassing high percentage of livestock loss might be explained by very cold weather during winter and the outbreak of the Avian Influenza. Surprisingly, the

[8] In Vietnam, the Government classifies households once a year according to their living standard into one of five wealth classes: 'hungry', 'poor', 'average', 'better-off', or 'rich'. The ranking is based on the household's monthly cash income or rather in terms of rice in kg/month/person.

[9] No multiple choice questions, respondents answered questions openly.

[10] Including all family members aged 18 and older. The dependency ration (including those under the age of 15 and over the age of 64) of all households is 59%.

[11] For more details concerning the demand of livestock insurance and the utilization of social networks in case of a livelihood emergency, please refer to Fischer and Buchenrieder [49] and Fischer et al. [50] respectively.

Table 1 Main shocks occurring in the course of last year as well as two to five years prior to survey (in percent of households).

Shocks/Difficulties (Expenditures)	Last year					Two to five years prior to survey			
	Household classified as					Household classified as			
	Poor	Average	Better-off	Rich*	Total	Poor	Average	Better-off	Total
Death of livestock	*20*	*26*	*35*	..	*25*	2	4	7	4
Sickness of household members (working and non-working)	*17*	*13*	*13*	..	*13*	*14*	*20*	*14*	18
Production factor risks	12	11	8	*20*	11	*9*	*21*	*24*	*19*
Insufficient harvest - lack of food	7	9	5	7	9	*12*	8	3	8
Replacement of dead livestock	9	11	5		9	*12*	6	0	6
Rebuild house and repair damages	12	7	5	*13*	8	*33*	*18*	*17*	*21*
Expenditures for ceremonies	5	5	3	7	4	7	4	7	5
Crop loss (due to bad weather)	4	3	3	..	3	2	4	7	4

Source: Own data. Multiple answers were possible, thus percentage may not sum up to 100. * None of the "rich" households experienced any difficulties during the past two to five years prior to this survey.

figures for the previous period (two to five years prior to the survey) are different from the last-year-recall.

Obviously, most of the respondents remembered to have spent a lot of money to rebuild the house and repair damages (mainly caused by storm) as well as they faced production factor risks, including lack of capital, manpower, land.

According to Makoka [51], research "shows that wealthier households experience as many shocks as poorer households. However, the type of shocks that poor households face is often different from those of wealthier households". Overall, own empirical research confirmed this statement. Focusing on poor households, the top two difficulties during the last two to five years were expenditures to rebuild the house and repair damages (33%) and sickness of household members (14%). Furthermore, they were seriously affected by lack of food (12%) and expenditures to replace dead livestock (12%). At the same time, both, the average (21%) and the better-off (24%) households stated, that production factor risks are most prevalent. While sickness among household members is the second most common difficulty for the average households (20%), it is only ranked third (14%) for the better-off households. Vice versa, expenditures to rebuild the house and repair damages are second most frequent (17%) for the better-off and ranked third (18%) for the average households. Finally, focusing on last year, only the rich households' difficulties vary from the other wealth groups and the total results.

Next, we explore the risk management strategies of ethnic minority households in Northern Vietnam (Table 2). Following Makoka [52], there is scholarly evidence that households living in risky environments devise strategies to deal with the risk both before the shock occurs (ex-ante risk management) and after the shock has manifested itself (ex-post coping strategies) (cf. Alderman and Paxson [53], Dercon [48], Holzmann [54]).

The analysis of own empirical data revealed that accumulation of savings is so-far the only adaptive strategy that is applied by ethnic minority farmers in Northern Vietnam. Almost half (46%) of the surveyed households are saving. On average, savings are kept at home in cash (39%), in kind, either in crops (12%) or in livestock (43%) or both, in cash and kind (5%). Once again, the preferred savings behaviour varies among respondents of different wealth groups. While the majority of the poor (47%) and the average (49%) households prefer to save in kind (in livestock), the better-off (80%) and the rich (57%) households prefer to save in cash. Only one respondent mentioned to save in the bank, due to safety reasons. More than half

Table 2 Risk management strategies applied in the last year and two to five years prior to survey (in percent of households).

Risk management strategies	Last year					Two to five years prior to survey			
	Household classified as					Household classified as			
	Poor	Average	Better-Off	Rich*	Total	Poor	Average	Better-Off	Total
Adaptive *(ex-ante):*									
Withdrawal of savings/ Divestment	6	5	25	33	8	7	6	14	7
Coping *(ex-post):*									
Credit total					25				32
Credit from bank (formal)	9	13	3	20	11	21	13	14	15
Credit from family/relatives (informal)	17	7	..	..	8	21	12	7	13
Credit from other sources	9	5	3	13	6	9	2	3	4
Sale of crops/livestock total					31				33
Sale of crops	19	12	18	20	14	12	9	3	9
Sale of livestock	5	18	18	..	15	9	18	28	18
Sale of crops & livestock	1	2	3	7	2	0	9	3	6
Others	12	8	..	..	8	9	8	10	9
No strategies applied	*22*	*30*	*33*	*7*	*28*	*12*	*23*	*17*	*20*

Source: Own data. Percentage is calculated for applied strategies as well as a 'no strategies applied' option. Households that provided no information are not included in this table, thus N = 199.

of the respondents (57%), who mentioned to save financially, argued that the ability to cover expenses in case of an emergency, especially health problems, is the main motivation. Another 10% stated explicitly that the savings will be used to pay for medicine and hospital visits. While 7% will use their savings to buy food, 6% will spend it on education or livestock respectively. The other respondents are saving money to invest in consumer goods (5%) or spend it on inputs, livestock and consumer goods (4%). Finally, some households save to cover expenses for ceremonies (2%), construction/repair of houses/storages (1%) or other purposes (2%).

Usually, one of the most common risk management strategies in developing countries is income diversification. However, in the mountainous regions of Northern Vietnam, the members of the different ethnic minority groups only have limited options for income diversification. Hence, most households have to rely primarily on (ex-post) risk coping strategies, which enhance the long-term level of vulnerability. In the research area, the most common coping strategy is the sale of livestock (Table 2). While wealthier households are usually capable of covering high expenditures by selling big ruminants (i.e. cows or buffalo), poor households only possess some pigs or chicken, which can be sold in the case of a livelihood emergency. Empirical research revealed that revenues from selling cash crops, which are also mentioned as one of the main coping strategies in the last year, are often significantly reduced by debt-service payments (e.g. for inputs, rice for consumption) to traders and shops. The remaining money is mainly spent on school fees or on consumer goods. Once again, poor households are worse off, as they normally have less cropping area and higher debts (especially from buying rice to compensate the household's lack of food).

Further common strategies include formal loans from banks and informal credits from relatives and friends. The later are crucial for poor households that have no/not sufficient access to formal loans. Own research confirms findings from Barslund and Tarp [55] that formal loans in Vietnam are almost entirely for production and asset accumulation. Although the issue of the fungiblity of money might be raised, and the validity of some of the data might be questioned

(farmers are usually requested to sign a statement to the effect that the loan will be used for productive purposes only), a good share of formally borrowed money is used for productive purposes compared to other countries. Up to date, there are no formal loans available for healthcare or hospital visits. A complex administrative system and language difficulties are further constraints faced particularly by members of ethnic minority groups-especially women. Moneylenders play only a small role in the informal financial sector of rural Vietnam as most loans are given by relatives or friends and are interest-free. The main reasons why formal finance is rarely used to ease shocks, however, is that it takes time to apply for a loan and households are locally screened; any income or consumption shocks may be reported to the relevant credit officer and the credit is consequently denied.

As formal safety nets to balance arising shocks are not accessible or simply non-existent, alternative strategies must be adopted. The formation, maintenance and use of social networks is one of the most important risk-management strategies of vulnerable households in Northern Vietnam. Own research [50] revealed that these networks are able to provide basic support (e.g., by providing informal loans for relatives), but do not suffice to buffer a major crisis completely. Thus, the households are still forced to sell assets, primarily livestock, in the event of a livelihood emergency. The situation becomes even more acute where a household loses a debt-financed animal, which immediately increases the household's vulnerability, substantially limits its long-term livelihood strategies and very often directly consolidates poverty or makes them slip into poverty. As a consequence, poor households will remain vulnerable to shocks despite the presence of an informal insurance system.

Finally, focusing on the 'no strategies applied' option, own research suggests that on average, 20-30% of all shocks could not be mitigated. During the last two to five years prior to this survey, major difficulties - where no coping strategies were available-include lack of capital (29%), lack of food (24%) and loss of livestock (17%). During the year immediately prior to the survey, as many as 73% of all households were not able to cope with livestock loss and lack of capital (8%).

7. Conclusion and Policy Recommendations

Despite the well-known achievements of the doi moi reform process and the (partially) successful implementation of the MDGs, Vietnam's Northern Uplands are still severely underdeveloped. Research results suggest that limited endowment with and access to capital assets and service institutions, as well as human and economic risks are the main components affecting rural livelihoods. Poor and near-poor ethnic minority farm households endure considerable livelihood vulnerability due to various income shocks. To buffer these shocks, households apply different risk-management strategies. While better-off households often have access to so-called (ex-ante) adaptive risk management strategies, poorer households have to rely primarily on (ex-post) risk coping strategies, which enhance their long-term level of vulnerability. As formal safety nets are not accessible or simply non-existent, alternative strategies must be adopted. Besides 'dissaving', the formation and maintenance of social networks is currently one of the most important adaptive risk-management strategies. Focusing on risk coping strategies, households of all wealth strata mainly rely on sale of livestock and sale of crops, especially maize. Both strategies are disadvantageous for poorer households that lack sufficient capital assets. Usually, they neither possess adequate physical capital (e.g. livestock) nor natural resources (land).

Research results point to a number of policy issues that need to be addressed if household vulnerability to poverty is to be significantly reduced among ethnic minority households in Northern Vietnam. First of all, poverty reduction strategies and programmes need to consider a broader target group, not only the currently

poor but also those at risk of being poor in the future. According to Dercon [56], there is scope for assisting the poor in protecting themselves, either by promoting more self-insurance via savings or by supporting micro-credit. Key problems with existing self-insurance via assets is that they tend to be risky and may well be strongly covariate with incomes, limiting their effectiveness, while financial savings products are typically not tailored to the poor, offering low or negative returns, and involving prohibitive transactions costs.

Furthermore, own research revealed that the combination of credit and insurance, especially loans that are taken up to purchase livestock, might help rural farm households to decrease their vulnerability and save them from slipping into poverty. Such a scheme would nevertheless only help those households that were able to get the credit in the first place, thus excluding the poorest of the poor. These households can only be reached by means of a general social welfare scheme. To date, however, no functioning rural social security schemes exist in Northern Vietnam. It is assumed that an efficient and accessible health care system would be an important alternative for securing livelihoods, as the majority of the interviewed households had problems with high cost of illness treatment. In addition, improved extension services and knowledge transfer for all people, especially women, could support a sustainable future development of ethnic minority households and therefore, in the long-run, lead to poverty alleviation.

Acknowledgments

The research for this article was carried out within the framework of the German-Thai-Vietnamese Collaborative Research Centre ‘Sustainable Land Use and Rural Development in Mountainous Regions of Southeast Asia’ (SFB 564), also known as The Uplands Program. Funding from the Deutsche Forschungs gemeinschaft (DFG) is gratefully acknowledged. All errors and omissions are the responsibility of the authors.

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Journal of Agricultural Science and Technology A
Volume 1, Number 2, June 2011

David Publishing Company
1840 Industrial Drive, Suite 160, Libertyville, IL 60048
Tel: 1-847-281-9826; Fax: 1-847-281-9855
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ISSN 2161-6256
06
9 772161 625118