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Nematode Community Structure and Efficacy of the Free-Living Nematode *Metarhabditis andrassyana* as a Toxicological Assay Organism

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ABSTRACT: A survey of agricultural fields around Yamuna river in Faridabad, Haryana was conducted to study the community structure of the soil inhabiting nematodes. A total of 32 genera belonging to 8 orders and 21 families were recorded. In terms of number of genera, order Rhabditida was most frequent. The trophic diversity index (TDI) ranged from 1.2 – 1.4 (1.32 ± 1.11), Shanon's Diversity Index (H') varied from 1.1 – 2.9 (2.70 ± 0.15) and the maturity index (MI) ranged from 1.23 – 1.45 (1.36 ± 0.23). The low MI value indicated that it is a less stable and disturbed ecosystem. The efficacy of the free-living nematode *Metarhabditis andrassyana* as a toxicological assay organism was studied using lead, mercury, cadmium and copper as test substances. Testing of the toxic effect of the four heavy metals revealed that lead was the most toxic among all tested metals.

***Keywords*:** Nematode Community Structure, Agriculture, Maturity Index, Toxicity.

The functioning of soil ecosystem is dependent on biological processes that can be disrupted by soil contamination. Therefore, it is necessary to assess the toxicity of contaminated soils on soil dwelling organisms and combined effects of chemical substances that are usually present. Free-living, nonparasitic nematodes have an important role in the soil rich being the most abundant and species-richest metazoans (Andrassy, 1992; Yeates, 1981). By evolving various feeding types, these invertebrates have been able to occupy key positions in terrestrial food webs (Yeates *et al.,* 1993), thus influencing nutrient cycling in soils (Yeates *et al.*, 1982; Ingham *et al.*, 1985; Beare, 1997). The presence of nematodes and the structure of nematode communities are, therefore, important to agricultural production and sustainability (Fiscus and Neher, 2002; Ahalavat and Chaubey, 2017).

Accordingly, nematodes are suitable ecological indicators for monitoring and assessing agricultural areas (Neher, 2001). Nematodes are emerging organism group in the field of environmental sciences (Wilson and Kakouli-Duarte, 2009), offering a variety of molecular, ecotoxicological and ecological tools for an integrated risk assessment of soils. In environmental studies, the soil-dwelling bacterivorous nematode, *Caenorhabditis elegans*, has been successfully used as a test organism for investigating complex matrices,such as soils (Donkin and Dusenbery, 1993; Freeman *et al.*, 1999; Sochova *et al.*, 2007; Höss *et al.*, 2008) and freshwater sediments (Traunspurger *et al.*, 1997; Höss *et al.*, 2001; Comber *et al.*, 2008).

The aim of this study was to study the nematode community structure to assess the role of nematodes as indicators of soil conditions and evaluate the suitability of *Metarhabditis andrassyana* as a test organism for assessing the toxicity of potentially hazardous chemicals under standardized experimental conditions and the response is compared with control which has no effect on the viability of test organism.

MATERIALS AND METHODS

Soil samples from agriculture fields near Yamuna river area in Faridabad, Haryana were collected from a depth of 0-10 cm by using a hand spade. Samples were tagged, stored in sealed plastic bags and brought to laboratory for further processing. Nematodes were

extracted from 100 cc. of soil using Cobb's (1918) modified sieving and decantation and modified Bermann's funnel techniques. All the nematodes from each extracted sample were counted and identified to genus level. Trophic groups were allocated according to Yeates *et al.* (1993) and c-p groups were assigned following Bongers (1990).

Nematode diversity was described using the Shanon's diversity index calculated at generic level ($H^{'}$). Maturity index (MI) was calculated to estimate the relative state of two ecosystems studied (Bongers, 1990). Trophic diversity was calculated by the trophic diversity index (TDI) (Heip*et al.*, 1988). The channel index (CI) was calculated to indicate predominant decomposition pathways (Ferris *et al.*, 2001). Structure index (SI) and enrichment index (EI) were calculated to determine the relative stability of the ecosystem studied (Ferris *et al.*, 2001). All indices were calculated by using MS Excel.

The free living nematode, *Metarhabditis andrassyana* was extracted from manure collected from crop field near Yamuna river and cultured on nematode growth medium (NGM) supplemented with *E.coli* OP50 bacteria as food source. All the chemicals were obtained from Thermo Fisher Scientific (Powai, Mumbai, India). The studied metals were preferred because of their dominance in the environment. Five concentrations of four metals $HgCl_2$, $Pb(NO_3)_2$, $CuCl_2$, $CdCl_2$ solutions were used in the current investigation and were used at 10, 20, 50, 100, 150 ppm concentrations. For the lethality test, fresh nematodes were used in this experiment and bioassays were carried out *in vitro* in 12-well sterile polystyrene tissue culture plates (Cat. No. TPP12, HiMedia Laboratories, Mumbai, India).

From NGM, nematodes were washed twice with double-distilled water and then 20 ± 1 worms in 1ml sterile water were transferred in each well. One ml of double strength solution of chemical was added in each well, whereas distilled water was added to the control. The experiment was independently repeated thrice. The plates were kept in a Tupperware box and incubated at 25 ± 2^0C. Nematode mortality was recorded every 3 h till 100% mortality. Mortality data were used to calculate median lethal concentrations(LC_{50}) and median lethal time (LT_{50}) values.

Frequency (N): Frequency of nematode genus (i.e. the number of samples in which the genus was present).

Mean density (D): Number of nematode specimens of the genus counted in all samples / total number of the samples collected.

Shannon's diversity (H') = Σ (*pi* ln*pi*)

Trophic Diversity index (TDI) = 1 Σpi^2

Where pi^2 is the proportional contribution of ith trophic group.

Maturity Index (MI)

$$MI = \sum_{i=1}^{n} V(i).f(i)$$

Where Vi= cp value of the ith taxon. f(i) the frequency of that taxon in a sample. *Maturity index (MI) is calculated as the weighted mean of the individual c-p value.

Channel index (CI) = 100 x 0.8 Fu2 / (3.2Ba2 + Fu2)

Enrichment index (EI) = (e/e+b) x100

Structure index(SI) = (s/s+b) x100

Where e, b & s are sum products of assigned weights and number of individuals of all genera.

RESULTS

Nematode Diversity

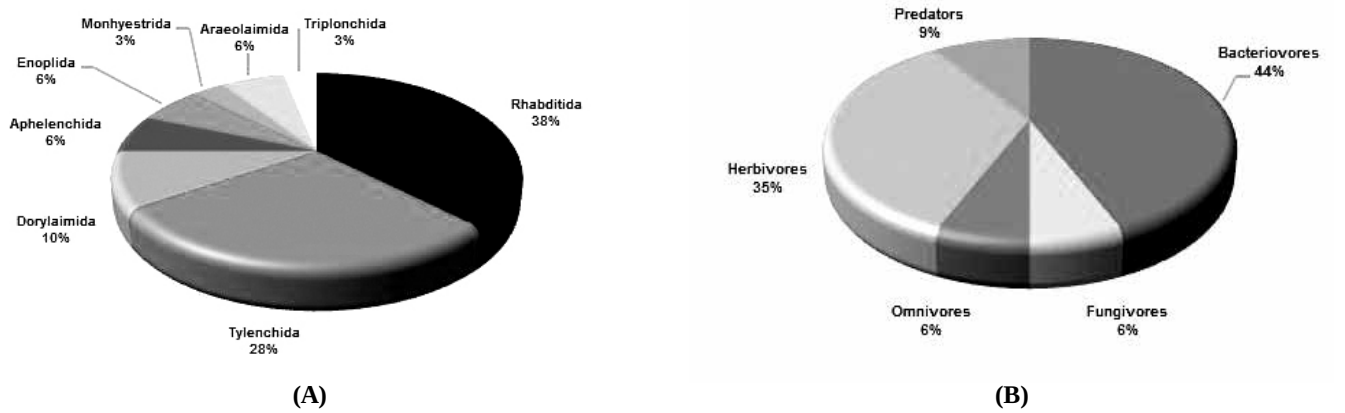
A total of 32 genera belonging to 8 orders and 21 families were recorded from the soil samples collected from agriculture fields near Yamuna river area in Faridabad, Haryana (Table 1). The number of genera varied from 3 to 11 per sample while in terms of abundance, the number varied from 142 to 1012 individuals per 100 cc of soil. *Meloidogyne* was the most abundant genus. In terms of number of genera (Fig. 1A), the Order Rhabditida was most frequent (38%) with 12 genera under 5 families, followed by Tylenchida (28%) with 9 genera under 7 families, Dorylaimida (10%) with 3

Table 1. Population structure of soil inhabiting nematodes, their mean abundance per 100 cc soil ± SD (N = 30).

Genera	c-pvalue	Order	N	Mean Abundance ± SD
Bacteriovores				
Bursilla	1	Rhabditida	4	2.6 ± 2.2
Mesorhabditis	1	Rhabditida	8	4.4 ± 5.3
Metarhabditis	1	Rhabditida	12	11.7 ± 3.2
Rhabditis	1	Rhabditida	1	0.30 ± 1.1
Acrobeles	2	Rhabditida	25	26.7 ± 21.5
Acrobeloides	2	Rhabditida	16	14.1 ± 10.1
Chiloplacus	2	Rhabditida	7	6.50 ± 7.87
Eucephalobus	2	Rhabditida	4	3.54 ± 13.7
Pseudacrobeles	2	Rhabditida	3	1.68 ± 5.98
Zeldia	2	Rhabditida	2	1.74 ± 3.78
Teratocephalus	2	Rhabditida	3	2.12 ± 2.87
Rhabdolaimus	2	Araeolaimida	2	1.34 ± 4.66
Chiloplectus	2	Araeolaimida	3	2.20 ± 3.88
Prismatolaimus	3	Monhysterida	6	5.54 ± 7.44
Fungivores				
Aphelenchoides	2	Aphelenchida	10	11.2 ± 19.40
Aphelenchus	2	Aphelenchida	12	14.9 ± 21.60
Omnivores				
Mesodorylaimus	4	Dorylaimida	4	2.1 ± 1.1
Minidorylaimus	4	Dorylaimida	3	1.7 ± 1.3
Herbivores				
Xiphinema	5	Dorylaimida	2	1.4 ± 0.7
Pratylenchus	3	Tylenchida	17	20.4 ± 12.46
Psilenchus	2	Tylenchida	3	5.50 ± 17.42
Helicotylenchus	3	Tylenchida	19	18.2 ± 21.24
Hemicriconemoides	3	Tylenchida	1	1.22 ± 3.12
Hoplolaimus	3	Tylenchida	21	20.2 ± 18.22
Meloidogyne	3	Tylenchida	27	29.5 + 22.1
Rotylenchulus	3	Tylenchida	22	20.9 ± 17.45
Tylenchorhynchus	3	Tylenchida	18	19.15 ± 19.5
Trichodorus	4	Triplonchida	2	1.40 ± 1.20
Basiria	2	Tylenchida	2	1.21 ± 3.45
Predators				
Tobrilus	3	Enoplida	3	3.2 ± 1.22
Mononchoides	1	Rhabditida	1	1.2 ± 0.4
Trypla	3	Enoplida	1	1.2 ± 4.2

genera under 2 families, Araeolaimida, Aphelenchida and Enoplida (6%) each with 2 genera under 2 families, while Monhysterida (3%) and Triplonchida (3%) were represented by 1 genus each.

In terms of trophic diversity, the bacteriovores (44%) constituted the most dominant group (Fig. 1, B) followed by herbivores (35%), predators (9%), omnivores (6%) and fungivores (6%). Among bacteriovores, *Acrobeles*



(A) **(B)**

Fig. 1. (A): Ordinal Diversity (Genera) and (B): Trophic Diversity of nematodes

was the most dominant genus while *Meloidogyne*, *Aphelenchus*, *Mesodorylaimus* and *Tobrilus* were most dominant genera among herbivores, fungivores, omnivores and predators, respectively.

Nematode Community Analysis

Shannon's diversity (H') and trophic diversity index (TDI) were calculated to assess diversity of nematode genera and trophic groups. The value of Shannon's diversity (H') was 2.70 ± 0.15 and trophic diversity index (TDI) was 1.32 ± 1.11 (Table 2). The Maturity index (MI) was calculated to assess the maturity of the agro-ecosystem and it was 1.36 ± 0.23 (Table 2). The structure index (SI) was calculated to assess the structure of the soil ecosystem. The values for SI was very low, indicating the polluted status of ecosystem (Table 2). The enrichment index (EI) gives the status of enrichment in the ecosystem due to contamination. The values of EI observed in the present study were very high at all the sites giving an idea of enriched ecosystem (Table 2). The higher values of channel index (CI) indicated a fungal dominated decomposition pathway while lower value indicated the bacteria based decomposition pathway. The values for CI in present work were mostly low (Table 2).

Table 2. Various ecological Indices for assessing the community dynamics.

Indices/Parameters	Values
Maturity Index (MI)	1.36 ± 0.23 (1.23 – 1.45)
Enrichment Index (EI)	71.65 ± 10.23 (63.21 – 73.26)
Structure Index (SI)	34.56 ± 14.24 (33.11 – 37.34)
Channel Index (CI)	16.66 ± 10.12 (10.17 – 16.98)
Trophic Diversity Index (TDI)	1.32 ± 1.11 (1.2 – 1.4)
Shannon's Diversity Index (H')	2.70 ± 0.15 (1.1 – 2.9)

Toxicological Assay

We calculated the median lethal concentration(LC_{50}) at 6 and 12h and the median lethal time(LT_{50}) for each concentration of the metal (Table 3 & 4). Only lead and mercury killed nematodes at 3h after inoculation at the lowest concentration. At later time points, mortality rate of *M. andrassyana* after treatment with various concentrations of lead, mercury, cadmium and copper were significantly different as compared with control as shown in Fig. 2 A & B. The effect of heavy metals (Hg, Pb, Cd and Cu) at 100 and 150ppm concentrations caused the severe ($p<0.05$) toxicity in nematodes as compared to control. Among these heavy metals lead was identified as the most lethal as it killed all the nematodes within 6h. At 6h, its LC_{50} was 12.65 ppm, significantly lower than all other tested metals (Fig. 2(A), Table 4). The least lethal metal was copper, showing

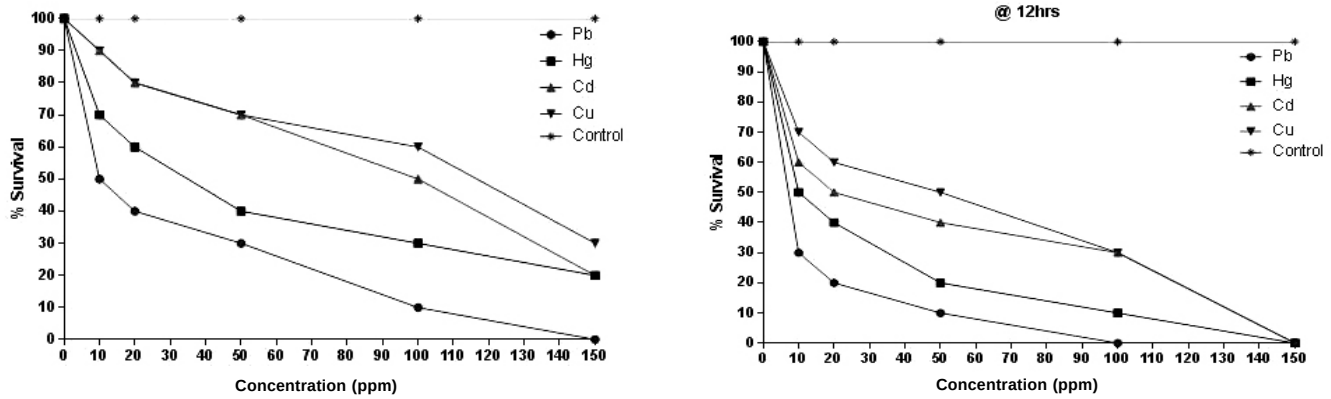


Fig. 2. Toxicity response curves of *Metarhabditis andrassyana* at (A) 6 h and (B) at 12 h to different concentrations of heavy metals

Table 3. Median lethal time (LT_{50}) values of heavy metals against the *Metarhabditis andrassyana.*

Dose (ppm)	LT_{50}			
	Cu	**Cd**	**Hg**	**Pb**
10	30	24	18	9
20	24	18	12	6
50	18	12	6	6
100	12	9	4.5	4.5
150	6	6	3	3
÷² (log-rank test)	42.97	49.45	44.87	43.40
p	< 0.0001	< 0.0001	< 0.0001	< 0.0001

higher LC_{50} value of 99.38 ppm @ 6h as compared to other metals (Fig. 2(A), Table 4).

The LT_{50} values for lead also showed that it was the quickest in killing *Metarhabditis andrassyana* with the lowest LT_{50} value of 9h at 10 ppm as compared to 30, 24 and 18h for copper, cadmium and mercury at 10 ppm (Table 3).

DISCUSSION

Soil organisms depend on each other for carbon and energy. The structure and function of below-ground food webs are disrupted by hydrocarbon and heavy-metal contaminants, mineral fertilizers and pesticides, and by physical disturbance. However, the results of such

Table 4. Median lethal concentration (LC_{50}) and LC_{90} values of heavy metals against the *Metarhabditis andrassyana*. (Numbers in parenthesis represent 95% confidence limits).

Metals	At 6h		At 12h	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Cu	12.65 (1.33 – 24.53)	96.18 (47.37 – 1499.18)	5.55 (0.001 – 12.83)	33.00 (15.34 – 853.53)
Cd	30.86 (6.21 – 71.83)	410.39 (131.46 – 482981.13)	11.95 (1.51 – 22.65)	79.03 (40.93 – 758.40)
Pb	74.81 (42.72 – 193.75)	459.78 (182.67 – 12712.86)	20.33 (3.11 – 40.40)	208.05 (84.44 – 16749.12)
Hg	99.38 (51.68 – 652.52)	892.50 (250.24 – 797020.10)	29.64 (11.98 – 54.49)	205.65 (93.94 – 2980.04)

disruptions are unpredictable because they are influenced by the heterogeneity of the soil, fluctuations in abiotic conditions, chemical and physical buffering capacity, and by other biotic and abiotic interactions (Bongers and Ferris, 1999). Because nematodes occupy key positions as primary and intermediate consumers in soil food webs, evaluation and interpretation of the abundance and function of their faunal assemblages or community structure offers an *in situ* assessment of disruptive factors. The soil environment significantly impacts on soil dwelling nematode communities. Therefore, soil nematode communities and their structural changes were found to be one of the best biological tools for assessing soil processes and plant conditions in terrestrial ecosystems (Wang *et al*., 2009; Pen-Mouratov *et al*., 2010).

Recent research indicated that simple analyses of *in situ* nematode faunae at generic level provide a wealth of information on the nature of decomposition pathways and soil nutrient status (Ettema, 1998). The analyses also indicate effects of agricultural practices and contaminants on the functioning of the soil food web (Lau *et al.*, 1997). They provide a basis for environmental management, remediation and conservation decisions. Nematodes respond differently to soil disturbance and therefore changes the nematode community composition (Gupta and Yeates, 1997; Yeates and Pattison, 2006; Ahalavat and Chaubey, 2017).

A low percentage of dorylaims (c-p 4 &5) in the crop field (10 %) clearly indicated that the soil is more disturbed as cropping always involves ploughing and/or tilling together with addition of fertilizers, organic matter and pesticides/weedicides. The dorylaims appeared to be susceptible to these activities as also shown by Ahalavat and Chaubey (2017). Hence, the sensitivity of the dorylaims is a good indicator of soil disturbance (Neher, 2001). In this study *Acrobeles* was the most abundant genus and confirms with the work of Yeates and Bongers (1999) and Gomes *et al*. (2003) where it was found that cephalobids were the most abundant bacterial feeders present in cropping systems. Shannon's diversity index ($H^{'}$) reflected diversity of nematodes in an ecosystem. Higher values of $H^{'}$ showed highly diverse ecosystem while low values showed the contrary. Hanel (1995) found $H^{'}$ in crop fields to vary between 2.66-2.83. In present work, the value of $H^{'}$ was 2.70 ± 0.15. This is in perfect agreement to earlier records where crop fields are found to be highly diverse in comparison to other ecosystems (Ahalavat and Chaubey, 2017). The MI has been used successfully as indicators for disturbances (Gorgieva *et al*., 2002; Kumar and Ahmad, 2017). Various case studies (Bongers *et al.*, 2001; Kumar and Ahmad, 2017) suggested that the MI is decreased by disturbances but increases during the colonization process. The low value of MI in present study indicated a disturbed environment due to agricultural practices.

Food web indices like EI, SI and CI provide an excellent means for studying the stability of ecosystem, weather it is stressed, enriched or structured and provide information on the dynamics of the soil food web (Ferris *et al.*, 2001). EI is generally known to reflect availability of resources to the soil food web and response of primary decomposers to the resources (Ferris *et al.*, 2004). Present study, revealed that this region was highly enriched (EI 71.65 ± 10.23). SI describes whether soil ecosystem is structured / matured (high SI) or disturbed (low SI). The value of SI during present study was low (SI 34.56 ± 14.24) that agreed with earlier studies (Ferris *et al*., 2001; Berkelmans *et al.*, 2003) which argued that low values of SI indicated disturbed conditions of the ecosystems studied. The higher values of channel index (CI) indicate a fungal dominated decomposition pathway while lower value indicated the bacteria based decomposition pathway. The values for CI in present work were mostly low, which indicated that greater participation of bacteriovores in the breakdown of soil organic matter.

The efficacy of the free-living nematode *M. andrassyana* as a toxicological assay organism was studied using lead, mercury, cadmium and copper as test substances. Testing of the toxic effect of the four heavy metals revealed that lead was the most toxic metal among all tested metals that agreed with earlier studies (Freeman *et al.*, 1999; Peredney and Williams, 2000; Dayong and Xiaojuan, 2008).

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Induction of Defence Enzymes using Bio-Agents in Tomato Infected with Root-Knot Nematode, *Meloidogyne incognita*

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ABSTRACT: An experiment was carried out in root-knot nematode infested soil (2 J_2/g soil) to assess the induction of defence enzymes PO, PPO, PAL and SOD by bio-agents against *Meloidogyne incognita* in a susceptible variety of tomato. Bio-agents *Trichoderma viride, T. harzianum, Pochonia chlamydosporia* and *Paecilomyces lilacinus* each @ 2g and 4g per kg soil and *Pseudomonas fluorescens* @ 4g/kg soil (standard check) were added to soil. Application of bio-agents increased the level of PO, PPO, PAL and SOD in tomato roots. Among all the treatments application of *T. viride* and *P. fluorescens* @ 4g/kg soil were found to be the best treatments to enhance enzymatic activity. Among enzymes the PO was found highest in tomato roots during different times of observation followed by SOD, while PPO and PAL were observed in low quantity. The enzyme activity showed gradual increase from 7 DAI onwards to 28 DAI in plant roots treated with bio-agents as compared to untreated ones. The lowest enzyme activity was recorded at 60 DAI in untreated control plant roots. *T. viride* @ 4g/kg soil was found to improve plant growth of tomato as also reducing number of galls per plant (20.50), number of egg masses per plant (14.17), number of eggs per egg mass (80.00), nematode population/200cc soil (180.33) and total nematode population (2036.00) in tomato and was at par with *P. fluorescens*.

Key words: Bio-agents, defence enzymes, root-knot nematode, tomato

Among plant parasitic nematodes, the root-knot nematodes are a severe constraint and causing major economic damage to vegetable production including tomato around the world (Anwar *et al*., 2007; Williamson and Hussey, 1996). The formation of galls by root-knot nematode grossly affect nutrient partitioning and water uptake in the host (Anwar and Van Gundy, 1993) thus affecting yield of the crop (Reddy, 1985 and Anwar and McKenry, 2012). In order to obtain effective control, nematicides are often applied at higher doses, which may be costly, uneconomical and phyto-toxic and may cause residue problems which may create ecological disturbance in the nature. However, the use of nematode resistant varieties remains the most viable option.

Plant resistance is one of the eco-friendly options for the management of nematode diseases. A series of biochemical and physical reactions occur in plants in response to root-knot nematode infection. Plants synthesize certain compounds that are toxic to root-knot nematode. Resistance is usually associated with hypersensitive reaction (HR), a rapid and localized cell death in the infected plant in response to nematode attack. Reactive oxygen species (ROS) play an important role in plant defence and during pathogen attack, levels of ROS detoxifying enzymes like peroxidase (PO) and catalase (CAT) are often suppressed in resistant plants (Klessing *et al.*, 2000). As a result, plants produce more ROS and accumulation of these components leads to HR in plant cells. For example, hydrogen peroxide plays a major role in triggering HR in incompatible interactions. Antioxidant enzymes such as PO, PAL, PPO, SOD, POD and CAT are considered to be the main protective enzymes engaged in the removal of free radicals and activated oxygen species (Blokhina *et al.*, 2003, Devi *et al.*, 2000; Chawla *et al.*, 2013). Oxidative enzymes such as PO, PAL, PPO, SOD and CAT are reported to be involved in the mechanism of disease resistance.

So far substantial work has been done on various aspects of *M. incognita* on tomato, however, there is not much information available on role of bio-agents in determining the defence enzymes against root-knot nematode, *M. incognita*, thus, the present investigation was undertaken.

MATERIAL AND METHODS

The studies were conducted at Rajasthan College of Agriculture, MPUAT, Udaipur. The experiment was laid out in pot filled with root-knot nematode infested soil having 2 J_2/g of soil obtained from the pure culture plots of Department of Nematology, RCA, Udaipur. Utmost care was taken right from sowing to till harvest of the experiment for proper growth and development of plants.

The experiment was carried out in 6" earthen pots filled with 1 kg steam sterilized soil inocultaed with *M. incognita* @ 2 J2/g soil. The inoculation of J2 was done on one month old seedling of tomato (1 seedling/pot). Talc–based formulations of *T. viride*, *T. harzianum, P. chlamydosporia* and *P. lilacinus* were added to soil each @ 2g and 4g per kg soil. Each treatment was replicated three times. Untreated and standard check (*Pseudomonas fluorescens* @ 4g/kg soil) was also maintained for comparison. So in all there were ten treatments.

Assessment of the induction of defence enzymes, peroxidase, polyphenol oxidase, phenylalanine lyase and superoxide dismutase by bio-agents against *M. incognita* in a susceptible variety of tomato was done on every 7days interval after transplanting (7, 14, 21, 28 and 60) days after transplanting. The experiments were harvested 60 days after transplanting. Observation on enzyme analysis and various growth parameters *viz*., fresh root and shoot weight, shoot and root length were recorded whereas for studying nematode infestation, the plant tissues were stained in 0.1% acid fuchsin in lacto phenol at 80ºC for 2-3 minutes (McBeth *et al.*, 1941). Then after gentle wash, roots were kept in clear lactophenol for 24 hrs and then examined under microscope for nematode infection and observation of number of galls/plant, number of egg masses/plant and number of eggs/egg mass were recorded. Final soil population/200 cc soil and total population were also calculated.

ENZYME ANALYSIS

Determination of peroxidase (PO) enzymes in tomato roots

The method proposed by Hammerschmidt *et al.* (1982) was adopted for assaying the activity of peroxidase (EC 1.11.1.7). A 20% homogenate was prepared in 0.1M phosphate buffer (pH 7.0) from the root parts of the plant. The homogenate was centrifuged at 16,000 rpm at 4°C for 15 min and the supernatant was used for the assay. The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1 per cent H_2O_2. The reaction mixture was incubated at room temperature (28 ± 2 °C). The changes in absorbance at 420 nm were recorded at 30s intervals for 3 min. The enzyme activity was expressed as changes in the absorbance min^{-1} mg^{-1} protein

Determination of polyphenol oxidase (PPO) enzymes in tomato roots

Polyphenol oxidase (EC 1.10.3.1) activity was estimated simultaneously by the method of Mayer *et al.*, (1965). The enzyme extract was prepared by homogenizing 0.5 g of plant tissue in 2.0 ml of the extraction medium 0.1M sodium phosphate buffer (pH 6.5). The homogenate was centrifuged at 16,000 rpm for 15 min at 4 °C and the supernatant was used for the assay. The reaction mixture consisted of 200 µl of the enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5). To start the reaction 200 µl of 0.01 M catechol was added and the activity was expressed as changes in absorbance at 495 nm min^{-1} mg^{-1} protein.

Determination of phenylalanine ammonia lyase (PAL) enzymes in tomato roots

The activity of phenylalanine ammonia lyase (EC 4.3.1.5) was measured following the method of Dickerson *et al*., (1984). Root samples (1g) were homogenized in 3 ml of ice cold 0.1 M sodium borate buffer, pH 7.0 containing 1.4 mM of 2-mercaptoethanol and 0.1 g of insoluble polyvinyl pyrrolidine. The extract was filtered through cheese cloth and the filtrate was centrifuged at 16,000 rpm for 15 min. The supernatant was used as enzyme source. PAL activity was determined as the rate

of conversion of L-phenylalanine to trans-cinnamic acid at 290 nM. A sample containing 0.4 ml of enzyme extract was incubated with 0.5 ml of 0.1M borate buffer, pH 8.8 and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30°C. The amount of trans-cinnamic acid synthesized was calculated using its extinction coefficient of 9630 m-1. Enzyme activity was expressed as nmol trans-cinnamic acid min^{-1} mg^{-1} protein

Determination of super oxide dismutase (SOD) enzymes in tomato roots

SOD was assayed according to the method of Beauchamp and Fridovich (1971). The activity of SOD (EC 1.15.1.1) was assayed by measuring its ability to inhibit the photochemical reduction of NBT. The roots (0.5g) were homogenized with 3.0ml of potassium phosphate buffer, centrifuged at 2000 rpm for 10 minutes and the supernatants were used for the assay. The 3 ml reaction mixture contained 50 mM phosphate buffer pH 7.8, 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 1.0 mM EDTA and 20 µl enzyme extract. Riboflavin was added last and the reaction was initiated by placing the tubes 30 cm below 15 W fluorescent lamps. The reaction was started by switching on the light and was allowed to run for 10 min. Switching off the light stopped the reaction and the tubes were covered with black cloth. Non-illuminated tubes served as control. The absorbance at 560 nm was read. One unit of SOD is the amount of extracts that gives 50% inhibition rate of NBT reduction.

RESULTS AND DISCUSSION

The enzymatic activity of selected bio-agents was assayed in tomato roots infested with the root- knot nematode *M. incognita*. Application of bio-agents increased the level of PO, PPO, PAL and SOD in tomato roots. Among all the treatments application of *T. viride* and *P. fluorescens* @ 4g/kg soil was found to be the best treatments to enhance enzymatic activity. Among enzymes, the PO was found highest in tomato roots during different times of observations followed by SOD, while PPO and PAL were observed in low quantity. The enzyme activity showed gradual increase from 7 DAI onwards to 28 DAI in plant roots treated with bio-agents as compared to untreated ones. The lowest enzyme

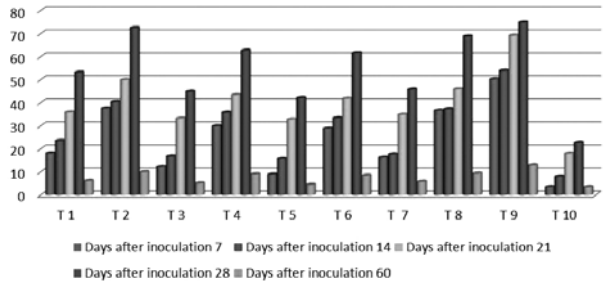

Fig. 1. Effect of bio-agents on peroxidase activity in tomato roots infected with *M. incognita*

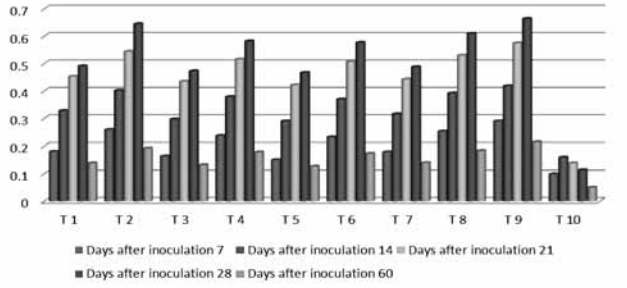

Fig. 2. Effect of bio-agents on polyphenol oxidase activity in tomato roots infected with *M. incognita*

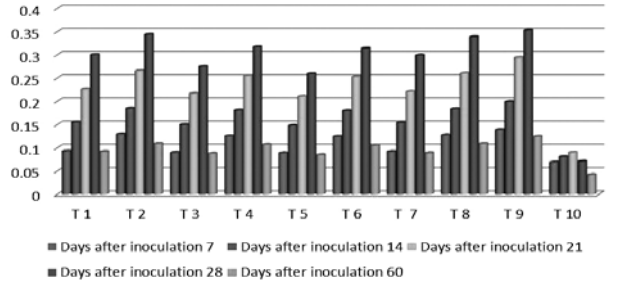

Fig. 3. Effect of bio-agents on phenylalanine ammonia lyase activity in tomato roots infected with *M. incognita*

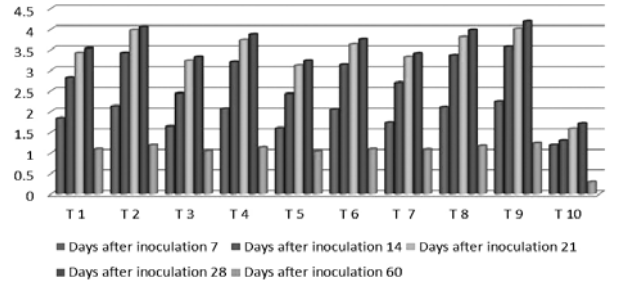

Fig. 4. Effect of bio-agents on super oxide dismutase activity in tomato roots infected with *M. incognita*

Table 1: Effect of bio-agents on plant growth characters of tomato infected with *M. incognita*

Treatments		Shoot length (cm)			Shoot weight (g)			Root length (cm)			Root weight (g)		
		Ist Year	IIst Year	Pooled	Ist Year	IIst Year	Pooled	Ist Year	IIst Year	Pooled	Ist Year	IIst Year	Pooled
T 1	*T. viride* @ 2 g/kg soil	28.57	28.43	28.50	27.40	27.17	27.28	34.40	34.60	34.50	24.23	24.13	24.18
T 2	*T. viride* @ 4 g/kg soil	34.13	34.38	34.26	34.90	35.00	34.95	43.17	43.48	43.33	29.43	29.67	29.55
T 3	*T. harzianum* @ 2 g/kg soil	27.03	26.90	26.97	25.57	25.30	25.43	31.03	31.18	31.11	22.20	22.33	22.27
T 4	*T. harzianum* @ 4 g/kg soil	31.17	31.39	31.28	32.20	32.83	32.52	41.27	41.40	41.33	26.67	26.83	26.75
T 5	*P. chlamydosporia* @ 2 g/ kg soil	26.27	26.42	26.34	23.27	23.48	23.38	29.93	30.08	30.01	20.37	20.87	20.62
T 6	*P. chlamydosporia* @ 4 g/ kg soil	30.93	30.80	30.87	29.77	29.50	29.63	39.07	38.90	38.98	25.67	25.98	25.83
T 7	*P. lilacinus* @ 2 g/kg soil	27.60	27.80	27.70	26.37	26.37	26.37	32.20	32.30	32.25	23.87	24.05	23.96
T 8	*P. lilacinus* @ 4 g/kg soil	32.53	32.90	32.72	32.83	33.07	32.95	42.60	42.42	42.51	27.51	27.63	27.57
T 9	*P. fluorescens* (Standard check) @ 4 g/kg soil	36.93	36.82	36.88	36.43	36.25	36.34	44.90	45.10	45.00	30.18	30.38	30.28
T 10	Control	10.37	10.22	10.29	4.50	4.27	4.38	8.37	8.58	8.48	2.50	2.67	2.59
SEm±		0.212	0.234	0.220	0.25	0.235	0.22	0.22	0.275	0.24	0.19	0.197	0.17
CD at 5%		0.624	0.689	0.648	0.743	0.695	0.660	0.653	0.811	0.714	0.546	0.582	0.509
CV		1.283	1.414	1.331	1.60	1.49	1.42	1.11	1.37	1.21	1.38	1.46	1.28

Note: (1) Data are average value of three replications, (2) Initial inoculums level 2 J2/g soil.

Table 2: Effect of bio-agents on nematode reproduction of tomato infected with *M. incognita*

Treatments	No. of galls/ plant			No. of egg masses / plant			No. of eggs and larvae / egg mass			Larval population / 200cc soil			Total population		
	Ist Year	IIst Year	Pooled	Ist Year	IIst Year	Pooled	Ist Year	IIst Year	Pooled	Ist Year	IIst Year	Pooled	Ist Year	IIst Year	Pooled
T 1	25.33	26.33	25.83	21.67	21.00	21.33	102.67	100.33	101.50	232.33	227.33	229.83	3389.67	3247.33	3318.50
T 2	20.33	20.67	20.50	13.67	14.67	14.17	79.33	80.67	80.00	182.00	178.67	180.33	1994.67	2077.33	2036.00
T 3	28.33	29.00	28.67	23.67	24.67	24.17	114.00	117.33	115.67	296.00	302.00	299.00	4180.00	4405.33	4292.67
T 4	22.67	21.67	22.17	16.33	17.00	16.67	95.00	93.67	94.33	206.00	199.00	202.50	2582.67	2578.67	2580.67
T 5	32.33	31.67	32.00	26.33	27.33	26.83	127.67	129.00	128.33	308.33	312.00	310.17	4905.67	5087.00	4996.33
T 6	24.67	24.33	24.50	19.33	19.00	19.17	101.00	97.00	99.00	219.00	215.33	217.17	3048.33	2918.33	2983.33
T 7	26.67	25.33	26.00	22.00	21.67	21.83	106.33	105.00	105.67	271.67	269.00	270.33	3697.00	3620.33	3658.67
T 8	22.33	22.00	22.17	15.67	14.67	15.17	83.67	82.33	83.00	195.00	191.67	193.33	2286.00	2166.00	2226.00
T 9	18.67	18.00	18.33	9.33	8.67	9.00	70.67	68.67	69.67	160.33	158.00	159.17	1461.00	1384.67	1422.83
T 10	61.67	63.33	62.50	44.67	47.33	46.00	209.67	211.33	210.50	981.00	989.00	985.00	14274.33	14955.00	14614.67
SEm±	0.73	0.73	0.60	0.98	0.97	0.81	1.59	1.86	1.45	1.57	3.14	2.04	154.53	160.54	123.51
CD at 5%	2.16	2.14	1.77	2.88	2.85	2.39	4.69	5.48	4.28	4.64	9.28	6.01	455.85	473.59	364.36
CV	4.47	4.45	3.68	7.95	7.75	6.54	2.52	2.97	2.31	0.89	1.79	1.16	6.40	6.55	5.08

Note:(1) Data are average value of three replications, (2) Initial inoculums level 2 J2/g soil.

activity was recorded at 60 DAI in untreated control plant roots. (Fig. 1, 2, 3 & 4).

Among all the treatments application of *T. viride* @ 4g/kg soil was found to be the best treatments to improve plant growth characters of tomato as well as in reducing number of galls per plant (20.50), number of egg masses per plant (14.17), number of eggs per egg mass (80.00), nematode population/200cc soil (180.33) and total nematode population of *M. incognita* (2036.00) in tomato. However, standard check *P. fluorescens* @ 4g/kg soil was found to be superior in improving plant growth as well as in reducing nematode population (Table 1 & 2).

Application of a talc-based formulation of bio-agents as soil treatment significantly reduced the root-knot nematode population in tomato roots. This is in agreement with the report that the level of infestation of *M. incognita* on tomato was reduced with fewer galls and egg masses in the root following root dipping with *P. fluorescens* strain (Pf l) (Santhi and Sivakumar, 1995). The studies on induced defence mechanisms revealed significant accumulation of PO, PPO, PAL and SOD in treated tomato plants inoculated with *M. incognita*. Accumulation of these enzymes began seven days after inoculation with the nematode and gradually increased up to 28 days. Among all the treatments application of *T. viride* @ 4g/kg soil was found to be the best treatments to enhance enzymatic activity. However, standard check *P. fluorescens* @ 4g/kg soil was found equally effective to increase the enzyme activity against *M. incognita* in tomato roots. Chen *et al.* (2000) reported greater activities of PO and PPO in cucumber root tissues treated with *Pseudomonas corrugate* challenged with *P. aphanidermatum*. Anita *et al*. (2004) studied accumulation of defence enzymes, *viz.* peroxidase, polyphenol oxidase, chitinase, phenylalanine ammonia lyase and catalase, in tomato root tissue treated with *P. fluorescens* isolate Pfl in response to invasion by the root knot nematode *M. incognita*. Activities of all the enzymes were significantly higher in bacterized tomato root tissues. Duan *et al.* (2011) showed that the *A. niger* could reduce the root-knot index and nematode populations, compared with untreated control in tomato. The activities of defense enzymes were enhanced significantly, such as phenylalanine ammonia, polyphenol oxidase, peroxidase, superoxide and catalase. Mohamed and Abo-Elyousr (2011) proved that application of different bio-control agents (*P. fluorescens, P. lilacinus* and *P. guilliermondii*) not only had a lethal effect on nematode, but also enhanced the plant growth, supplying many nutritional elements and induced the systemic resistance in plants.

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Ecofriendly Management of Wilt Complex in Black Pepper (*Piper nigrum* L.)

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ABSTRACT: Black pepper (*Piper nigrum* L.) is prone to attack by burrowing nematode, *Radopholus similis* and fungal wilt pathogen, *Phytophthora capsici* causing wilt complex and considerable yield loss. The field experiment was carried out in the farmer's field, with a view to evaluate bioagents (*Trichoderma harzianum, Purpureomyces lilacinum, Pseudomonas fluorescens* and *Bacillus subtilis*), organic amendment (Neem cake) and chemicals (Carbofuran and Bordeaux mixture) separately and in combination for the management of *R. similis* and *P. capsici.* The final population of *R. similis* in soil was lowest in Bordeaux mixture (1 %) + *P. lilacinum* (50 g) (302.66/ 200 cc soil), followed by Carbofuran 3G (15 g) (335.33 nematodes/200 cc soil), Bordeaux mixture (1%)+ Carbofuran- 3G (15 g) (349.33 nematodes/200 cc soil) and *P. lilacinum* (50 g) (371.33 nematodes/200 cc sol) as compared to untreated control (922.00 nematodes/ 200 cc soil) respectively. The final root population of *R. similis* was minimum in Bordeaux mixture (1 %) + *P. lilacinum* (50 g) (150.66/ 5 g roots) followed by Carbofuran 3G (15 g) (178.00 /5 g roots nematodes) as compared to untreated control (478.00/5 g roots) respectively. The lowest foliar yellowing, defoliation and lesion indices were observed in the treatment Bordeaux mixture (1 %) + *P. lilacinum* (50 g) (1.33, 1.00 and 1.00) followed by Bordeaux mixture (1 %) + *T. harzianum* (50 g) (1.66, 1.33 and 1.00) and these two treatments were at par with each other. Vines treated with Bordeaux mixture (1 %) spray + *T. harzianum* (50 g) recorded maximum dry berry weight of 2.27 g / vine and it was on par with vines treated with Bordeaux mixture (1 %) + *P. lilacinum* (50 g) with 2.05 g/vine. However, untreated control vines recorded lowest dry berry weight (0.63 g) and it was at par with Carbofuran 3G (15 g) (1.04g).

Keywords: Burrowing nematode, *Phytophthora capsici*, wilt complex, black peper

Black pepper (*Piper nigrum* L.), (Family *Piperaceae*) known as the "King of Spices" has remained most precious and valuable spice in the world. It is also called as "Black gold" due its durability and value. It is playing a vital role in International trade. It is said that the European invaded India primarily for this very spice. Black pepper is native to India and is extensively cultivated in tropical regions. Currently, Vietnam is the world's largest producer and exporter of black pepper, producing 34 per cent of the world's demand (Anon., 2005).

The cultivation of black pepper is mainly confined to India, Brazil, Indonesia, Malaysia, Thailand, Sri Lanka and Vietnam. During 2013-14, 21,250 tonnes of black pepper worth Rs. 94,002 lakhs were exported to various countries (Devasahayam *et al*., 2015). Black pepper is cultivated to a large extent in Kerala, Karnataka and Tamilnadu and to a limited extent in Maharashtra, north eastern states and Andaman & Nicobar Islands and Pondicherry. Kerala and Karnataka account for a major portion of production of black pepper in the country and to a lesser extent, in Maharashtra, Andhra Pradesh, Tamil Nadu and north eastern regions (Anon., 2005).

Slow wilt of black pepper is a debilitating disease where the affected plants survive for several years and death of plant occurs gradually over a period of 3-4 years. This disease has been referred to under different names such as slow wilt or slow decline in India, yellow or yellows disease in Indonesia.

The drastic drop in the black pepper production in India has been attributed mainly for pronounced mortality of vines by the dreaded foot rot caused by *Phytophthora capsici* and nematodes, *Meloidogyne incognita* and *Radopholus similis.* The other major constraints for low

production of black pepper are old gardens occupied with traditional cultivars having poor genetic potential, non-adoption of improved package of practices and bad management of gardens.

R. similis and *M. incognita* are the primary incitants of slow decline in black pepper, though *P. capsici* can also induces similar symptoms (Ramana *et al.,* 1992). Slow decline causes up to 32 per cent crop loss in Indonesia (Sitepu & Kasim, 1991) and about 30 per cent vines are damaged annually in Guyana by this disease (Biesser, 1969). Unfortunately, in spices still nematicides have been the primary option for growers in managing nematodes. There is an urgent need for the development of a non chemical and eco friendly control options. Such work is necessary to develop eco friendly management practices and minimize chemical use while maintaining high production standards. By keeping these factors the present study was taken to manage effectively by using biocontrol agents.

MATERIAL AND METHODS

The present experiment was conducted in the garden of farmer field. The field was heavily infested with *R. similis* with population density of more than one nematode per g soil i.e., 864 nematodes per 200cc soil. Farm was situated in Agro climatic zone-9 (Hilly region Zone) of Karnataka state at varied elevations (900-950msl) and rainfall (2500 to 3000mm) with 11^0 56' and 15^0 46' N latitude and 74^0 31' and 76^0 46' E longitude. Further the experiment was conducted in the existing 10-12 years of old orchard, which was grown as mixed crop-black pepper (Panniyur-1) along with arecanut and forest plants. Farm Yard Manure @ 10 kg /vine was commonly applied for all the treatments and normal package of practices like irrigation, fertilizer application and weeding was done uniformly to all the plants.

Treatment Details

T_1: Bordeaux mixture spray (1%), T_2: T_1 + *Trichoderma harzianum* (50g), T_3: T_1 + *Pseudomonas fluorescens* (50g), T_4: T_1 + *Purpureomyces lilacinum* (50g), T_5: T_1 + *Bacillus subtilis* (50g), T_6: T_1 + Carbofuran 3G–15gms, T_7: T_1 + Neem cake 2 kgs, T_8: Neem cake 2 kgs, T_9: *Pseudomonas fluorescens* (50g), T_{10}: *Trichoderma harzianum* (50g), T_{11}: *Purpureomyces lilacinum* (50g), T_{12}: *Bacillus subtilis* (50g), T_{13}: Carbofuran 3G–15g, T_{14}: Untreated Control. Each treatment was replicated thrice.

The talc-based formulations of *T. harzianum*, *P. lilacinum P. fluorescens*, and *B. subtilis* were obtained from Indian Institute of Horticultural Research (IIHR), Bangalore.

The experiment was conducted in two seasons' *viz*., 2014-15 and 2015-16 and biocontrol agents were applied before and after monsoon along with 10 kgs of farm yard manure. Organic amendments and Bordeaux mixture were sprayed before and after monsoon every year.

Observations recorded

1. **On host:** Number of runner shoots, number of spikes, spike length, spike weight, dry weight (per vine)

2. **On nematode:**

I. Initial nematode population before the treatment imposition
II. Nematode population at 60 days intervals
III. Final nematode population
IV. Number of lesions per plant
V. Root lesion index

3. **On wilt complex incidence:**

1. Number of wilted plants
2. Per cent defoliation index (1-4)
3. Per cent leaf yellowing index (1-4)
4. Per cent root necrosis (0-5)
5. Leaf lesion index (0-4)
6. Root lesion index (1-5)

The total number of plants showing foliar yellowing symptoms and defoliation were recorded by using the following scale (Mohandas and Ramana, 1991).

Foliar Yellowing Index (FYI): 1-4 scale

Scale	Descriptions
1	No leaves showing yellowing
2	Up to 20 per cent of leaves showing yellowing
3	20-60 per cent leaves showing yellowing
4	More than 60 per cent leaves showing yellowing

Defoliation Index (DFI): 1-4 scale

Scale	Descriptions
1	Less than 10 per cent defoliation
2	More than 10 per cent upto 30 per cent defoliation
3	More than 30 per cent upto 60 per cent defoliation
4	More than 60 per cent defoliation

The virulence rating of *P. capsici* in green house condition was done at 10 days interval till the termination of the experiment using the following disease rating scale by Turner (1973) based on per cent root necrosis.

Grade	Root necrosis (%)
0	0
1	1-10
2	11-25
3	26-50
4	51-75
5	76-100

Lesion Index: The root lesion index was calculated using the lesion index rating scale (Ramana *et al*., 1987).

Lesion number and size	Lesion index
No lesions	1
Few up to 1mm diameter (1-20 lesions)	2
Many up to 1mm diameter (21-50 lesions)	3
Many upto 1cm diameter (51-100 lesions)	4
Very severe 1 cm diameter (>100 lesions)	5

While collecting soil and root samples lesion characters were also recorded. The wilt disease incidence in the fields was calculated by using following formula.

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants infected}}{\text{Total Number of plants observed}} \times 100$$

Soil sample of 200 cc was washed thoroughly and processed using combined Cobb Sieving and Baermann's funnel technique (Ayoub, 1977).

RESULTS AND DISCUSSION

The field experiment was carried out in the farmer's field for two years, with a view to evaluate bioagents (*T. harzianum*, *P. lilacinum*, *P. fluorescens* and *B. subtilis*), organic amendment (Neem cake) and chemicals (Carbofuran and Bordeaux mixture) separately and in combination for the management of *R. similis* and *P. capsici*.

Observations on plant growth parameters like Number of runner shoots, Number of spikes, Spike length, Spike weight, and dry weight (per vine) was calculated and presented in the Table 1.

Number of runner shoots: Among combined treatments of bioagents, organic amendments and chemicals, maximum number of runner shoots was observed in plants with Bordeaux mixture (1 %) spray + soil application of *T. harzianum* (50 g) with 27.33 followed by Bordeaux mixture (1 %) + *P. lilacinum* (50 g) recorded 25.00 and it was at par with 24.33 number of runner shoots in Bordeaux mixture (1 %) + *P. fluorescens* (50 g). lowest number of runner shoots was observed in Bordeaux mixture (1 %) + Carbofuran-3G (15 g) treated vines with 19.66 number of runner shoots and it was at par with Bordeaux mixture (1 %) + Neem cake (2 kg) with 20.33 number of runner shoots.

Among the individual treatments, maximum number of runner shoots (16.33) was observed in *T. harzianum* (50 g) and lowest number of runner shoots (7.33) in untreated control.

Table 1: Influence of various treatments on growth and yield parameters of black pepper under field conditions (Season-I)

Treatments	Number of runner shoots	Number of spikes	Spike length (cm)	Spike weight (g)	Dry berry weight/ vine (Kg)	Yield Kg/ha
$T_{1:}$ Bordeaux mixture (1 %) spray	11.66	183.33	10.33	1316.33	1.27	254.66
$T_{2:}$ T_1 + *T. harzianum* (50 g)	27.33	321.33	17.66	2992.00	2.27	453.33
T_3: T_1+ *P. fluorescens* (50 g)	24.33	285.66	16.66	2516.66	1.91	381.33
$T_{4:}$ T_1+ *P. lilacinum* (50 g)	25.00	294.66	17.66	2760.66	2.05	411.33
$T_{5:}$ T_1+ *B. subtilis* (50 g)	22.33	284.66	15.66	2309.33	1.70	340.66
$T_{6:}$ T_1+ Carbofuran-3G (15 g)	19.66	213.33	11.66	2016.00	1.59	317.33
$T_{7:}$ T_1+ Neem cake (2 kg)	20.33	280.00	14.33	2189.33	1.83	366.66
T_8: Neem cake (2 kg)	11.00	179.33	9.33	1316.33	1.36	271.33
T_9: *P. fluorescens* (50 g)	13.33	191.33	9.00	1511.00	1.48	296.66
$T_{10:}$ *T. harzianum* (50 g)	16.33	214.66	12.33	1968.00	1.59	319.33
$T_{11:}$ *P. lilacinum* (50 g)	14.66	203.33	11.33	1778.66	1.41	282.66
$T_{12:}$ *B. subtilis* (50 g)	12.33	163.33	8.66	1164.66	0.82	164.66
$T_{13:}$ Carbofuran 3G (15 g)	10.66	151.33	8.33	1241.66	1.04	208.00
$T_{14:}$ Untreated	7.33	89.33	7.66	1072.66	0.63	127.33
S. Em ±	0.52	24.18	0.92	135.72	0.27	2.93
CD @ 5 %	1.50	70.29	2.67	394.63	0.77	8.54
CV (%)	5.29	19.18	13.06	12.39	29.35	1.69

Number of spikes: Highest number of spikes was produced in the vines treated with Bordeaux mixture (1 %) +*T. harzianum* (50 g) with 321.33 followed by Bordeaux mixture (1 %) + *P. lilacinum* (50 g) which recorded 294.66 number of spikes and lowest number of spikes was produced in untreated control plots (89.33) followed by 151.33 in Carbofuran-3G (15 g).

Spike length (cm): With respect to spike length, all the treatments were at par with each other**.** Vines treated with Bordeaux mixture (1 %) +*T. harzianum* (50 g) and Bordeaux mixture (1 %) + *P. lilacinum* (50 g) recorded maximum spike length of 17.66 cm respectively and it was at par with vines treated with T_3: Bordeaux mixture (1 %) + *P. fluorescens* (50g) (16.66 cm) and Bordeaux mixture (1 %) + *B. subtilis* (50 g) (15.66 cm). Lowest spike length was observed in vines with untreated control (7.66 cm) and it was at par with Carbofuran 3G (15 g) (8.33 cm) and *B. subtilis* (50 g) (8.66 cm).

Spike weight: Highest spike weight was recorded in Bordeaux mixture (1 %) + *T. harzianum* (50 g) with 2992.00 g followed by Bordeaux mixture (1 %) + *P. lilacinum* (50 g) with 2760.66 g and lowest spike weight was recorded in untreated control (1072.66 g) and it was at par with Carbofuran 3G (15 g) (1241.66 g).

Dry berry weight per vine (kg): Vines treated with Bordeaux mixture (1 %) spray + *T. harzianum* (50 g) recorded maximum dry berry weight of 2.27 g / vine and it was at par with vines treated with Bordeaux mixture (1 %) + *P. lilacinum* (50 g) with 2.05 g/vine. However, untreated control vines recorded lowest dry berry weight (0.63 g) and it was at par with Carbofuran 3G (15 g) (1.04 g).

Effect of various treatments on nematode population in soil and roots of black pepper infected by *R. similis* and *P. capsici* at different intervals (Season-I)

Nematode population in soil and roots

The *R. similis* population in soil and roots was recorded at 60 days interval from treatment imposition to harvest. The population of *R. similis* in soil differed significantly among all the treatments compared to control. The data is presented in Table 2.

Among the individual treatments, lowest multiplication of *R. similis* was observed in Carbofuran 3G treatment followed by *P. lilacinum* and *T. harzianum*, the same trend was followed in all the intervals of sampling till the harvest of the crop. Among combined treatments, the T_2: Bordeaux mixture (1 %) + *T. harzianum* (50 g), T_3: Bordeaux mixture (1 %) + *P. fluorescens* (50 g), T_4: Bordeaux mixture (1 %) + *P. lilacinum* (50 g), T_5: Bordeaux mixture (1 %) + *B. subtilis* (50 g), T_6: Bordeaux mixture (1 %) + Carbofuran-3G (15 g) and T_7: Bordeaux mixture (1 %) + Neem cake (2 kg) the soil was maximum in *Bacillus subtilis* treated vines followed by *P. fluorescens* at all the intervals.

The population of *R. similis* in roots was differed significantly among the treatments compared to control. The same trend was observed in root population as that of soil population.

Table 2: Effect of treatments on population of *R. similis* in soil and roots of black pepper at different intervals under field conditions

Treatments	Nematode population (200 cc soil) Days after treatment				Nematode population (5 g roots) Days after treatment			
	60	120	180	240	60	120	180	240
T_1 : Bordeaux mixture (1 %) spray	632.00	618.00	558.66	543.33	303.33	324.00	338.00	290.66
T_2 : T_1 + *T. harzianum* (50 g)	594.00	516.66	482.00	389.33	237.33	276.00	241.33	179.33
T_3: T_1 + *P. fluorescens* (50 g)	610.66	552.66	510.66	451.33	278.00	300.66	281.33	203.33
T_4 : T_1 + *P. lilacinum* (50 g)	555.33	505.33	418.00	302.66	222.66	267.33	249.33	150.66
T_5 : T_1 + *B. subtilis* (50 g)	626.00	596.00	501.33	417.33	280.00	317.33	304.00	230.66
T_6 : T_1 + Carbofuran-3G (15 g)	489.33	432.00	402.66	335.33	179.33	235.00	214.66	184.66
T_7 : T_1 + Neem cake (2 kg)	608.66	530.00	476.00	412.00	256.00	291.33	265.33	191.33
T_8 : Neem cake (2 kg)	618.00	561.33	434.00	430.00	260.00	297.33	273.33	196.00
T_9 : *P. fluorescens* (50 g)	600.00	570.66	503.33	444.66	276.33	308.66	287.33	210.00
T_{10} : *T. harzianum* (50 g)	583.33	531.33	494.66	401.33	252.00	282.00	259.33	190.00
T_{11} : *P. lilacinum* (50 g)	578.66	493.33	464.66	371.33	230.66	251.33	232.66	163.33
T_{12} : *B. subtilis* (50 g)	619.99	583.33	520.00	463.33	264.00	309.33	296.66	219.33
T_{13} : Carbofuran 3G (15 g)	519.33	477.33	384.66	349.33	215.33	244.00	226.66	178.00
T_{14} : Untreated	699.33	814.00	883.33	922.00	338.66	405.33	435.33	478.00
S. Em ±	3.89	31.45	3.92	2.75	17.91	1.80	1.49	2.96
CD @ 5 %	11.33	91.47	11.39	8.01	52.09	5.25	4.34	8.61
CV (%)	1.13	9.77	1.35	1.07	12.08	1.06	0.93	2.34

INP: 864 J_2/200 cc soil

The final population of *R. similis* in soil was lowest in T_4: Bordeaux mixture (1 %) + *P. lilacinum* (50 g) (302.66/200 cc soil), followed by T_6: Carbofuran 3G (15 g) (335.33 nematodes/200 cc soil), T_{13}: Bordeaux mixture (1%)+ Carbofuran- 3G (15 g) (349.33 nematodes/200 cc soil) and T_{11}: *P. lilacinum* (50 g) (371.33 nematodes/200 cc sol) as compared to untreated control (922.00 nematodes/200 cc soil) respectively.

The final root population of *R. similis* was minimum in T_4: Bordeaux mixture (1 %) + *P. lilacinum* (50 g) (150.66/5 g roots) followed by T_{13}: Carbofuran 3G (15 g) (178.00 /5 g roots nematodes) as compared to untreated control (478.00/5 g roots) respectively.

Effect of various treatments on wilt complex incidence in black pepper infected with *R. similis* and *P. capsici*

Influence of bioagents, organic amendments and chemicals on foliar yellowing, defoliation and leaf lesion indices were recorded before harvest and the data is presented in Table 3. The lowest foliar yellowing, defoliation and lesion indices were observed in the treatment T_4: Bordeaux mixture (1 %) + *P. lilacinum* (50 g) (1.33, 1.00 and 1.00) followed by T_2: Bordeaux mixture (1 %) + *T. harzianum* (50 g) (1.66, 1.33 and 1.00) and these two treatments were at par with each other. Highest foliar yellowing, defoliation and lesion indices were observed in untreated control (4.00, 4.00 and 4.00) followed by T_{13}: Carbofuran 3G (15 g) (4.00, 3.66 and 3.66) respectively.

Effect of various treatments on multiplication of nematodes and per cent root necrosis on black pepper infested with *R. similis* and *P. capsici*

Influence of bioagents, organic amendments and chemicals on number of lesions, lesion index and percent root necrosis on black pepper was analyzed before harvest and the data is presented in Table 4.

Table 3: Effect of treatments on foliar yellowing, defoliation and lesion indices on balck pepper under field conditions

Treatments	Foliar yellowing (1-4)	Defoliation index (1-4)	Leaf Lesion index (0 - 4)
T_1: Bordeaux mixture (1 %) spray	2.66	2.33	1.33
T_2: T_1 + *T. harzianum* (50 g)	1.66	1.33	0.66
T_3: T_1 + *P. fluorescens* (50 g)	3.00	3.00	1.66
T_4: T_1 + *P. lilacinum* (50 g)	1.33	1.00	1.00
T_5: T_1 + *B. subtilis* (50 g)	4.00	3.33	2.33
T_6: T_1 + Carbofuran-3G (15 g)	4.00	3.66	2.33
T_7: T_1 + Neem cake (2 kg)	2.66	2.33	2.00
T_8: Neem cake (2 kg)	3.33	2.66	2.33
T_9: *P. fluorescens* (50 g)	3.33	2.66	2.66
T_{10}: *T. harzianum* (50 g)	2.33	1.66	2.66
T_{11}: *P. lilacinum* (50 g)	2.00	1.66	2.00
T_{12}: *B. subtilis* (50 g)	4.00	3.00	3.00
T_{13}: Carbofuran 3G (15 g)	4.00	3.66	3.66
T_{14}: Untreated	4.00	4.00	4.00
S. Em ±	0.23	0.23	0.31
CD @ 5 %	0.69	0.67	0.91
CV (%)	13.60	15.96	24.00

Table 4: Effect of various treatments on *R. similis* and *P. capsici* under field conditions

Treatments	No. of lesions	Lesion index (1- 5)	Root necrosis (%)	Wilt incidence (%)	% decrease over control	Yield/ Vine (Kg)	Per cent increase over control	B: C Ratio
T_1: Bordeaux mixture (1 %) spray	116.00	5.00	32.00	28.33	64.28	1.27	101.58	1.19
T_2: T_1 + *T. harzianum* (50 g)	60.00	4.00	26.67	13.33	83.19	2.27	260.03	2.92
T_3: T_1 + *P. fluorescens* (50 g)	73.66	4.00	39.33	38.00	52.09	1.91	203.17	1.61
T_4: T_1 + *P. lilacinum* (50 g)	51.33	3.66	23.00	13.66	82.78	2.05	225.39	2.48
T_5: T_1 + *B. subtilis* (50 g)	97.00	4.00	64.66	48.66	38.66	1.70	169.84	1.33
T_6: T_1 + Carbofuran-3G (15 g)	65.00	4.00	35.67	23.00	71.00	1.59	152.38	1.24
T_7: T_1 + Neem cake (2 kg)	89.67	4.00	48.66	33.66	57.56	1.83	190.47	1.60
T_8: Neem cake (2 kg)	106.00	5.00	74.00	55.66	29.83	1.36	115.87	1.46
T_9: *P. fluorescens* (50 g)	106.66	5.00	69.33	61.33	22.69	1.48	134.92	1.30
T_{10}: *T. harzianum* (50 g)	96.66	4.00	53.00	42.00	47.06	1.59	152.38	1.38
T_{11}: *P. lilacinum* (50 g)	80.67	4.00	44.66	42.33	46.66	1.41	123.80	1.24
T_{12}: *B. subtilis* (50 g)	110.33	5.00	75.33	69.33	12.60	0.82	30.15	0.73
T_{13}: Carbofuran 3G (15 g)	82.66	4.00	59.00	74.66	5.89	1.04	65.07	0.91
T_{14}: Untreated	146.67	5.00	83.66	79.33	0.00	0.63	0.00	0.71
S. Em ±	1.16	0.09	0.68	0.82	-	0.27	-	0.02
CD @ 5 %	3.37	0.26	1.98	2.38	-	0.77	-	0.05
CV (%)	2.19	3.50	2.26	3.18	-	29.35	-	2.12

Number of lesions and lesion index

There were significant differences observed between the treatments in number of lesions on roots and the lowest number of lesions and lesion index was observed in the T_4: Bordeaux mixture (1 %) + *P. lilacinum* (51.33 and 3.66), T_2: Bordeaux mixture (1 %) +*T. harzianum* (60.00 and 4.00) and T_6: Bordeaux mixture (1 %) + Carbofuran (65.00 and 4.00) respectively. Highest number of lesions and lesion index were observed in untreated control (146.00 and 5.00) followed by Bordeaux mixture (1 %) (116.00 & 5.00).

Per cent root necrosis

Similar trend was also followed in per cent root necrosis and lowest root necrosis was recorded in T_4: Bordeaux mixture (1%) + *P. lilacinum* (23.00 %), followed by T_2: Bordeaux mixture (1 %) +*T. harzianum* (26.67 %) and these two treatments were at par with each other. Highest per cent root necrosis was observed in untreated control (83.66 %) followed by T_{12}: *B. subtilis* (75.33 %).

Per cent wilt incidence

The results indicated that plants treated with T_2: (Bordeaux mixture (1 %) + *T. harzianum* (50 g) recorded very less wilt incidence of 13.33 % compared to the maximum wilt incidence (79.33 %) in untreated control. The next best treatment was plant inoculated with T_4: (Bordeaux mixture (1 %) + *P. lilacinum* (50 g), which recorded the wilt incidence (13.66 %) which was at par with T_2: Bordeaux mixture (1 %) +*T. harzianum* reduced the wilt incidence compared to individual application of bioagents (Table 4).

Influence of various treatments on yield and economic benefit of disease management in black pepper infested with *R. similis* and *P. capsici*

All the treatments recorded increased yield and B: C ratio with decreased wilt incidence and the data is presented in Table 4.

Individual application of *P. lilacinum* (1.41 kg), *T. harzianum* (1.59 kg), *P. fluorescens* (1.48 kg), *B. subtilis* (0.82 kg), neem cake (1.36 kg), Bordeaux mixture (1 %) spray (1.27 kg) and Carbofuran (1.04 kg) and these treatments did not significantly differ in yield per vine and the lowest yield was recorded in untreated control (0.63 kg/vine).

Among the combined treatments, maximum yield (2.27 kg) was recorded in plants treated with Bordeaux mixture (1 %) +*T. harzianum* (50 g) followed by 2.05 kg in Bordeaux mixture (1 %) + *P. lilacinum* (50 g) treated plants.

The economic analysis, for integrated management of *R. similis* and *P. capsici* wilt on black pepper under field conditions was carried out. The results revealed that, the T_2: Bordeaux mixture (1 %) +*T. harzianum* (50 g) recorded maximum B: C ratio (2.92) with wilt lowest incidence of 13.33 per cent followed by T_4: Bordeaux mixture (1 %) + *P. lilacinum* (50 g) recorded B: C ratio (2.48) and per cent disease incidence (13.66 %) and these two treatments were at par with each other and superior over other treatments.

It was apparent that, the integrated application of Bordeaux mixture (1 %) +*T. harzianum* (50 g) or Bordeaux mixture (1 %)+ *P. lilacinum* was most effective in improving plant growth parameters, berry yield and in reducing nematode population, per cent yellowing, defoliation, leaf lesion indices and per cent root necrosis and also maximum B: C ratio.

Integrated disease management would be the ideal strategy to tackle the complex and elusive soil borne problems like foot rot of black pepper, since single approach would be of little consequence to contain the disease. Nursery hygiene, phytosanitation and other cultural practices, chemical control, biocontrol measures coupled with host resistance are important components of integrated disease management that would reduce the pesticide load into the environment. Out of the various components of integrated disease management, biocontrol programmes are of high priority in managing soil borne plant pathogens. An integrated approach with cheap and efficient plant protection technology is of great relevance to check plant parasitic nematodes and *P. capsici.*

The results revealed that combination of Bordeaux mixture (1%)and *T. harzianum* (50 g) application or combined applications of Bordeaux mixture (1%) + *P. lilacinum* (50 g) provided the maximum growth and yield of black pepper infested with *R. similis* and *P. capsici* under filed conditions.

The present findings are in confirmation with findings of Haffeez *et al*. (2001) who reported that the addiction of *Paecilomyces lilacinus* and *T. harzianum* as nematophagous fungi separately along with organic manure to the infested field sufficiently retarded the pathogenic activity of *M. incognita* and increased the plant vigor. Thankamani *et al*. (2005) who reported that number of roots and biomass production were higher with combined application of *P. fluorescens* (thrice) and *T. harzianum* which was at par with application of *P. fluorescens* thrice.

Use of a biocontrol agent (*T. harzianum*) for *Phytophthora* foot rot in black pepper, parameters such as yield increase (*i.e*. quantity saved), change in cost of cultivation and improvements in economic returns were used to assess the impact of the project. Adoption of the technology resulted in maximum proportionate productivity increase of 11.6 % and the net proportionate reduction in cost per ton output was 78.3 % (Madan *et al*., 2005).

Combined treatment of *P. putida* ($2X10^{-6}$/ml) and *P. lilacinus* ($2X10^{-6}$/ml) was more effective when compared with all other treatments. These treatments increased the plant growth and rhizospheric colonization of both bioagents significantly and reduced the disease incidence of *M. incognita* and *F. oxysporium* f. sp. *gladioli* by 66 per cent and 57 per cent respectively. There was also a significant increase in the yield of the crop which was to the tune of 23 per cent (Sowmya and Rao, 2013).

Dipel (*B. thuringiensis*) & Bio-nematon (*Paecilomyces lilacinus*) showed their superiority on the shoot, root length and root weight (Mohamed, 2013). This performance of fungal bioagents in the present study may be attributed to their strong fungicidal and nematicidal property against both the pathogens which provided maximum defense with improved plant growth. The various phytoharmones produced by PGPR play a major role in growth promotion and many bacteria have the ability to produce auxins, gibberlines and cytokinines and ethylenes (Bottini *et al*., 2004). Similarly, *T. harzianum* is potential biocontrol agent which poses growth hormone and all these combinations resulted in improved plant growth parameters. The *T. viridae* has been reported to be a natural source of enzymes and plant hormones provide additional support to plants for its better growth development and immunity.

Nematode population and wilt incidence

With respect to nematode population in soil plants treated with Bordeaux mixture (1 %) + *P. lilacinum* (50 g) recorded lowest nematode population compared to other treatments and it was significantly superior over other treatments.

Ramana (1994) reported the efficacy of *P. lilacinus* in suppressing *M. incognita* and *R. similis* infestations in black pepper (*Piper nigrum*). Though the fungus could not affect absolute control of nematodes, it significantly suppressed nematode infestation and increased total root mass production.

Biocontrol agent *T. harzianum* along with potassium phosphonate has recorded highest disease suppression with least foliar yellowing (Kumar *et al.,* 2000)

It was demonstrated that *P. lilacinum* is an effective biocontrol agent against *R. similis* in banana and can be an important component of integrated pest management strategies (Mendoza *et al*., 2007).

Less foliar yellowing (13.52 %), less defoliation (15.28 %), less death of vines (4.72 %) and highest green berry yield of 2.46 Kg per vine was recorded when vines were treated before onset of monsoon (May), during rainy season (June- July) and during 2nd fortnight of August with potassium phosphonate (0.3 %) as spray (2 l/vine) and drenching of *T. harzianum* 50g per vine with 1kg of neem cake to the root zone. This was followed by chemical check with application of (1.0 %) Bordeaux mixture spray (2 L /vine) and copper oxychloride (0.1 %) as drenching (3l/vine) wherein less foliar yellowing (16.6%), less defoliation (20.25 %), less death of vines (5.40 %) and green berry yield of 2.08 kg per vine were recorded (Raja Kumar *et al*., 2012).

The antagonistic organisms viz., *T. viride*, *T. harzianum*, *Laetiseria arvalis*, and *Bacillus subtilis* were tested against *P. capsici* in pot culture by adding infected material to healthy vine. Among the four bioagents tried, *T. viride* and *T. harzianum* were effective in reducing the incidence of the disease as compared to *L. arvalis* and *B. subtilis*. The disease incidence was maximum in untreated vines (Lokesh *et al.,* 2013).

Devasahayam *et al.* (2015) have given integrated disease management of wilt complex in black pepper and reported that foliar spraying of 1 % Bordeaux mixture during and May and June followed by drenching and spraying with same fungicide during October coupled with soil application of *T. harzianum* around the base of the vine @ 50 g/vine during May - June and October months.

The reasons for the reduced wilt incidence and increased yield may attributed to the *Trichoderma* spp. involved in the reduction of *P. capsici* by mycoparasitism, spatial and nutrient competition, antibiosis by enzymes and secondary metabolites and induction of plant defense system. The developing *P. lilacinum* kills the nematode by feeding on its body content and in effect *P. lilacinum* acts as a parasite on all the stages of nematode.

The combined effects of these two bioagents against *R. similis* and *P.capsici* helped tremendously for the management of wilt complex in black pepper under field conditions. The amount of disease suppression obtained with a biological control agent depends on the density of the agent, the density of the pathogen and how efficiently individual units of the agents render units of the pathogen ineffective.

Finally, it may be concluded that, soil borne pathogens like *P. capsici* and *R. similis* cannot be controlled with just a single management strategy. In the present study, an integrated approach was attempted to manage this disease, with mixtures of biocontrol formulations which showed significant reduction in the disease incidence. The application of *P. lilacinum* and *T. harzianum* in combination with neem cake and farm yard manure is highly useful in managing *R. similis* and *P. capsici* wilt complex.

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Effect of Age on Pharyngeal Pumping in Two Species of Free-Living Nematodes

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ABSTRACT: Pharynx is a neuromuscular pump present at the anterior end of the alimentary tract. Feeding rate as well as the precise timing of pharyngeal movements, is required for efficient feeding and for survival in nature. Described here are the age-related changes in pharyngeal behaviour of two rhabditid nematodes *viz*., *Metarhabditis andrassyna* and *Teratorhabditis palmarum*. In both the species the average pharyngeal pulsation were more in the presence of bacteria than in its absence. Pulsation rates declined with age in both species in both the conditions. Deterioration in the structure of the basal bulb was also observed.

Keywords: Age, behavior, pharynx, rhabditid.

A bacteriovorous nematode consumes bacteria and its feeding apparatus, the pharynx, is neuromuscular organ that functions as a pump (Avery *et al*., 2003). The cycle of contraction and relaxation that draws food from the environment and filters bacteria from liquid is referred to as pharyngeal pumping.Old adults pump and defecate more slowly as compared to young adults (Bolanowski *et al*. 1981; Kenyon *et al*. 1993; Duhon & Johnson 1995). Feeding motions are monitored by the presence of food in the nematodes environment. The rate of pumping decides the amount of food intake and the rate of growth. The overall structure and function of the pharynx muscles can be easily monitored under a light microscope. Garigan *et al*. (2002) and Herndon *et al*. (2002) showed that ageing is also associated with structural deterioration of the pharynx muscles. Hence the pharynx suffers both structural and functional declines. The main function of pharynx is to pump food against the internal body pressure and prevent gut contents from being regurgitated (Bennet-Clark, 1976). The basic mechanism of pharyngeal pumping is accomplished by sequential contraction of muscle fibres (striated) that leads to wave of dilation which propagates along the lumen from anterior to posterior region (Lieven, 2003). We studied the pharyngeal pumping and structural changes in the basal bulb.

MATERIAL AND METHODS

Metarhabditis andrassyna and *Teratorhabditis palmarum* were cultivated on NGM agar plates with the *Escherichia coli* strain OP50 following standard protocols (Brenner, 1974).

Pharyngeal pumping: Pumping assays were performed on agar plates at room temperature using a Zeiss DiscoveryV20 Stereomicroscope. Pumping rate was determined as the number of contractions and relaxations in the grinder of terminal bulb in one minute. For this purpose, two sets of NGM plates were prepared. One set of plates were inoculated with *E.coli* OP50 and another set was maintained without *E. coli*. Nematodes of different age groups were selected i.e., 2, 6 and 10 days old and placed separately on both sets of dishes with or without *E.coli* OP50. Twenty replicates were maintained to ensure accuracy. The nematodes were allowed to acclimatize for ten minutes and then the number of pumping were observed over a two minute period. From this, average pumping rate was calculated. If no pumping was seen in two minute, the worm was recorded as non-pumping.

Metacorpus structure: 10-20 individual nematodes of each age were mounted on 2% agarose pads and paralyzed with alcohol. The pharynx was studied and photographed using an Olympus BX 40 DIC microscope with aProgRes C3 camera mounted.

Data Analysis

All the data were statistically analyzed by one-way ANOVA using GraphPad Prism 7 to reveal significant difference between mean values of different age groups. All values are presented as mean ± deviation (SD). The probability levels of 0.01 were considered statistically significant.

RESULTS

Pharyngeal pumping declined gradually with age in the presence or absence of bacteria. When bacteria were absent, pumping rate was significantly lower as compared to when bacteria were present in all age groups of both species. In *M. andrassyna* pharyngeal contraction declined from 268±26.4 pumps per min in 2 days old reduced to 88±7.63 pumps per min in 10 days old in the presence of bacteria. In the absence of bacteria pulsation declined from 199 ± 15.4 in 2 days old to 56 ± 11.2 in 10 days old individuals. Similarly in *T. palmarum* contractions declined from 248± 16.20 pumps per min to 108± 9.14 pumps per min in 2 & 10 days old. Here also in the absence of bacteria pulsation declined from 196 ± 13.6 in 2 days old to 86 ± 9.5 in 10 days old individuals (Fig. 1). Among the two genera, rate of pulsation was found to be high in *T. palmarum*. It was found that muscles weakening during aging resulted in bends in the length of isthmus (Fig. 2A, B & C). In some cases, the muscle swelled due to either bacterial infection at old stage (Fig. 2F) and in most other cases they shrunk, distorting the overall look (Fig. 2C& F). The surface morphology of the basal bulb was smooth in young adults (Fig. 3A & D) as compared to an irregular and rugged appearance in aged worms (Fig. 3C & F). The shape also appeared to change being rounded and rhomboidal in young individuals (3A) and flattened-oval (3C) in old worms. In aging adults, muscle cells at the basal region appeared deformed and shrunken that lead to surface deterioration (Fig. 3H) as compared to younger worms (Fig. 3G). It was also observed that feeding ability was

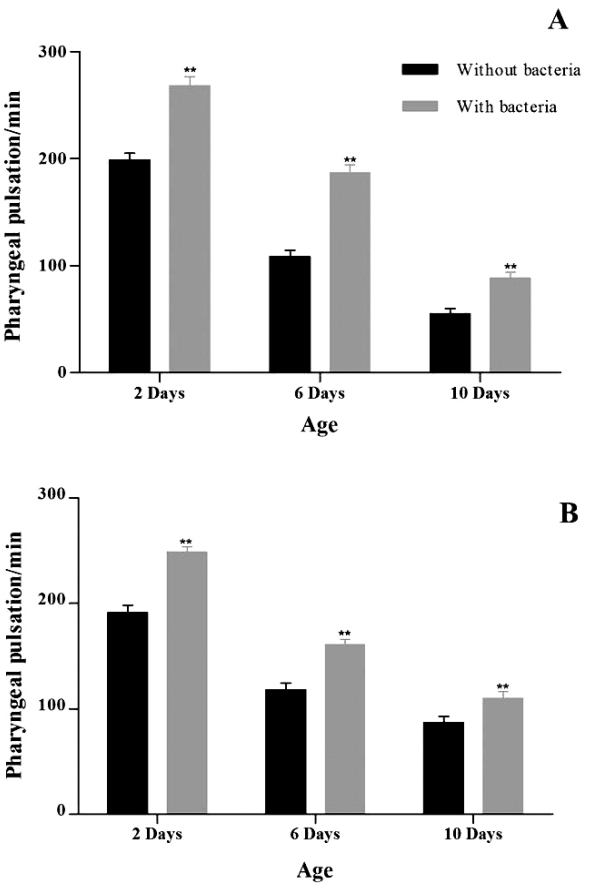

Fig. 1. Pharyngeal pumping rates of different age groups of*M. andrassyna* (A)and *T. palmarum* (B)

higher in younger worms that lead to bacterial clogging in grinder region (Fig. 2D & E) while due to weak musculature of grinder in older worms may also lead to bacteria clogging (Fig. 3I). In both cases it would lead to slower pumping rate.

DISCUSSION

The decline in the rate of pharyngeal pumping with age in *M. andrassyna* and *T. palmarum* is consistent with earlier observations on *C. elegans* (Kenyon *et al*., 1993). While Croll and Smith (1978) and Horvitz *et al*., (1982) also observed that the feed-bacteria stimulated the pumping activity, it was also observed in both nematodes that the rate of pumping declined with or without food. In young adults of *C. elegans*, the pharynx pumps, approximately 200-300 times/min and this pumping

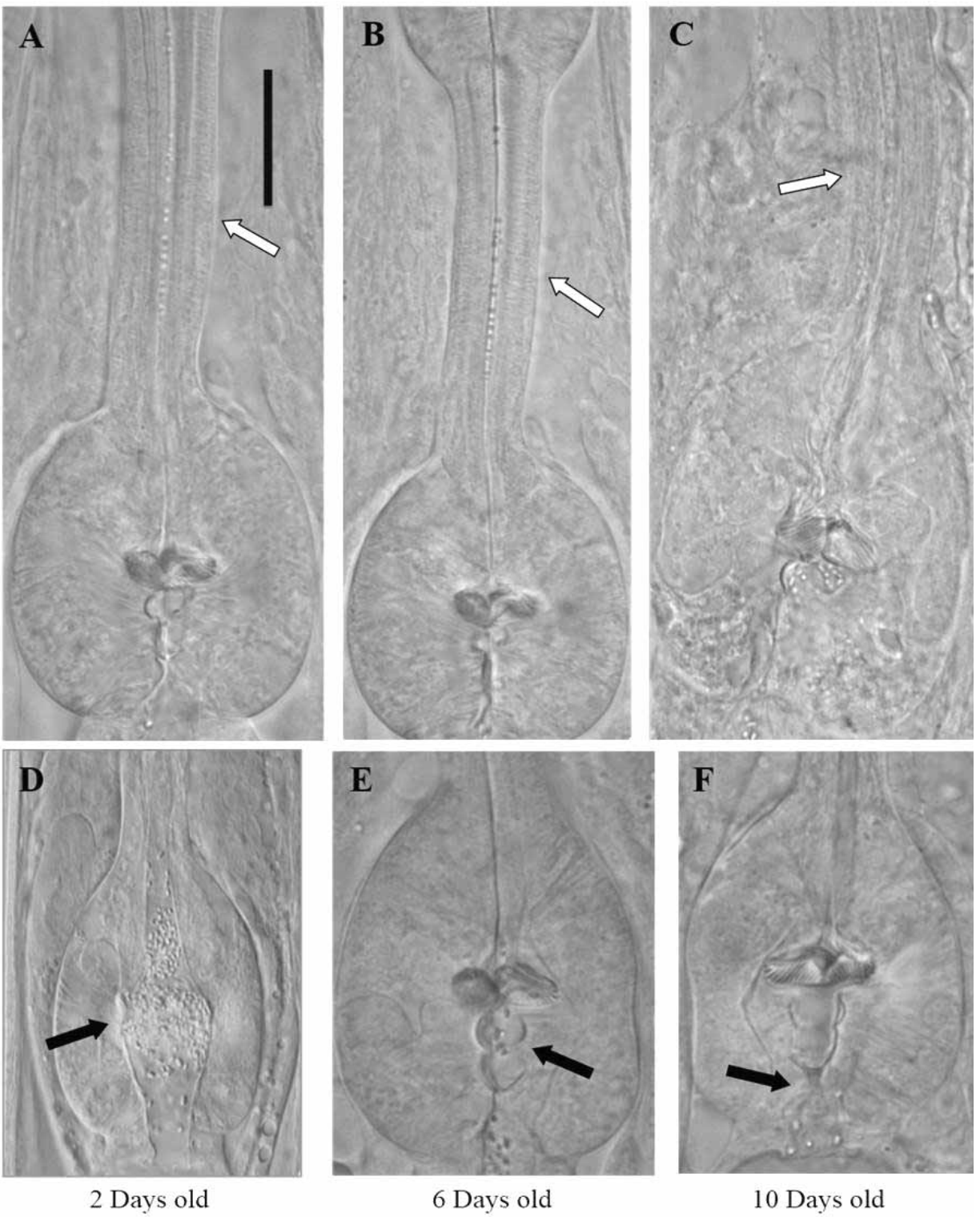

Fig. 2. Showing bend in isthmus length with age (A, B & C). Bacterial clogging in basal bulb of aging nematode (D, E& F). Scale bar: A, B, C, D, E & F = 20 ìm. (Black arrow shows bacterial clumping & White arrow represent bend in isthmus).

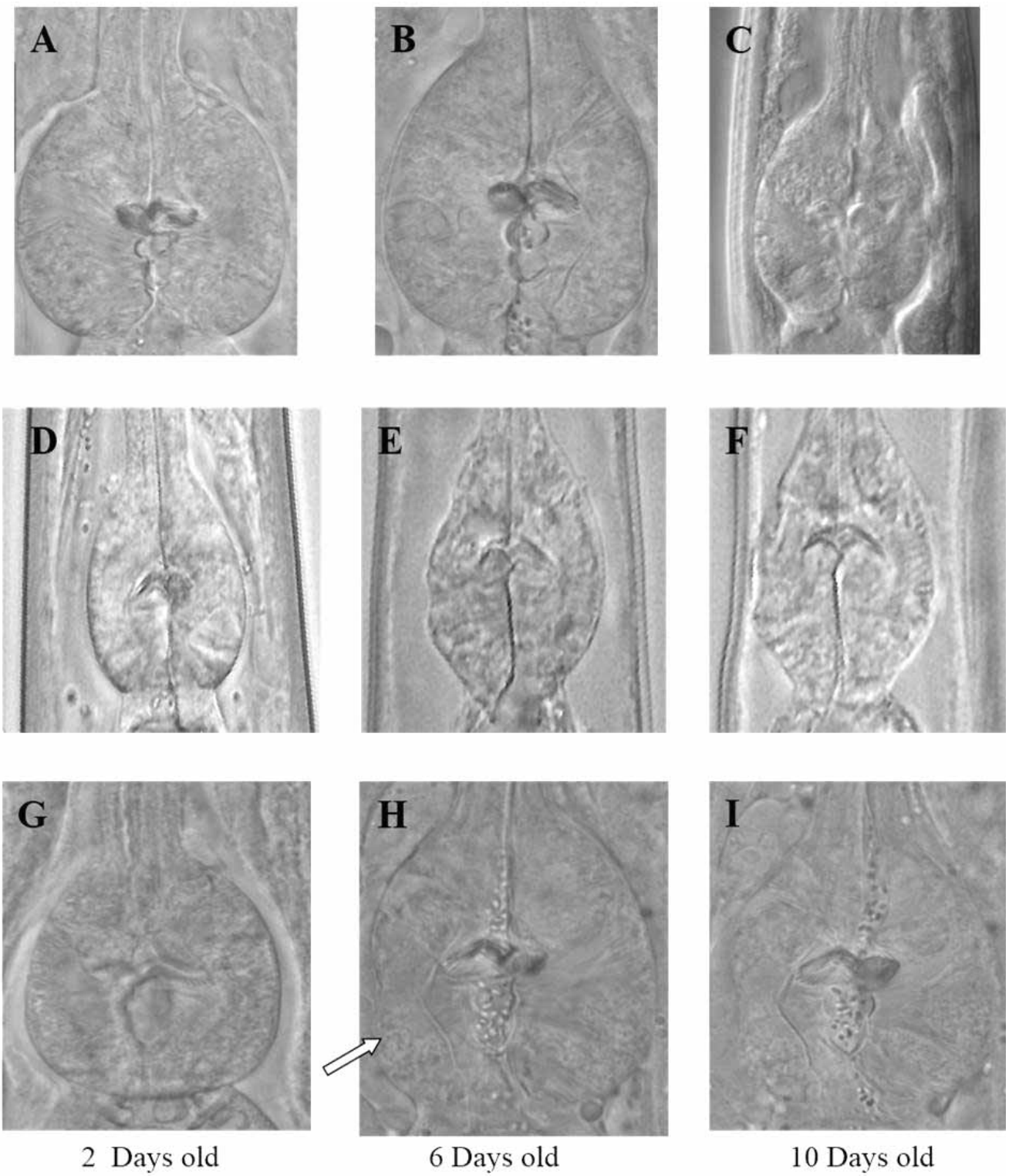

Fig. 3. Structural and morphological observation in different age nematodes (A, B, C, G, H & I) in *T.palmarum*and (D, E & F) in *M.andrassyna*. Scale bar: A, B, C, D, E, F, G, H & I = 20 ìm. (White arrow show surface breakdown).

rate declined gradually with aging (Bolanowski *et al*., 1981; Huang *et al*., 2004). Similarly, we also observed progressive rate of decline in both nematodes species. The rate of muscle contraction appeared to be correlated with structural and functional aspects of pharynx.

The damage in large muscles cells of the basal bulb was observed and isthmus appeared bent in old individuals of *T. palmarum*. The muscles weaken and hence rate of muscles contraction decreases. In mutants of *C. elegans* with slower pharyngeal pumping rates the muscle structure was better preserved (Chow *et al*., 2006). However, structural decline was not significantly delayed during aging in slow pumping nematodes, perhaps indicating a diagnostic effect in the progression of muscle damage. Age-related pharyngeal functional decline is most likely intrinsic to the pharynx and not due solely to microbial invasion or toxicity (Chow *et al*., 2006; Zhao *et al*., 2017). Moreover, one of the possible reasons of swollen pharynx (pharyngeal muscle near the grinder) was due to bacterial accumulation in that region. Normal pharynx contained either no invading *E. coli* or small, membrane-bound bacterial inclusions usually near the grinder region. Further, muscle contraction in the weakened tissue may cause it to deteriorate. We believe that contraction-related injuries are an accessible factor only during young and middle age, when the muscles contract actively. Beyond this phase, further impairment of muscle contraction may be compounded by other physiological factors, viz, the nutritional state of the individual or impaired nervous stimulation or retarded physical mobility.

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Survey of Major Bitter Gourd Growing Areas of Punjab to Determine the Incidence and Prevalence of Root-Knot Nematode

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ABSTRACT: In the present study, with the aim to study the nematode infestation and to identify the prevalent root-knot species, major bitter gourd growing areas were surveyed in different districts of Punjab. From these fields one hundred eighteen samples were collected out of which sixty six samples were found to be infested showing overall 57.27 percent mean disease incidence. District wise maximum disease incidence (75%) was recorded in Ferozepur followed by Sangrur (65%) and the minimum disease incidence was seen in Ludhiana (44.16%). The observation recorded on cropping sequence followed by the farmers revealed that fields where crops like brinjal, okra, chilli and cucumber were grown in rotation with the bitter gourd crop, nematode infestation was higher as compared to the fields where marigold, garlic and onion crops were included in the crop rotation. Further, it was noticed that fields with wild bitter gourd/*Jhaar Karela* (*Momordica balsamina*) grown areas showed less soil nematode population as compared with fields where cultivated bitter gourd (*M. charantia*) was grown. Morphological identification using perineal pattern studies and the PCR based detection using species specific primers revealed *Meloidogyne incognita* as the most prevalent species.

Keywords: *Momordica charantia, M. balsamina*, *Meloidogyne* spp., survey.

Bitter gourd is one of the most popular cucurbitaceous vegetable grown in India. Among cucurbits, bitter gourd has the highest nutritive value and is good source of proteins, minerals, vitamins and carbohydrates. It possesses various antioxidant, anti-hepatotoxic, antimicrobial and antiviral properties and have ability to lower blood sugar. The genus *Momordica* consists of 60 species (Schaefer and Renner, 2011); out of which seven species are known to occur in India but only *Momordica balsamina* (commonly known as *Jhaar Karela*), *M. diocia* (spine gourd), *M. cochinchinensis* (sweet gourd of Assam) and *M. charantia* (cultivated bitter gourd) are major cultivated species available in India (Yadav *et al.,* 2004) with *M. charantia* as most widely grown specie. At country level crop is being cultivated over an area of 95 thousand ha with production of 1030 metric tons/ ha (Anonymous, 2016-17)

In the Punjab state, farmers are taking two bitter gourd crops; one is sown in Feb.- March and crop remains in the field up to the month of July and second crop is sown in June-July and continues till the month of October. Therefore, bitter gourd crop remains in the field for about 8-9 months. Like other cucurbitacious vegetable crops, bitter gourd is susceptible to several pathogens including viruses, fungal pathogens and plant parasitic nematodes. Among the plant parasitic nematodes, root-knot nematodes *Meloidogyne* spp. are considered as first among the ten most important genera of plant parasitic nematodes causing economic losses to the crops throughout the world (Kayani *et al.,* 2013).

For any management strategy to be successful, correct identification of the pathogen is a pre-requisite. The information on prevalence of root knot nematode species associated with bitter gourd crop under Punjab conditions was lacking. Therefore, in order to quantify and document the occurrence and prevalence of root knot nematode species associated with bitter gourd crop survey of the major bitter gourd growing areas in different districts was conducted during the crop season. Identification of the *Meloidogyne* species associated with the bitter gourd crop was done on the basis of morphology and morphometric study of the mature

females and confirmation was done using species specific molecular markers.

MATERIAL AND METHODS

Prevalence, incidence and severity of root knot nematodes

Surveys were conducted in major bitter gourd growing areas falling in different districts of Punjab in order to assess the incidence and prevalence of root knot nematode in the year 2015-16. Soil and root samples of plants were taken from rhizospheric region showing nematode symptoms were brought to the laboratory for further nematode examination and population estimation. The root gall index as given by (Bridge and Page, 1980) was used for the determination of severity of root-knot nematodes. The percent root-knot nematode incidence of individual bitter gourd fields was determined as follows:

$$\text{Percent incidence} = \frac{\text{Number of infected sites}}{\text{Total number of sites surveyed}} \times 100$$

Soil samples were analyzed for the estimation of root knot nematode population using Cobb's sieving and decanting technique (Cobb, 1918; Schnidler, 1961).

Morphological identification of root knot nematode species

Morphological identification of prevailing *Meloidogyne* species associated with bitter gourd was done on the basis of perineal pattern studies of the mature females extracted from the infested root samples collected from the farmers' fields. Identification of root knot nematode specie(s) was done on the basis of female perineal patterns as described by Taylor and Netschler (1974). Further, morphometric observations of perineal patterns were also recorded to study the variation among the populations collected from different districts. Perineal pattern of ten females was studied per population and distribution of each *Meloidogyne* species was calculated in each district.

Confirmation of root knot nematode species using molecular markers

For further confirmation of most prevalent root knot nematode species associated with bitter gourd in Punjab, total DNA was extracted from root knot nematode population collected from the farmer's fields using a Proteinase K enzyme method (Williamson *et al.,* 1997). About 50 egg masses were transferred to a 1.5-ml micro tube containing 50 µl nematode lysis buffer (1X PCR buffer with 100 ìg/ml proteinase K), and crushed using a conical micro pestle before freezing at -20°C for 1 hr. Incubation was performed at 60°C for 1 hr, followed by inactivation of proteinase K by incubation at 94°C for 10 min. Centrifugation was done at 13,000 rpm for 2 min. and supernatants (DNA extracts) were taken in separate sterile tubes. Two volumes of cold absolute ethanol (-20°C) were added to the supernatants and left in the freezer at -20°C for 1 hr. The precipitated DNA was pelleted by centrifugation at 14,000 rpm for 3 min., washed with 70% ethanol, air dried for 2 hours at room temperature (25°C). The DNA pellet was re-suspended in 50 µl of tris extraction (1xTE) buffer and spectrophotometer (Thermo Scientific NanoDrop™ 1000) was used for quantitative and qualitative assessment of nucleic acid. primers specific to three main *Meloidogyne* species, *M. incognita, M. javanica,* and *M. arenaria.* The amplified PCR product was run in 1 percent agarose gel. It was then visualized and documented using UV trans-illumination (Alphaimager, USA). The results were verified against DNA marker.

RESULTS

Survey of fields for prevalence of root knot nematode in Punjab

Samples were collected from five districts; Ludhiana, Patiala, Sangrur, Ferozepur and Gurdaspur of Punjab state for prevalence and incidence of root knot nematode infecting bitter gourd crop during the year 2015-2016. A total of 118 samples were collected from the different fields (Table 1). In Ludhiana district, total thirty samples were collected out of which, fifteen samples were found to be infested with root knot nematode showing overall 44.16 percent mean incidence. Maximum nematode

Table 1. Prevalence and incidence of root knot nematode in bitter-gourd growing areas in different districts of Punjab State

S. No.	District/Villages	Crop rotation followed by the farmer	Percent incidence (%)	Mean soil population/ 250cc soil sample	RGI (0-10) scale	RKN spp. prevalent
	District Ludhiana					
1.	Punjab Agricultural University	Brinjal-Bitter-gourd- Tomato	80.00	390	6.5-8.0	*M. incognita* & *M javanica*
2.	Abbuwal	Chilli-Bitter-gourd-Okra	60.00	265	4.0-6.5	*M. incognita*
3.	Abbuwal	Cucumber-Bitter-gourd-Tomato	80.00	307	5.0-6.5	*M. incognita*
4.	Boparai kalan	Brinjal-Bitter-gourd-Onion	33.33	62	50-75	*M. incognita*
5.	Boparai kalan	Chilli-Bitter-gourd-Okra	66.66	295	5.6-6.0	*M. incognita*
6.	Boparai kalan	Okra-Bitter-gourd-Aloevera	33.33	115	2.0-4.0	*M. incognita*
7.	Dhatt	Onion-Bitter-gourd-Chilli	nil	nil	nil	
8.	Dhatt	Garlic-Bitter-gourd-Aloevera	nil	nil	nil	
		Overall mean	44.16	179		
	District Sangrur					
9.	Malerkotla	Bitter-gourd-Okra	80.00	375	7.0-8.0	*M. incognita* & *M. javanica*
10.	Malerkotla	Bitter-gourd-Chilli	80.00	385	6.0-8.0	*M. incognita*
11.	Sandhaur	Brinjal-Bitter-gourd-Tomato	100.00	525	8.0-8.5	*M. incognita*
12.	Bhani kalan	Brinjal-Jhaarkarela-Onion	nil	Nil	nil	
		Mean	65	321		
	Gurdaspur					
13.	Kot(dhar)	Bitter-gourd-Chilli	80.00	345	6.0-7.0	*M. incognita*
14.	Kot(dhar)	Bitter-gourd- Okra	60.00	262	5.0-6.0	*M. incognita*
15.	Khanpur	Bitter-gourd-Cucumber	100.00	405	7.0-8.0	*M. incognita*
16.	Khanpur	Bitter-gourd-Marigold	nil	nil	nil	
17.	Azampur	Brinjal-JhaarKarela-Tomato	33.33	82	2.0	*M. incognita*
18.	Baheri	Chilli- JhaarKarela-Onion	nil	Nil	nil	
		Overall mean	45.55	182		
	Ferozepur					
19.	Behak	Bitter-gourd-Chilli	100.00	420	6.0-8.0	*M. incognita*
20.	Behak	Bitter-gourd-Okra	100.00	395	7.0-8.0	*M. incognita*
21.	Ittanwali	Potato-Bitter-gourd	80.00	405	7.0-8.0	*M. incognita*
22.	Ittanwali	Bitter-gourd-Onion	20.00	72	2.0	*M. incognita*
		Overall mean	75		323	
	Patiala					
23.	Pattran	Bitter-gourd-Cucumber	75.00	317	6.0-7.0	*M. incognita*
24.	Pattran	Bitter-gourd-Chilli-Onion	25.00	65	2.0	*M. incognita*
25.	Amarpur	Bitter gourd-Marigold	nil	nil	nil	
26.	Amarpur	Bottle gourd-Bitter-gourd-Chilli	80.00	395	7.0-7.5	*M. incognita*
27.	Sanaur	Bitter-gourd-Brinjal-Chilli	80.00	352	6.0-7.0	*M. incognita*
28.	Banaur	Chilli-Potato-Bitter-gourd	80.00	346	6.0-7.0	*M. incognita*
		Overall mean	56.66	246		

infestation was observed in the samples collected from the Vegetable Research Farm, PAU, Ludhiana in terms of percent disease incidence (80%), soil population (360-420 J_2/250cc soil) and root gall index (RGI) (6.5-8.0) followed by samples collected from the village Abbuwal with percent disease incidence 80 percent, soil population 170-375 J_2/250cc soil and RGI ranging from 4.0-6.5. In the village Boparai kalan infested fields showed percent disease incidence ranging from 33.3 to 66.6 percent, soil nematode population ranging from 50-310 J_2/250cc soil and RGI from 2.0-6.0. Negligible root-knot nematode incidence was observed in the samples collected from village Dhatt, Ludhiana. Both the fields were found free from nematode infestation. This might be due to the cropping sequence as in both these fields garlic and onion crop was a succeeding crop to bitter gourd.

In Sangrur district, total eighteen samples were collected and twelve samples were found to be infested with root knot nematode showing mean disease incidence of 65.00 percent. Location wise maximum nematode incidence was recorded in Sandhaur village showing 100 percent disease incidence, soil population (480-570 J_2/ 250cc soil) and root gall index (RGI) (6.0-8.0). This was followed by Malerkotla, with disease incidence 80 percent, soil population ranging from 300-470 J_2/250cc soil and RGI from 8.0-8.5. In the village Bhani kalan, all the samples collected were found to be free from nematode attack. The less infestation may be due to the cultivation of wild bitter gourd, *M. balsamina i.e. Jhaar karela* in these fields which is reported to possess resistance against root knot nematode.

Total twenty six samples were collected from Gurdaspur district, out of which thirteen samples were found to be infested with nematode showing 45.55 percent mean disease incidence in this district. Maximum disease incidence was seen in village Khanpur, with soil population (370-440 J_2/250cc soil) and RGI from 7.0-8.0 followed by samples collected from the village Kot , Dhar with 70 percent disease incidence, soil population ranging from 230-365 J_2/250cc soil) and RGI ranging from 5.0-7.0. In the village Azampur all the fields surveyed were found to be infested however percent disease incidence recorded was 33.3 percent, soil nematode population ranged from 75-90 J_2/250cc soil with RGI (2.0). Negligible root knot nematode attack was recorded in the samples from village Baheri, Gurdaspur. The less infestation in both (Azampur and Baheri) villages might be due to the cultivation of *Jhaar karela* (*M. balsamina*) as a crop rotation with chilli, tomato, brinjal and onion.

In Ferozepur district out of twenty samples taken, fifteen samples were found to be infested showing 75 percent mean incidence. The village Behak showed maximum nematode incidence with percent disease incidence 80 percent, soil population (340-500 J_2/250cc soil) and RGI from 6.0-8.0 followed by samples collected from Ittanwali with percent disease incidence ranging from 20-80 percent, soil population from 60-440 J_2/250cc soil and RGI ranging from 2.0-8.0.

Fifteen soil samples were found to be infested with root knot nematode out of twenty eight samples collected from Patiala district showing overall 56.66 percent incidence in the district. Village Amarpur showed maximum infestation in terms of percent disease incidence (80%), soil population (380-410 J_2/250cc soil) and root gall index (RGI) (7.0-7.5) followed by village Sanaur with 80 percent disease incidence, soil population 320-385 J_2/250cc soil and RGI 6.0-7.0. In the village Pattran, lowest nematode attack was recorded showing percent disease incidence (25 to 75 percent), soil nematode population (55-345J_2/250cc soil) and RGI from 2.0-7.0. Low root knot nematode infestation was observed in the fields where Marigold crop was taken in the cropping sequence by the farmers.

Samples collected from Ludhiana (Vegetable Farm of PAU) and Sangrur (Malerkotla) showed the presence of both *M. javanica* and *M. incognita*. In all other soil samples *M. incognita* was found to be most prevalent root knot nematode species associated with bitter gourd. However, the samples collected from different districts during the survey showed variation regarding morphometric characters of perineal patterns (LVS= Length of vulval slit, AVS= Anus to vulval slit, ATT= Anus to tail terminus) (Table 2). LVS was recorded to be highest (18.79µm) in Patiala population followed by Ferozepur population (16.18 µm) whereas, Gurdaspur populations showed minimum LVS (14.10 µm). The length of anus to vulval slit (AVS) was maximum (19.79µm) in Ludhiana population followed by Ferozepur

Table 2: Morphometric characters of perineal pattern of populations of *M. incognita* collected during the survey (Mean±SD; range) (µm)

Characters	Ludhiana	Patiala	Ferozepur	Sangrur	Gurdaspur
LVS (µm)	14.21±1.09 (14.10-16.45)	18.79±1.87 (15.98-20.83)	16.18±1.56 (14.74-18.46)	15.69±1.98 (12.45-17.46)	14.10±1.24 (13.46-16.46)
AVS (µm)	19.79±1.38 (17.45-21.05)	15.11±3.21 (10.87-19.73)	17.54±3.09 (13.45-20.67)	12.16±2.26 (10.46-15.84)	13.66±2.52 (10.57-16.47)
ATT (µm)	14.83±1.19 (12.65-15.76)	18.18±2.43 (14.64-20.65)	18.44±1.61 (16.46-20.89)	16.37±3.03 (12.46-20.75)	11.83±1.13 (10.57-13.58)

Character ranking (CR): LVS= Length of vulval slit, AVS= Anus to vulval slit, ATT= Anus to tail terminus

population (17.54µm). The value of ATT was maximum (18.44µm) in Ferozepur population followed by Patiala population (18.18µm) while minimum (11.83µm) in Gurdaspur population.

Molecular identification

Confirmation of morphologically characterized most prevalent root knot nematode sp. was done by PCR amplification of DNA with SCAR (sequence characterized amplified regions) primers Finc/Rinc specific to *M. incognita* (Zijlstra *et al.,* 2000). The DNA extracted from *M. incognita* populations collected during survey was subjected to PCR amplification with *M. incognita* specific primer pairs Finc/Rinc along with negative control. An expected size amplicon of ~1200bp with Finc/Rinc primer was observed from all the samples while no amplification was seen in negative control (Fig. 1) which further confirmed the prevalence and association of *M. incognita* sp. with bitter gourd crop in Punjab.

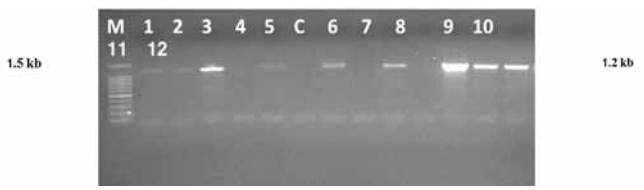

Fig. 1. 1% agarose gel showing amplicon of 1.2kb with SCAR Finc/Rinc primer pair from Samples: 1-2 (Sangrur); 3-5 (Patiala); 6-7 (Ludhiana); 8-9 (Gurdaspur); 10-11 (Ferozepur), C- control, M-marker (100bp)

DISCUSSION

Results showed that out of total twenty eight bitter gourd fields surveyed from five different districts of Punjab state, nineteen fields were found to be infested with root knot nematode. From the total one hundred eighteen samples collected, sixty six samples were found to be infested showing overall 57.27 percent mean disease incidence. About 60 percent fields were found to be highly infested with root knot nematode showing soil population >250J_2/250cc soil sample. District wise, Ferozepur showed maximum mean percent disease incidence (75.0%) with 323 J_2/ 250 cc soil followed by Sangrur (65.00%) with 321 J_2/250 cc soil, Patiala (56.66%) with 246 J_2/250 cc soil, Gurdaspur (45.55%) with 194 J_2/250 cc soil and Ludhiana (44.16%) with 171 J_2/ 250 cc soil.

Further, it was observed that more nematode infestation was recorded in the fields where susceptible vegetables like cucumber, okra, chilli and brinjal *etc*. were grown in rotation with the bitter gourd crop as compared to the fields where crops like marigold, garlic and onion crops were included in the cropping sequence. It was also observed that *Jhaar Karela* (*M. balsamina*) cultivated areas showed less nematode attack as compared with areas where cultivated bitter gourd (*M. charantia*) was grown. *Jhaar Karela* is reported to acquire resistance against root knot nematode (Pofu *et al.,* 2010). Pofu *et al.,* (2015) also revealed that *M.*

balsamina cultivated areas showed less nematode population and incidence. So this might be the reason for less nematode incidence in field where *M. balsamina* was cultivated. Marigold (*Tagetus* spp.) is well known to produce compounds such as á-terthienyl which are reported to have allelopathic properties against different species of plant parasitic nematodes including root knot nematodes (Siddiqui *et al.,* 1988; Natarajan *et al.,* 2006). Recently, Xie *et al.,* (2017) reported that for the control of root knot nematode in angelica (*Angelica sinensis*) both crop rotation and intercropping with marigold are effective however, crop rotation was more effective than intercropping. Garlic (*Allium sativum*) is known to produce allicin which have both antimicrobial and nematicidal properties (Gupta and Sharma, 2008; El-Nagdi and Youssef, 2013).

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New Distributional Record of *Steinernema hermaphroditum* (Rhabditida: Steinernematidae) from Kerala, India

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ABSTRACT: A total of 141 soil samples were collected from coconut gardens in various tracts of Alappuzha, Kollam, Pathanamthitta, Idukki and Eranakulam districts of Kerala for the natural occurrence of EPN using soil-baiting techniques with greater wax moth larvae, *Galleria mellonella*. Among these samples, 13.5% were found positive for EPN, which included three steinernematids and 16 heterorhabditids identified based on the characteristic colour of the infected cadavers. Three heterorhabditids and two steinernematid isolates were further subjected to molecular characterization by sequencing ITS region of the ribosomal DNA using 18s and 26s primers. All the three heterorhabditid species intercepted in the survey, were found to have 99% sequence similarity with *Heterorhabditis indica*. Both the steinernematid isolates were found to be belonging to the *glaseri* group. The steinernematid isolate CPCRI0804 was found non-homologous with any of the described species and, therefore, presumed to be a new species for identification. The presence of long and highly curved spicule is the striking feature of this unidentified species. One of the steinernematids (CPCRI0905) was 99% homologous with *Steinernema hermaphroditum* and is the first report of this species from South India. The identity of this isolate was confirmed based on the morphological and morphometric characters as well as the presence of first generation hermaphroditic females, which is a unique characteristic feature of *S. hermaphroditum*.

Key words: Entomopathogenic nematodes, Biological control, Hermaphroditism

The members of nematode families, Steinernematidae and Heterorhabditidae, which are lethal insect pathogens, are commonly referred as entomopathogenic nematodes (EPN) (Gaugler, 2002). The bacteria, *Xenorhabdus* sp. and *Photorhabdus* sp. respectively are symbiotically associated with these nematodes. While most of the bio-control agents are slow acting in terms of host mortality, EPNs with the help of their symbiotic bacteria are extremely quick to induce insect mortality within one or two days attracting them in wider use in pest management. Third stage juveniles, commonly known as infective juveniles (IJs) are non-feeding and only functional survival stage in the life cycle of EPN. They are naturally present in almost all types of soils and when they intervene with the suitable insect host, they enter the body either through natural openings like mouth, anus or spiracles or by direct penetration through thin cuticle. Once IJs reach the insect haemocoel, they release the bacteria, where the bacteria proliferate and induce septicemia by the production of various toxins and enzymes, which ultimately leads to host mortality. The nematodes complete its life cycle by feeding on the metabolites produced by the bacteria. EPNs are absolutely safe for human, animal or plant health and devoid of any environmental or ecological ill effects and can be used without any registration procedure. In this context EPN forms an excellent candidate in biological pest suppression especially against soil and cryptic pests (Lacey and Georgis, 2012).

Rao and Manjunath (1966) initiated the work on steinernematids in India, by importing DD-136 strain of *S. carpocapsae* for insect pest management in rice and sugarcane. Initially many researchers used exotic strains of *S. glaseri* (NC 34), *S. feltiae* and *Heterorhabditis bacteriophora* and reported bio-control potential against a wide spectrum of insect pests (Ganguly *et al*., 2006). Extensive and exploratory searches through soil-baiting technique were undertaken across the country to identify indigenous strains of EPN, as they could have enhanced adaptability, survival and virulence invading insect pests

of national importance (Ganguly *et al*., 2006; Banu *et al*., 1998). Keeping this in view, an elaborate probing for the detection of EPN was undertaken in coconut plantations of Kerala, India a state enjoying a wide distribution of rainfall and undisturbed forest niche to ascertain the presence of any new virulent species of EPN that could be commercially exploited for mass production and successfully employed in biological control of coconut pests.

MATERIAL AND METHODS

Survey and soil sample collection

Entomopathogenic nematodes occur naturally in all soil types, but they can reproduce only in the haemocoel of infected insect hosts. Soil sampling followed by baiting of samples with *Galleria mellonella* larvae (greater wax moth) is the method followed for the isolation of infective stage juveniles of EPNs (Bedding and Akhurst, 1975, Mracek, 1980). Survey was conducted in various tracts of coconut gardens in Alappuzha, Kollam, Pathanamthitta, Idukki and Eranakulam districts of Kerala for the natural occurrence of EPN during August and September 2017. Soil samples were ccollected at a depth of 5 to 15 cm using a soil auger and transferred to the laboratory in a plastic bag with appropriate labeling. All the debris from the collected soil samples were removed off before being bagged. Around 100 cc of soil was baited with three larvae of the greater wax moth, *G. mellonella* in a clean plastic container with a lid. These plastic containers were turned upside down up for a period of 15 days and regularly observed for any EPN infection every 2-3 days, Dead insects showing symptoms of EPN infection if any, were removed, cadavers rinsed in sterile distilled water and placed them on a modified White trap for recovery of nematode progeny from infected cadavers.

In vivo multiplication and storage of EPN

The recovered isolates of EPN from Kerala soil were multiplied in greater wax moth larvae and harvested using modified White trap method (Kaya and Stock, 1997). The EPN was stored in sterile double distilled water at 15 °C.

Morphological characterization

The nematode isolate intercepted from Pathiyoor, Alappuzha district, Kerala, India was subjected to morphological characterization. Twenty specimens from each life stages (Infective juveniles and adult males and females of first generation) were randomly collected from ten *G. mellonella* cadavers. Nematodes were examined live as well as after heat-relaxed in Ringer's solution at 60°C. Nematodes were fixed in triethanolamine formalin (TAF) (Courtney *et al.*, 1955) and processed to anhydrous glycerine for mounting (Seinhorst, 1959). Observations were made from live and mounted specimens using Nikon Eclipse Ni microscope equipped with differential interference contrast optics. Specimen measurements were made with a stage micrometer. Selection of morphometric characters was done according to Hominick *et al.* (1997).

Molecular characterization and phylogenetic analysis

Extraction of DNA

DNA was extracted from infective juveniles using the method reported by Hominick *et al*. (1997). The nematodes were crushed with micro pestle in 20 µl of lysis buffer (50 mM KCL, 10 mM Tris pH 8.3, 2.5 mM $MgCl_2$, 0.45% Tween 20 and 60 µg ml^{-1} proteinase K) in a sterilized 1 ml micro-centrifuge tube on ice. The tube was frozen at -20°C for 10 min, incubated at 65°C for 60-90 min, followed by 95°C incubation for 8 min. The tube was cooled on ice and centrifuged at 11600 g for 2 min. The supernatant containing the DNA was collected and kept at -20°C (Nguyen and Hunt, 2007).

PCR amplification

PCR amplification was carried out based on the method described by Nguyen and Hunt (2007). The amplification of ITS region of the ribosomal DNA was carried out in a 25 µl reaction tube by the polymerase chain reaction (PCR). Tubes were set up on ice and each tube was added with: 2.5 µl of 10X PCR buffer, 1 µl of dNTP mixture (10 mM each), 1 µl of 10 pM forward primer, 1 µl of 10 pM reverse primer, 1.5 µl $MgCl_2$ (50

mM), 0.02 µl of Taq DNA polymerase (5 U μl^{-1}), 15 µl of distilled water, and 2.5 µl of DNA. The 18S: 5'-TTGATTACGTCCCTGCCCTTT-3' (forward) and 26S: 5'-TTTCACTCGCCGTTACTAAGG-3' (reverse) primers were used (Vrain *et al*. 1992). All PCR reactions were run in a Thermocycler with the cycling profile suggested by Nguyen *et al*. (2004): 1 cycle of 94°C for 7 min followed by 35 cycles of 94°C for 60 s, 50°C for 60 s, 72°C for 60 s. The last step is 72°C for 10 min. The presence of DNA was confirmed by running 5 µl of the PCR product on a 1% agarose gel in 0.5X TBE buffer for 30-40 min and visualized by ethidium bromide staining (Maniantis *et al*., 1989).

Sequencing and Phylogenetic analysis

PCR products are purified with a QIAquick PCR purification kit. Purified DNA was sequenced in both directions using 18S and 26S primers. Molecular characterization was done by analysis of ITS region of ribosomal DNA sequences. An existing library of more than 30 *Steinernema* spp. was used for sequence comparisons and phylogenetic interpretation. Multiple sequence alignments were made using ClustalW. A phylogenetic tree was constructed with the ITS sequences using Mega ver. 6.0 by the neighbor joining method with 1000 replications for bootstrap analysis.

RESULTS

Soil sample collection and isolation of EPN

Soil samples were collected from various tracts of Alappuzha, Kollam, Pathanamthitta, Idukki and Eranakulam districts of Kerala and were processed using soil-baiting techniques with greater wax moth larvae, *G. mellonella*. Out of 141 soil samples baited, 19 (13.5%) were found positive for EPN, which included three steinernematids and 16 heterorhabditids identified based on the characteristic colour of the infected cadavers. Soil samples from Alappuzha and Pathanamthitta districts yielded EPNs with a recovery of 22.5% and 4.8%, respectively. However, steinernematids were isolated only from Pathiyoor and Krishnapuram of Alappuzha districts. All the steinernematids isolated were characterised by the presence of long infective juveniles with mean body length in the range 800 to 1000µ, which is the characteristic feature of members of *feltiae* group. The nematode isolate retrieved from Pathiyoor, Alappuzha district was identified to be *S. hermaphroditum* CPCRI0905 based on the molecular and morphological characteristics. Steinernematid obtained from ICAR-CPCRI, Regional Station, Krishnapuram, Kayamkulam (*Steinernema* CPCRI0805) did not match with any of the identified species. The details of the sampling location and isolates recovered are given in table 1.

Morphology and morphometrics

Infective juveniles

Long and slender body, gradually tapering anteriorly from the pharynx posteriorly from the anal region. Long and narrow pharynx. Average body length is 920 µ. with 73 µ long tail.

First generation male

Posteriorly curved body which took J shape when heat relaxed. Testis was single and reflexed. Paired,

Table 1. Details on the location of soil sample collection and recovery of EPNs

Sl No.	Location	No. of samples	Positive samples	EPN Species
1.	Alappuzha	80	18 (22.5%)	*Heterorhabditis* sp. (15), *Steinernema* sp. (3)
2.	Pathanamthitta	21	1 (4.8%)	*Heterorhabditis* sp. (1)
3.	Kollam	30	0 (0.0%)	
4.	Ernakulam	5	0 (0.0%)	
5.	Idukki	5	0 (0.0%)	
	Total	**141**	**19 (13.5%)**	*Heterorhabditis* sp. (16), *Steinernema* sp. (3)

brown colored and curved spicule with rectangular manubrium, short calomus, wide lamina and short velum. Gubernaculam is two third of spicule length.

Second generation male

Body length and diameter, spicule and guberanculum length were less than first generation males. Excretory pore was more anteriorly located as compared to the first generation.

First generation hermaphroditic females

Body assumed C-shape when heat relaxed. Cephalic region was truncate to slightly round and continuous with body. Stoma was short and broad with inconspicuous sclerotised walls. Excretory pore located just anterior to nerve ring. Pharynx was set off from the intestine with cylindrical procorpus, slightly swollen metacorpus, distinct isthmus and pyriform basal bulb with reduced valve. Nerve ring surrounded the isthmus or anterior part of the basal bulb. Opposed and reflexed ovaries had a well developed oviduct. Glandular spermatheca was filled with spermatozoa. Vulva located almost middle of the body with asymmetric and protruding vulval lips. Digitate tail with mucron and post anal swelling.

Second generation amphimictic females

Shorter than first generation hermaphroditic females. Spermatheca was absent and vulva with slightly protruding vulval lips. Round conoid tail without mucron and post anal swelling. All other characters were same as in first generation.

Molecular characterization

Three heterorhabditids and two steinernematid isolates were subjected to molecular characterization by sequencing ITS region of the ribosomal DNA using 18*s* and 26*s* primers. All the three heterorhabditid species intercepted in the survey, were found to have 99% sequence similarity with *Heterorhabditis indica*. Phylogenetic reconstruction of both the steinernematid isolates indicated to be belonging to the *glaseri* group. One of the steinernematids (CPCRI S0905) showed 99% identity with *Steinernema hermaphroditum* (Genbank Acc. No. JQ687355, MF663703). Ribosomal sequences for *S. hermaphroditum* (CPCRI S0905) was deposited in GenBank under the accession number MH802516.

The steinernematid isolate CPCRI S0804 showed only 92% identity with the described species and, therefore, presumed to be a new species for identification. The phylogenetic relationships between 21 species of *Steinernema* with new isolates are presented in Fig. 2. The species in the *glaseri*-group (*S. apuliae, S. arenarium, S. glaseri, S. hermaphroditum, S. guangdongense, S. longicaudum, S. lamjungense, S. khoisanae, S. diaprepesi, Steinernema* S0905 CPCRI and *Steinernema* S0804 CPCRI*)* form a monophyletic group. However, *Steinernema* S0804 CPCRI did not group with any other species, but showing more relation with members of *glaseri* group.

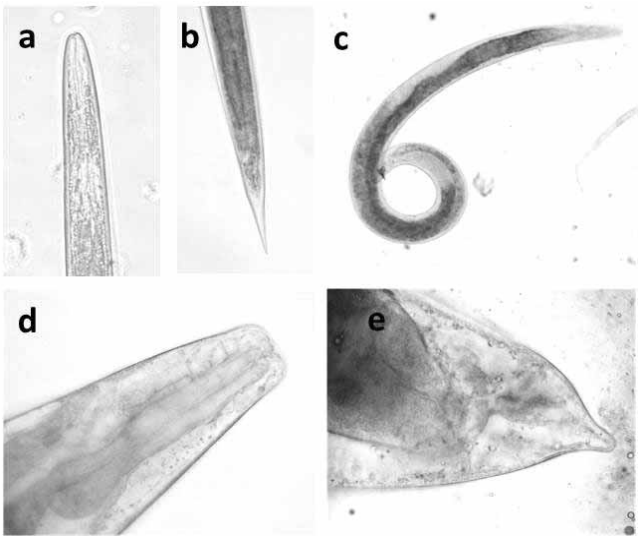

Fig. 1. a & b: Infective juvenile (a-anterior region; b-tail); c - first generation male; d & e - first generation hermaphroditic female (d-anterior region; e-tail).

DISCUSSION

Extensive surveys have been conducted worldwide as well as in India and a spectrum of EPN belonging to Steinernematids and Heterorhabditids have been reported (Josephrajkumar and Sivakumar, 1997; Nguyen *et al*., 2007; Hatting *et al*., 2009). Interception of EPN could be observed in different soil types as well as in soils with

Table 2. Morphometrics of *Steinernema hermaphroditum* CPCRI S0905. Measurements are in µm and in the form: mean ± SD (Range).

Character	Male (First Generation)	Hermaphrodite female (First Generation)	Infective juveniles
Length	2368 ± 71	8365 ± 211	922 ± 76
a (L/MBD)	-	-	24 ± 4
b (L/ES)	-	-	6.3 ± 1
C (L/T)	-	-	11.7 ± 1
c' (T/ABD)	-	-	4.0 ± 0.3
Vulval aperture	-	58 ± 4	-
Max. body diam.	130 ± 8	289 ± 11	34 ± 3
Excretory pore	86 ± 6	135 ± 9	72 ± 3
Nerve ring	128 ± 4	152 ± 5	102 ± 2
Esophagus	182 ± 9	217 ± 12	134 ± 5
Tail length	40 ± 3	68 ± 3	73 ± 2
Anal body diam.	49 ± 2	69 ± 2	18 ± 1
Spicule length	65 ± 2	-	-
Spicule width	14 ± 1	-	-
Gubernaculum length	49 ±1	-	-
Gubernaculum width	7.4 ± 1	-	-
D% (EP/ES X 100)	47 ± 4	-	53 ± 3
E% (EP/T X 100)	-	-	99 ± 6
SW% (SL/ABD X 100)	133 ± 30	-	-
GS% (GL/SL X 100)	75 ± 2	-	-
Hyaline tail	-	-	37 ± 2
H% (H/T X 100)	-	-	51 ± 1

varied content of organic matter. However, the prevalence of EPN in coastal sandy soil as compared to soil with high clay content is reported by several workers (Hara *et al*., 1991; Amarasinghe *et al*., 1994; Mason *et al*., 1996; Griffin *et al*., 2000; Jawish *et al*., 2015). The output of the present survey was in agreement with these findings as 18 out of 19 isolates were from coastal sandy soil. The exact reason for the prevalence of EPNs in coastal sandy soil is not completely understood. It is presumed to be the coastal location or sandy texture or both of them (Griffin *et al*., 2000). Sand fraction of the soil favours the survival and optimum movement of nematodes, where as clay content restricts mobility (Stock *et al*., 1999; Molyneux and Bedding 1984; Kung *et al*., 1990). Thus the host finding process becomes comparatively easier in sandy soil. Prevalence of higher insect population in washed up marine detritus which ensures availability of hosts for the survivability of EPN could favour the prevalence in marine habitat (Hominick *et al*., 1996; Griffin *et al*., 2000).

During the present survey, a total of 16 heterorhabditids and only 3 steinernematids were isolated. Predominance of Heterorhabditids over steinernematids

were reported from different parts of the world (Hara *et al*., 1991; Roman and Figueroa, 1995; Shamseldean and Abd-Elgawad, 1994; Amarasinghe *et al*., 1994; Rosa *et al*., 2000). However the dominance of Steinernematids were also documented (Garcia del Pino, 1996; Griffin *et al*., 1991; Hominick *et al*., 1995; Steiner, 1996; Sturhan and Liscova, 1999; Yoshida *et al*., 1998). The relative prevalence of any one of the genera over the other is attributed to the various soil and environmental parameters as well as the altitude of the sampling site (Rosa *et al*., 2000). Heterorhabitids were reported to be more abundant at lower altitudes, where as steinernematids prefers higher elevations (Hara *et al*., 1991). Coastal sandy habitat is reported to be most preferred habitat for heterorhabditids (Hominick *et al*. 1996; Griffin *et al*., 2000).

Over all EPN recovery (13.5%) was comparatively on higher side and most of the positive sites were from the coastal sandy belt. Natural occurrence of EPN in this part of the world could one of the reasons that the soil borne white grubs are scanty and these EPN could naturally suppress the white grub population beyond certain level. In addition, the weather factors prevailing would encourage survival of IJs in soil for a long period of time. Organic farming popularized by the Government policy adds further boon for the survival and prevalence of such natural bio-agents in the system and Kerala is known for one of the Biodiversity hotspots. Unscientific application of soil insecticides in coconut gardens is another advantageous feature to mark this accomplishment.

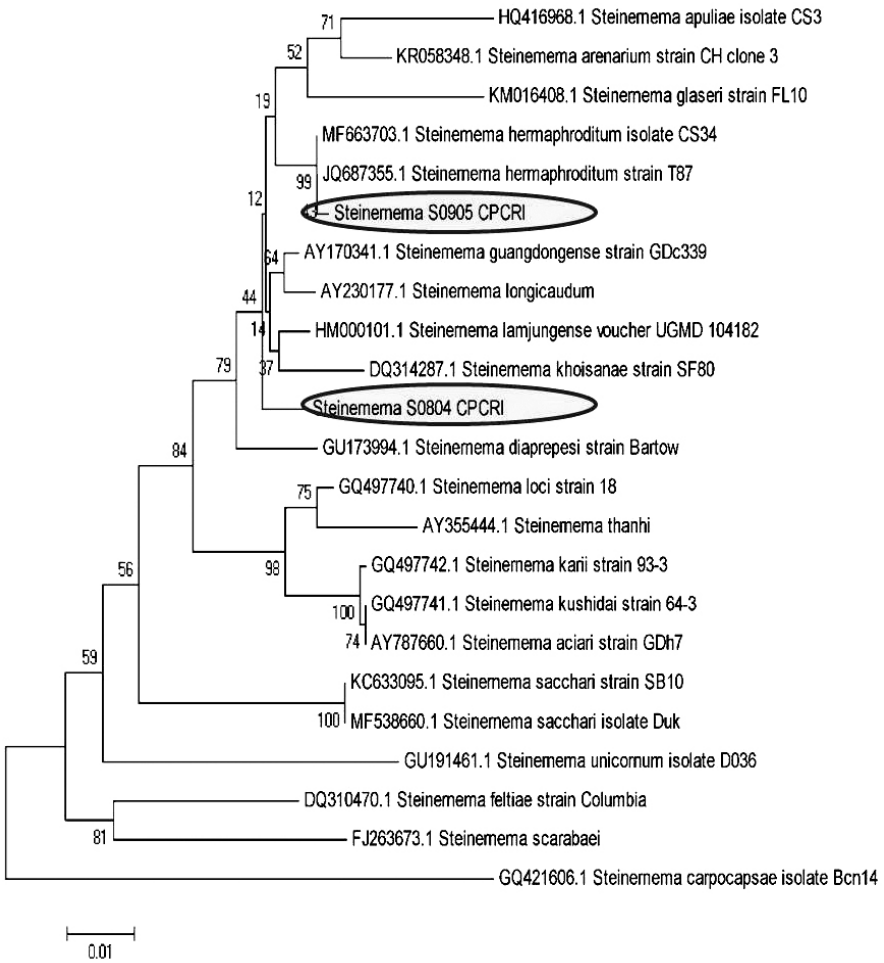

Fig. 2. Phylogenetic relationships of 23 species of Steinernema based on analysis of ITS rDNA regions. Numbers at the nodes represent bootstrap proportion for MP (below) and neighbour joining (above) (50% or more).

One of the most important achievement of this survey was the isolation and identification of *S. hemaphroditum* from coconut garden at Pathiyoor, Alappuzha district. Surveys conducted in India could not report the occurrence of this species so far and this marks the first report in the country based on morphological and molecular characterization. The identity of the isolate was initially indicated by the presence of hermaphroditic females and very rare frequency of males in the first adult generation. The morphometric characters of infective juveniles of new isolate showed more closeness with the members of feltiae group which are characterised by the length of IJs in the range between 800 to 1000µ. However, morphometrics of other stages of this isolate could delineate it from this group. Nevertheless, phylogenetic analysis indicated the closeness of this species with the members of *glaseri* group. Since all the morphological, morphometric and molecular characteristics of this isolate were in agreement with the original description, the identity of the new isolate was confirmed as *S. hermaphroditum*. This species was originally described by Patricia Stock *et al*. (2004) from Moluccan islands, Indonesia. This species is characterized by the presence of hermaphrodites in the first adult generation. Approximately 1% of the IJ developed into males and males were also present in the second adult generation, but at a very low level (1-6%) (Griffin *et al.*, 2001). Because of the hermaphroditism in this species, even the entry of a single individual in to the host insect can lead to progeny production and host mortality, which is otherwise a common characteristic feature among Heterorhabditids. Key morphological diagnostic characters of this species are: a digitate tail with a mucro and a glandular spermatheca filled with sperm in the first generation hermaphrodite; the value of D%; the morphology of the male spicules and gubernaculum and the number and arrangement of the genital papillae; the values of D%, E% and the pattern of the lateral field of the third-stage infective juvenil (Patricia Stock *et al*., 2004).

CONCLUSION

The steinernematid isolated from coconut garden of Pathiyoor, Alappuzha district was identified as *S. hermaphroditum* (CPCRI 0905) based on the molecular, morphological and morphometric characters. The sequence information of ITS region of ribosomal DNA of this isolate exhibited 99% homology with two available sequences of the species in NCBI. The presence of first generation hermaphroditic females and very rare frequency of males in both the generations is unique characteristic feature of this species. Morphological and morphometric characters are in agreement with the original description of the species. This forms the first report of this species from Peninsular India. Widespread natural occurrence of EPN from Kerala indicates the favourable niche prevailing in the region for biological suppression of soil pests in a sustainable manner. Organic Farming policy adopted by the Government adds further positive tone to this approach. All the local isolates of EPN encountered were found to be effective against red palm weevil infesting coconut, which can be effectively employed for their management.

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Efficacy of Fungal Bioagents for the Management of *Meloidogyne graminicola* Infecting Paddy

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ABSTRACT: The field experiments were carried out during summer, 2010 to 2015 to know the efficacy of bio agents viz., *Trichoderma viride, Paecilomyces lilacinus and Pochonia chlamydosporia* against root knot nematode, *Meloidogyne graminicola*. The bioagents were applied as seed treatment @10g/kg seed and in one treatment paddy seeds were treated with carbosulfan @ 3% w/w at the time of sowing. All the three bioagents were again applied in the field @ 2.5 kg/ha after 45 days of sowing of paddy seeds in the treatments where respective bioagents were applied earlier as seed treatment. In case of the treatment with Carbosulfan @ 3% w/w, Carbofuran was applied @ 1 kg *a.i.*/ha after 45 DAS. The data of all the years were pooled. The results revealed that all the treatments were significantly superior to untreated control with respect to nematode population, RKI, yield and ICBR. Treatment combination of seed treatment with Carbosulfan followed by soil application of Carbofuran at 45 DAS was most effective in reducing the nematode population, galls per plant and increasing yield (22.72 q/ha) except *T. viride* @10g/kg seed + soil application @2.5 kg/ha at 45 DAS which was at par in respect of yield. Among the bioagents, *T. viride* @10g/kg seed as seed treatment and soil application @ 2.5 kg/ha at 45 DAS was found to be most effective in reducing nematode population, galls per plant and increasing yield (22.29 q/ha). The ICBR in *T. viride* @10g/kg seed as seed treatment + soil application @2.5 kg/ha at 45 DAS (1:4.96) was found to be more as compared to Carbosulfan as seed treatment followed by soil application of Carbofuran at 45 DAS (1:1.62). This was because of the lower cost of the bioagents as compared to the chemicals.

Key words: *Meloidogyne graminicola, Trichoderma viride, Paecilomyces lilacinus, Pochonia chlamydosporia,* Carbofuran, Carbosulfan

Asia is the main rice-growing region of the world, responsible for about 90% of global rice production, which is estimated at around 740 million tons of paddy rice annually (FAOSTAT, 2013). In addition, rice is the staple food for more than 50% of the population in Asia. In India, total rice production is 104.23 million tonnes in 2015-16 from an area of 43.86 million hectares. While in Assam the total area under rice is 2.28 million hectares with the production of 4.86 million tones in 2014-2015(Anonymous, 2015).

However, various pests and diseases which constitute important constraints in the successful crop production among which plant parasitic nematodes play an important role and more than 200 species of PPNs have been reported to be associated with rice (Prot, 1994). Out of the various plant parasitic nematodes, *Meloidogyne graminicola* is the dominant species infecting rice and is a serious pest of both cereals across rice–wheat rotation areas of South Asia's Indo-Gangetic plain and of rice producing areas of Southeast Asia. *M. graminicola* has been found to infect rice in different parts of India namely, Assam, Andhra Pradesh, Karnataka, West Bengal, Orissa, Kerala, Tripura and Madhya Pradesh (Prasad *et al*., 1987) and is a serious pest of upland rice and nurseries world over in well drained soils (Rao *et al*., 1986; Gaur & Pankaj, 2010; Pankaj *et al.,* 2010).

Field-based yield loss studies have indicated that the nematode can cause yield losses between 20 and 80% (Bridge and Page, 1982 and Padgham *et al*., 2004). Netscher and Erlan (1993) reported that *M. graminicola* caused 28 to 87% yield loss in upland rice in Indonesia. Jairajpuri and Baqri (1991) reported grain yield losses from 16 to 32%. Soriano *et al*. (2000) recorded 11 to 73% yield losses by this nematode under simulation of intermittently flooded rice, whereas under simulated upland conditions, yield loss varied between 20 and 98%

(Tandingan *et al*., 1996). Padgham *et al* (2004) also reported 16 to 20% yield loss caused by *M. graminicola* in low land rainfed rice in Bangladesh. In Assam, this nematode has been reported to cause an avoidable loss in yield to the tune of 20.60 per cent ((Anonymous, 2013).

Use of chemical nematicide is commonly adopted method for managing *M. graminicola* in rice. Several studies supported the effectiveness of carbofuran to control *M. graminicola* in rice (Dang-ngoc Kinh *et al*., 1982 and Prasad and Rao, 1985). However, nematode management strategies have moved towards partial or complete avoidance of chemicals due to groundwater contamination and other environmental hazards. This necessitates efforts to find alternative methods of nematode management in rice. Presently, field application of biological control agents is emerging as a promising alternative strategy in the management of nematodes and also regarded as an important component of integrated nematode management system. Among the various biocontrol agents reported to manage plant parasitic nematodes, the chitinolytic microbes such as *Trichoderma viride* (Priya, 2015) and egg parasitic fungi, *Purpureocillium lilacinum* (Thom.) Samson, appear to be ideal agents for the control of rice nematodes because they are known to survive better under clay soils (Seenivasan, 2011 and Priya, 2015) in which the irrigated rice is normally grown. Although soil nematicides are effective and fast-acting, they are currently being reappraised with respect to the environmental hazards and human health (Wachira *et al*., 2009). In addition to that, they are relatively unaffordable to many small scale farmers. Hence an eco-friendly and environmentally safe approach was aimed at through incorporation of bioagents in the management of root knot nematode, *Meloidogyne. graminicola* in direct seeded ahu rice under field conditions.

MATERIAL AND METHODS

The field experiments were carried out during summer, 2010 to 2015 as direct seeded ahu rice in a field naturally infested with *M. graminicola* at ICR Farm, Dept. of Nematology AAU Jorhat-13, Assam. The initial nematode population of *M. graminicola* during the years recorded to be 228 (2010), 216 (2011), 217 (2012), 212 (2013) 224 (2014) and 216 (2015) J2/200 cc of soil. The susceptible variety Luit was used for the studies. The experiments were laid out in a randomized block design (RBD) with five treatments replicated four times. The bioagents were used both as seed treatment as well as soil application. The treatments were: T_1- Untreated control, T_2- *T. viride* @10g/kg seed + soil application @2.5 kg/ha at 45 DAS, T_3- *P. chlamydosporia* @10g/kg seed + soil application @2.5 kg/ha at 45 DAS, T_4- *P. lilacinus* @10g/kg seed + soil application @2.5 kg/ha at 45 DAS and T_5- Seed treatment with Carbosulfan @ 3% w/w + soil application of Carbofuran @1 kg *a.i.*/ha at 45 DAS. *T. viride, P. chlamydosporia* and *P. lilacinus* @ 10g/kg of seed were applied as seed treatment at the time of sowing and in one treatment paddy seeds were treated with carbosulfan @ 3% w/w at the time of sowing. All the three bioagents were again applied in the field @ 2.5 kg/ha after 45 days of sowing of paddy seeds in the treatments where respective bioagents were applied earlier as seed treatment. The bioagents were applied to the soil in the form of FYM after enriching with the bioagents @ 1% w/w. For this, the required amount of bioagent was mixed with well dried FYM, moistened and incubated for 10 days by covering with a gunny sheet. Thereafter, it was mixed thoroughly before applying in the soil. In case of seeds treated with carbosulfan @ 3% w/w, carbofuran was applied @ 1 kg *a.i.*/ha after 45 DAS. The observation on final nematode population in 200 cc soil and in 5g root, number of galls/root system and grain yield per plot were recorded and incremental cost benefit ratios (ICBR) in relevant treatments were calculated. The soil population of juveniles of *M. graminicola* was determined using modified Cobb's sieving and decanting technique and root knot index at harvest was recorded according to the number of galls per root system Taylor and Sasser (1978). The data of all the years were pooled and subjected analysis of variance (ANOVA), using Microsoft office Excel Worksheet, and significant means separated, using critical difference (CD) at 5%.

RESULTS AND DISCUSSION

From the results of pooled data (Summer, 2010 to 2015) as presented in Table 1 and Table 2 it was observed that seed treatment with carbosulfan followed by soil application of Carbofuran at 45 DAS (T_5) was

most effective in reducing the soil and root nematode population (49.94 % and 45.58 % respectively), root knot index (RKI) (28.75 %) and increasing yield (23.28 %) as compared to untreated control (T_1). This treatment was significantly superior to rest of the treatments except the treatment, *T. viride* @10g/kg seed + soil application @2.5 kg/ha at 45 DAS (T_2) which was at par. There was decrease in soil and root nematode population (45.96 % and 40.25 % respectively), RKI (25.75 %) and increase in yield (20.94 %) as compared to untreated control (T_1). The yield in the treatments, T_5 and T_2 were recorded to be 22.72 q/ha and 22.29 q/ha respectively as against 18.43 q/ha in untreated control (T_1). Although the yields in the treatments T_2 and T_5 were at par, the ICBR in T_2 (1:4.96) was found to be much higher as compared to the treatment T_5 (1:1.62). This was because of the lower cost of the bioagents as compared to the chemicals.

Although the chemical nematicides are always found to provide quick and better control of plant parasitic nematodes, because of their adverse effect on human health and environment there has been growing reluctance for its use. On the other hand, the bioagents are self propagating under favorable conditions and therefore may remain in soil for long period and also produce enzymes such as chitinases which are capable of rupturing nematode egg shells that result in reduced multiplication of nematodes (Gortari and Hours, 2008). *T. viride* is known and considered as an economically viable and ecofriendly alternative to chemical nematicides against root knot nematode in various crops (Pathak *et al*., 2005). The present study indicated all the bioagents to be effective against *M. graminicola*, *T. viride* was found to be the best and was comparable to the chemicals. Priya (2015) reported that soil application of *T. viride*

Table1. Efficacy of fungal bioagents against *M. graminicola* on rice, (Pool of summer, 2010 to 2015)

Treatment	Galls in 20 seedlings	% decrease over control	Nematode population			
			Soil (200cc)	% decrease over control	Root (5g)	% decrease over control
T1	85.33		285.00		81.17	
T2	51.00	40.23	154.33	45.96	48.50	40.25
T3	56.17	34.17	174.67	38.71	57.17	29.57
T4	55.00	35.54	165.83	41.81	52.50	35.21
T5	44.67	47.65	142.67	49.94	44.17	45.58
S. Ed ±	2.35		5.93		2.62	
CD (0.05)	5.09		12.86		5.68	

Table2. Efficacy of fungal bioagents against *M.graminicola* on rice, (Pool of summer, 2010 to 2015)

Treatment	RKI at harvest	% decrease over control	q/ha	% increase over control	ICBR
T1	4.00		18.43		-
T2	2.97	25.75	22.29	20.94	1:4.96
T3	3.37	15.75	20.69	12.26	1:2.82
T4	3.15	21.25	20.86	13.18	1:3.11
T5	2.85	28.75	22.72	23.28	1:1.62
S. Ed ±	0.18		0.52		
CD (0.05)	0.39		1.13		

and *P. fluorescens* increased the yield of *Withania somnifera* and were found to be at par with carbofuran. In addition to direct antagonism, the biocontrol agents increase the activity of various defense-related enzymes and chemicals in response to pathogen infection. All plants are known to be endowed with defense genes, which are quiescent in nature and require the appropriate stimulation signals to activate them. It has been reported that biocontrol agents trigger/activate latent plant defense mechanisms in response to pathogen infection. Inducing the plant's own defense mechanism by applying biological agents is a novel strategy in nematode management. Application of bioagents was found to increase chitinase enzyme accumulation in rice plants (Vidyasekaran *et al.* 2001, Nandakumar *et al.* 2007 and Seenivasan, 2011). Chitinase is a hydrolytic enzyme that degrades chitin (a polymer of β-1,4-linked N-acetylglucosamine), a structural component of nematode egg shells (Muzzarelli, 1977). Plant chitinase have been proposed to play an important role in defense against plant parasitic nematodes (Cohn and Spiegel, 1991). Qiu *et al.* (1997) also reported that higher chitinase activity in roots was associated with resistance to *M. incognita* in soybean. Annapurna *et al*. (2018) reported increase in defense related enzymes such as peroxidase, polyphenol oxidase and phenyl alanine amoniaa lyase and total phenol in tomato plants inoculated with *T. harzianum*, *Purpureocillium lilacinum* and *Pochonia chlamydosporia* in tomato plants against *M. incognita*. They also reported highest enzymatic activity in plants inoculated with *T. harzianum*. Similar mode of action of the bioagents might be operative in the present investigation resulting in decreased nematode infection and multiplication with increased yield. In addition, the plant growth regulators, including gibberellins, cytokinins and indole – 3 acetic acid (IAA) produced by bioagents also reported to constitute a mechanism for plant growth promotion (Brown, 1974 and Lifshits *et al*., 1987). In the present investigation, it was observed that among the bioagents, *Trichoderma viride* @10g/kg seed as seed treatment and soil application @ 2.5 kg/ha at 45 DAS was found to be most effective in reducing nematode population, galls per plant and increasing yield (22.29 q/ha) with a favorable ICBR in ahu rice under Assam condition.

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Descriptions of a New and a Known Species of the Genus *Chronogaster* Cobb, 1913 (Chromadorea: Plectida: Chronogasteridae) from India

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ABSTRACT: Various sediment samples were collected from the edge of water bodies located to determine the diversity of aquatic nematodes. During the screening of sediment samples collected from Loktak Lake (a Ramsar Site) in Manipur and an estuary located in Vishakhapatnam a large number of nematodes were found. Of them, a new and a known species of the genus *Chronogaster* Cobb, 1913 were also collected. *Chronogaster loktakensis* sp.nov. is characterised by the presence of vacuolated bodies, crystalloids, faint longitudinal lines, 7-10 µm long cephalic setae, 16-28 µm long radial tubule arising from the base of stoma and tail with a terminal stout mucro surrounded by three spines. While, *Chronogaster citri* Khan & Nanjappa, 1973 is being reported with a difference found in the shape of stoma with additional information on body annules, presence of crystalloid and vacuolated bodies. A key to identification of Indian species has also been provided.

Keywords: *Chronogaster citri*; *Chronogaster loktakensis* sp.nov., India; Loktak Lake; Ramsar site.

The genus *Chronogaster* has been reported from a variety of habitats ranging from terrestrial to aquatic, freshwater to salty and thermal springs (Abebe *et al*., 2006). However, this genus has experienced a lot of lumpers and destructors of nematode taxonomy since its first description by Cobb (1913) with the type species *C. gracilis*. De Man (1921) described the genus *Walcherenia* with *W. typica* as type species. However, De Coninck (1935) synonymized *Walcherenia* with *Chronogaster* and accordingly *W. typica* was transferred to *Chronogaster*. Andrássy (1958) transferred *Cephalobus longicolis* to *Chronogaster*. Heyns and Coomans (1980) gave a detailed taxonomic history and morphology of the genus. They also reported four new species from South Africa. Later in 1983, they added further new species from West Africa, Brazil and Papua New Guinea. Further species were added to the genus by Gerlach (1956), Loof & Jairajpuri (1965), Khera (1972), Khan & Nanjappa (1972), Bajaj & Bhatti (1979), Chaturvedi & Khera (1979), Heyns and Coomans (1983), Maggenti *et. al*., (1983), Raski & Maggenti (1984), Tahseen *et al*., (1994), Saha & Lal (2001), Mounport, 2005 and Abebe *et al*., (2013).

Taxonomic keys to the genus have been proposed by Loof & Jairajpuri (1965), Heyns and Coomans (1983) and Raski & Maggenti (1984), all based on females only. Siddiqui (2003) proposed *Keralanema* with its nomino typical species *K. spinicorpus.* However, Holovachov (2004) synonymised *Keralanema* with *Chronogaster*. Holovachov and De Ley (2006) listed 48 valid species belonging to this genus.

The present paper deals with two species of *Chronogaster* collected from two aquatic habitats – freshwater and marine. *Chronogaster loktakensis* sp.nov. was collected from a freshwater habitat while, *Chronogaster citri* from a marine habitat which is a unique habitat for this species as earlier Khan & Nanjappa (1972) reported it from soil around citrus plant.

MATERIAL AND METHODS

The nematodes were extracted from moist soil samples by the sieving and decantation and modified Baermann's funnel techniques (Flegg, 1967). The extracted nematodes were killed and fixed in FA (4:1)

for 24h and then transferred to glycerine-alcohol (5 parts of glycerine: 95 parts 30% alcohol) for slow dehydration in a desiccator containing fused calcium chloride. Dehydrated specimens were mounted in anhydrous glycerine on glass slides using the wax ring method (de Maeseneer & d' Herde, 1963). All observations, drawing and photographs were made on an Olympus BX 50 DIC microscope.

Abbreviations used:

L	=	Total body length
a	=	Body length / greatest body diameter
b	=	Body length / distance from anterior end to the pharyngo-intestinal junction
c	=	Body length / tail length
c´	=	Tail length / anal body diameter
V	=	Distance of vulva from anterior end x 100 / body length
ABD	=	Anal body diameter
VBD	=	Vulval body diameter
diam	=	Diameter

RESULTS

Chronogaster loktakensis sp.nov.
Fig. (1, 2)

Measurements: In Table I

Females: Body ventrally curved upon fixation, tapering towards both the ends. Cuticle with prominent transverse striations. Striae 0.5-0.8 µm apart behind lip region, 1.0-1.2 µm at mid-body and 0.5 µm near tail tip. Lateral lines indistinct. Longitudinal lines faint. Lip region truncate, lips being separated at apex not completely fused. Cephalic setae 7-10 µm long. Amphidial apertures transverse, 3-4 µm wide, one to two annules from anterior end. Stoma 7-9 µm in length, cylindrical, with a 16-28 µm long radial tubule arising from the base of stoma. Pharynx cylindrical, terminating in a basal bulb with longitudinally serrated valve plates. Post-bulbular extension 20-35 µm long. Nerve ring at 40-55% of pharyngeal length from anterior end. Excretory pore present not visible. Cardia bean-shaped. Intestine with wide lumen. Crystalloids seen prominent in pharyngeal region. Vacuolated bodies a few, but a single specimen showed clustered vacuolated bodies below pharyngeal region. Female reproductive system mono-prodelphic, post-uterine sac short, less than one anal body diam. long. Ovary reflexed, on right side of the intestine, oocytes arranged singly in maturation zone while in two rows in germinal zone. Uterus muscular with a wide lumen, single uterine egg is seen in some specimens. Vagina swollen. Vulva transverse not sunken, vulval lips closed. Rectum 1.1-1.3 ABD long. Female tail elongate to conoid 9.6-13.3 VBD long, with three small spines surrounding one stout spike. Caudal glands and spinneret absent.

Type Habitat and locality: Sediment sample collected from eastern bank of Loktak Lake 24°33ÊN93°47ÊE, Phoubakchao, Manipur, INDIA.

Type specimens: Holotype female on slide *Chronogaster loktakensis* sp.nov./1; eight female paratypes on slides *Chronogaster loktakensis* sp.nov./2-6;deposited in the nematode collection of Department of Zoology, Aligarh Muslim University, Aligarh, India.

Diagnosis and relationship

Chronogaster loktakensis sp.nov. is characterised by a medium sized body, transverse amphids, a terminal stout mucro surrounded by three spines.

C. loktakensis sp.nov. resembles *C. spinicauda* Tahseen *et al*., 1994, *C. andrassyi* Loof & Jairajpuri, 1965 and *C. indica* Bajaj & Bhatti, 1979 in general morphology and morphometrics but it differs from *C. spinicauda* in having stouter body (*a*=17-27 *vs* 43-58), shorter pharynx (b=6-7 *vs* 4-5), crystalloid and vacuolated body (present *vs* absent) and in the number of spines at tail tip (three *vs* ten). The new species has been reported from an aquatic habitat while the *C. spinicauda* is a terrestrial species extracted from soil around roots of mango. From, *C. andrassyi* Loof & Jairajpuri, 1965 it differs in having fine annules (1-1.2 µm *vs* 2.5 µm), crystalloid and vacuolated body (present *vs* absent) and in number of spines at tail tip (3 *vs* 4). The new species further differs from *C. indica* Bajaj & Bhatti, 1979 in the

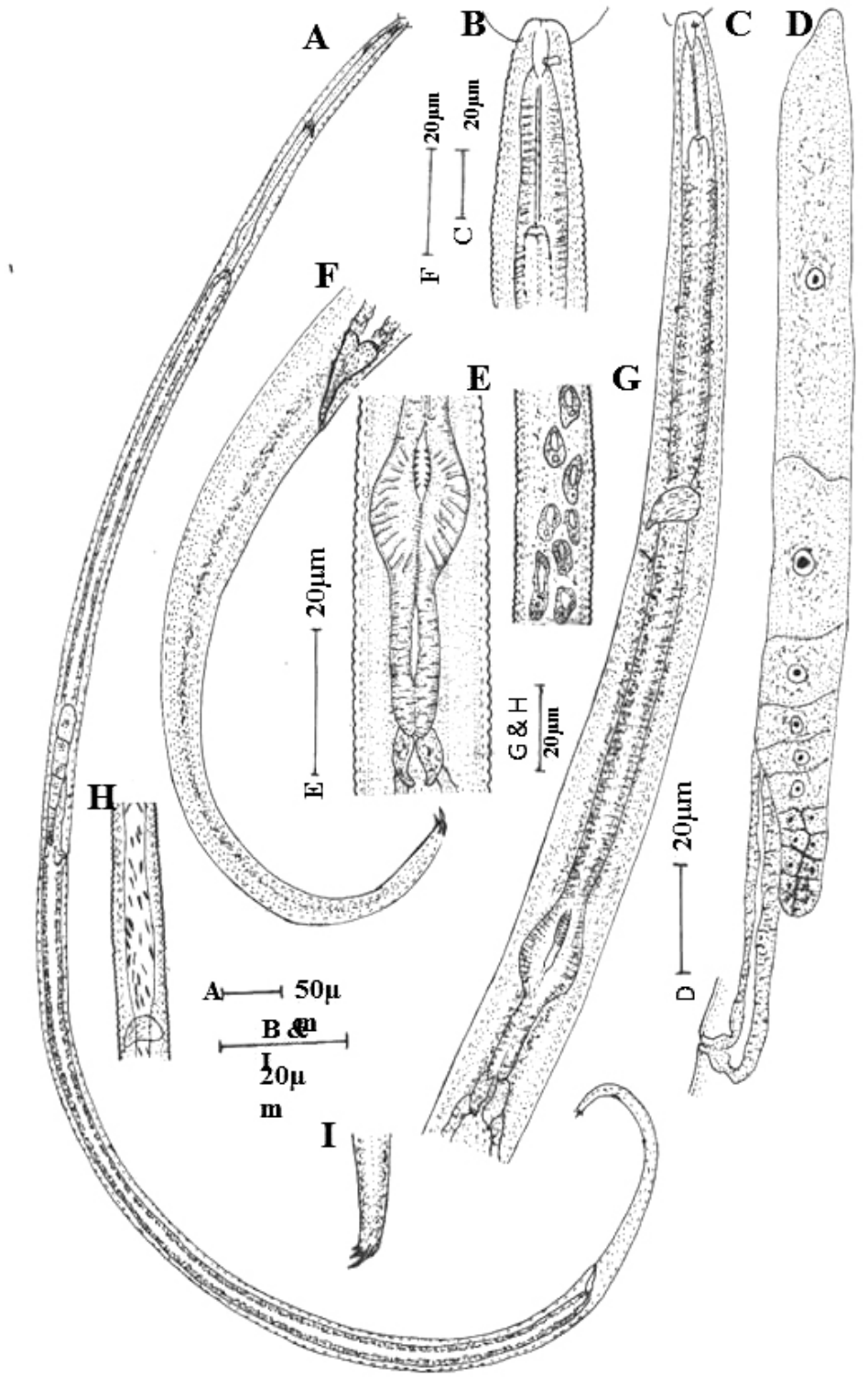

Fig.1. *Chronogaster loktakensis* sp. n. A. Entire female; B. Anterior region; C. Pharyngeal region; D. Female reproductive system; E. Basal bulb; F. Female posterior region; G. Vacuolated body; H. Pharyngeal region showing crystalloid; I. Tail tip

Table 1. Measurements (in µm) of *Chronogaster loktakensis* sp. nov.

Mean and S.D. given in parenthesis.

Characters	Holotype female	Paratype females (n = 8)
L	1372	1218–1605 (1462.5±139.3)
A	20	17–27 (22.5±3.5)
B	6.3	6–7 (6.7±0.3)
C	8.8	5–9 (6.8±0.8)
c¢	9.6	9.6–13 (11.4±1.1)
V	50	48–53 (50.0±1.5)
Maximum body width	68	59–79 (65.5±6.5)
Lip width	7	6–8 (6.5±0.8)
Lip height	2	2
Length of stoma	8	7–9(8.0±0.5)
Pharynx	218	190–290 (216.5±14.0)
Nerve ring from ant end	108	75-115 (85.4±7.3)
Pharynx base to gonad	270	220–360 (273.5±28.0)
Anterior gonad	160	100–250 (194.5±48.5)
VBD	20	17–26 (21.5±3.0)
Vulva – anus distance	520	440–755 (549.5±67.0)
Rectum	17	16–25 (18.5±1.5)
Tail	155	150–230 (174.5±16.5)
ABD	16	22-25 (23.3±1.1)

value of *a* (17-27 *vs* 43-52) and b (6-7 *vs* 4.3-5) and in tail tip (three small spines surrounding one stout spike *vs* three spines of equal length).

Etymology: The species is named after the place it was found.

Chronogaster citri Khan & Nanjappa, 1972

Fig. (2, 3)

Measurements: In Table II

Females: Body ventrally curved upon fixation, tapering towards both ends. Cuticle transversely striated. Longitudinal lines absent. Annules 1.5 µm apart behind the lip region, 1.8-2.0 µm at mid body and 1.0 µm near tail tip. Lateral lines indistinct. Lip region truncate, lips separated at apex but fused at base. Cephalic setae 8-13µm long. Amphidial apertures transverse, 3-4 µm wide, located at first annule from the anterior end. Stoma cylindrical, 6-8 µm long, radial tubule 22-30 µm long. Pharynx cylindrical terminating in a basal bulb. Post-bulbular extension 25-30 µm long. Pharyngeal lumen dilated to form a denticulated chamber with longitudinal rows of denticles in basal bulb. Nerve ring at 42-60% of pharyngeal length from anterior end. Excretory pore indistinct. Cardia elongate. Intestine with wide lumen. Crystalloids seen more in pharyngeal region. Vacuolated bodies a few. Female reproductive system mono-prodelphic. Post-uterine sac small, 0.5-0.8 VBD long. Ovary reflexed, on right side of the intestine. Oocytes arranged singly in maturation zone and in multiple rows in germinal zone. Uterus muscular with a wide lumen, single uterine egg seen in some specimens. Vagina swollen. Vulva transverse, not sunken, vulval lips closed. Rectum 1.3-2.0 ABDs long. Female tail elongate-conoid, 10-20 ABDs long. Tail tip with a stout, 2-3 µm long terminal mucro and about 1µm long two minute spines on each side. Caudal glands and spinneret absent.

Habitat and locality: Sediment sample collected from the edge of an estuary, near Rushikonda beach, Vishakhapatnam, Andhra Pradesh, India.

Voucher specimens: Seven females on slides *Chronogaster citri* Khan & Nanjappa, 1972 / 1-

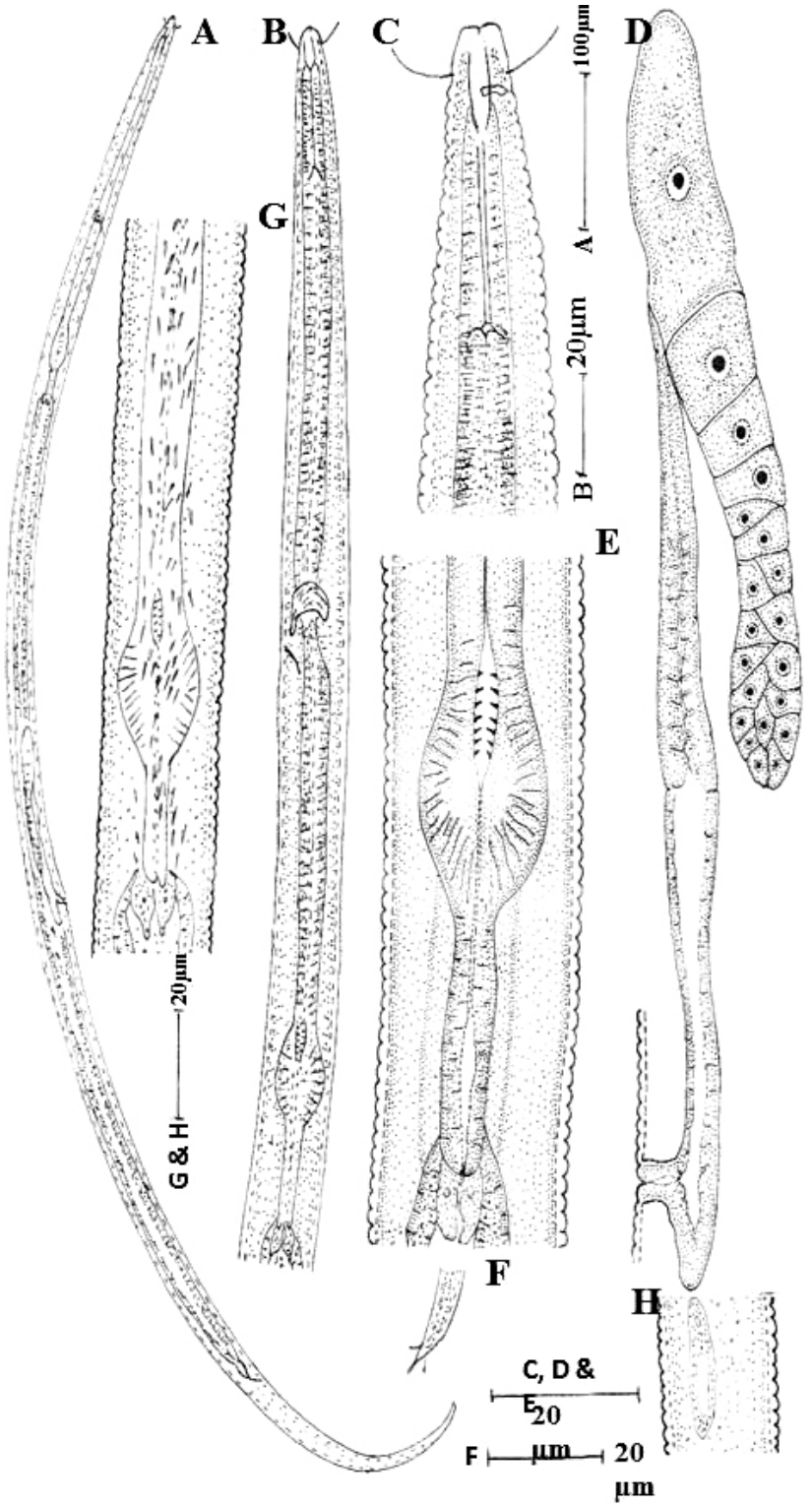

Fig. 2. *Chronogaster citri* Khan & Nanjappa, 1972. A. Entire female; B. Pharyngeal region; C. Anterior region; D. Female reproductive system; E. Basal bulb; F. Tail tip. G. Pharyngeal region showing crystalloid; H. Vacuolated body

Table 2. Measurements (in µm) of *Chronogaster citri* Khan & Nanjappa, 1972

Mean and S.D. given in parenthesis.

Characters	Females (n =8)
L	1170–1230 (1193±65.67)
a	43–55 (48±3.7)
b	4–5 (4.8±0.4)
c	4–5 (4.7±0.5)
c¢	10–20 (14.6±2.8)
V	47–52 (49.6±1.5)
Maximum body width	22–26 (24.0±1.5)
Lip width	6–8 (6.5±0.5)
Lip height	3
Length of stoma	10–15 (13±1.8)
Pharynx	195–300 (249.5±34.4)
Nerve ring from anterior end	100–130 (119±9.9)
Pharynx base to gonad	200–270 (228.0±24.5)
Anterior gonad	90–120 (105.5±9.5)
VBD	22–26 (24.9±1.5)
Vulva – anus distance	345–385 (372.5±14.5)
Rectum	16–25 (21.5±2.5)
Tail	145–240 (215.0±29.5)
ABD	12–16 (14.5±1.0)

5deposited in the nematode collection of the Department of Zoology, Aligarh Muslim University, Aligarh, India.

The present population resembles the type population of Khan & Nanjappa (1972) in general morphometric, morphological characters and body size but differs in shape of stoma (barrel shaped *vs* anteriorly constricted stoma), vulva (closed *vs* sunken). Crytalloid and vacuolated body was also observed in our population. Khan & Nanjappa, 1972 described the species as a terrestrial species extracted from soil around roots of grapevine (*vs* marine habitat).

Key to Indian species of *Chronogaster*

1. Lateral field with four lines……*C. chilkensis*
   Lateral lines indistinct……2
2. Tail with ventral mucro without spines...*C. neotypica*
   Tail with spines……..3
3. Tail tip (claw-like) with three spines of equal length .....*C. indica*
   Tail tip with mucro and spines……4
4. Tail tip with three spines and a mucro...*C. loktakensis* sp.n
   With ten spines and a mucro ……*C. spinicauda*
5. Amphidial apertures circular ……..…6
   Amphidial apertures stirrup-shaped……7
6. Tail tip a single mucro and two spines..*C. bengalensis*
   Single axial mucro …….....*C. vacouli*
7. Annules coarse, 2.4 µm at mid body, L= 1.25-1.37mm; terminus with four spines, conoid tail tip……..C. *andrassyi*
   Annules fine 2.0, L= 1.07-1.00mm; terminus with two spines, conoid tail tip……....*C. citri*
8. b=4-4.7, c=5-8……...9
   b=4.3-5.3, c=4.6-9……10
9. Longitudinal incisors 18, tail terminus finely rounded, L= 1.44mm.....*C. alata*
   Longitudinal incisors absent, tail terminus with a large dorsal and small ventral mucro; body length less than 0.8mm……*C. doai*
9. Annules coarse (<3µm), pbl<18,cephalic setae=7-8µm terminus a spine like extension ……*C. loofi*
   Annules fine (1-2 µm), pbl<10, cephalic setae=5 µm single mucro……*C. bigubernaculum*

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Bio-Efficacy of Phytotherapeutic Substances Against *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *cucumerinum* Affecting Cucumber in Polyhouse under Protected Cultivation

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ABSTRACT: The nematicidal effect of neem seed kernel extract (NSKE), neem, aak and castor leaves was tested against root-knot nematode, *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *cucumerinum*. Under the polyhouse conditions, all the treatments reduced the disease severity and enhanced the plant growth as compared to untreated inoculated check. Application of neem seed kernel extract (NSKE) was more effective as compared to neem leaves. Plant height, dry shoot weight, dry root weight, were significantly reduced as a results of infection with *M. incognita* and *F. oxysporum* f. sp. *cucumerinum*. However, application of phytotherapeutic substances recovered this reduction. Moreover, they enhanced the growth parameters viz., plant height, dry shoot weight, dry root weight compared to control. Our results proved that application of different phytotherapeutic substances, neem seed kernel extract (NSKE), neem, aak and castor leaves not only had a suppressive effect on the root knot nematode, but also enhanced the plant growth parameters, supplying nutritional elements to the plants. Among phytotherapeutic substances, *A. indica* seed kernel extracts were found to be most effective in suppressing galling and final population in soil and fungus wilt incidence after 30th day of germination followed by mustard and aak leaves as compared to untreated inoculated check.

Keywords: *Meloidogyne* spp., cucumber, neem seed kernel extract, neem leaves, aak leaves and castor leaves, fungus

Cucumber (*Cucumis sativus* L.) is widely cultivated crop in the gourd family Cucurbitaceae grown all over the world due to a good source of vitamins, minerals, fiber and roughages (Weng, 2011). Though in the polyhouses, crops are grown under protected conditions, yet they are not so protected. Polyhouse cultivation involves intensive cultivation of crops, optimum use of fertilizers and frequent use of irrigation. But due to continuous growing of the same crop with high day temperature and relative humidity within the greenhouse, polyhouse and low tunnel along with poor plant hygienic conditions inside and outside the greenhouse increase problem of soil borne pests and diseases including plant parasitic nematodes (Minuto *et al*., 2006) which results in the availability of ideal conditions for the growth and multiplication of these pests.

Under polyhouse cultivation, crops are attacked by a number of pests and diseases including nematodes which interfere with the successful cultivation under protected conditions ((Hanafi and Papasolomontos, 1999; Greco and Esmenjaud, 2004). Among the nematodes, root-knot nematode (*Meloidogyne* spp.) is the most damaging under polyhouse conditions, parasitizing almost all the polyhouses crops (Sharma *et al*., 2007). A frequency of occurrence of root-knot nematode under protected conditions was recorded to be 63.15% and population density range was 30-10000 j2/ 200cc soil (Patil *et al*. 2017). The damage becomes very severe in association with fungi (Abawi 1998). Though, yield loss due to this nematode is difficult to predict, approximate yield loss due to this nematode has been predicted by many authors in various crops (Kruger 2007). Another

important biotic stress to which the crop exposed is the fungus, *Fusarium oxysporum* f. sp. *cucumerinum.*

Generally, root knot nematode-fungus complex is considered to be one of the important factors responsible for the reduced crop production under field and polyhouse conditions. However, very little work has been done on the management of nematode-fungus disease complex in cucumber under polyhouses conditions. This research proposed to study the management nematode-fungus disease complex in cucumber under protected conditions by using different phytotherapeutic substances.

MATERIALS AND METHODS

Pure culture of root-knot nematode *Meloidogyne incognita* was maintained in the pots planted with tomato in the Nematology Laboratory. Fresh J2 were obtained from the eggs isolated from the infected roots and used in the experiment as required.

Pure culture of *F. oxysporum* f. sp. *cucumerinum*, isolated from the infested plants during random survey of polyhouses was maintained on PDA in petriplates at 27±1 °C. In order to produce mass-culturing pure culture of the fungus was grown on sand maize meal medium (700gm sand + maize meal 300gm + 150ml distilled water). The flasks and polypropylene bags were incubated in a BOD incubator at a temperature of 27±1°C for 15 days. During incubation, the flasks were shaken three times in a day, to ensure proper growth of the fungal mycelium on the sand maize meal medium.

Experimental procedure

Experiment was conducted in polyhouse (26.7±3°C, 73.5 % ± 11% Relative Humidity and 0.918 kPa) in earthen pots (18 cm diameter) filled with a mixture of autoclaved sandy loam soil (sand 70%, silt 22% and clay 8%, pH 7.5). Sterilized autoclaved soil was filled in pots (1 kg capacity). Autoclaved soil was subsequently infested with root-knot nematode (1000 J_2/kg soil) and fungus (50g /kg soil). Uninoculated pots and nematode + fungus inoculated pots served as controls. Chopped leaves of neem, aak, castor and neem seed kernel powder each @ 20 & 30 g/pot. Carbofuran 0.1 g/pot and Bavistin 2 g/1 water were used as chemical treatments. A waiting period of ten days was given before sowing for decomposition of leaves. Each pot was sown with cucumber cv. sania 5 seeds per pot. One plant per pot was retained after 30 days. The experiment was terminated 60 days after germination. Each treatment was replicated four times in a completely randomized block design during the months of April to June, 2015 in the polyhouse under protected conditions.

Evaluations were performed 60 days after sowing on plant growth parameters (shoot length, fresh and dry shoot and root weight). Observations were recorded on the root population, number of galls per plant, number of egg masses per plant, final nematode population per 200cc soil. Per cent wilt incidence due to fungus was also assessed. The data were subjected to one or two factorial Completely Randomized Design (CRD) using OPSTAT programme available on-line at CCS HAU, Hisar University website (www.hau.ernet.in).

RESULTS

Effect of different treatments on the plant growth parameters

The data (Table 1) indicated that shoot length in all the treatments were significantly better over untreated inoculated checks, nematode alone, fungus alone and nematode + fungus simultaneously. Among the various treatments, maximum shoot length was observed in neem seed kernel powder (NSKP) 30 g per kg soil, followed by neem leaves 30 g per kg soil irrespective of whether nematode or fungus inoculated individually or concomitantly. The data (Table 2) expressed that dry shoot weight in all the treatments was significantly better over untreated inoculated checks, nematode alone, fungus alone and nematode + fungus simultaneously. Among the various treatments, maximum dry shoot weight was observed in NSKP 30 g per kg soil, followed by neem leaves 30 g per kg soil irrespective of whether nematode or fungus inoculated individually or concomitantly. In plants inoculated with nematode and fungus concomitantly, dry shoot weight was maximum in case of NSKP followed by neem leaves as compared to untreated inoculated check.

Table 1. Effect of soil treatment with phytotherapeutic substances on shoot length (cm) of cucumber infested with *M. incognita* and fungus

Treatments	Shoot length (cm)			
	Nematode alone	Fungus alone	Nematode + fungus	Pooled Mean
T1: Neem leaves 20 g/ pot	133.3	128.8	126.1	129.4
T2: Neem leaves 30 g/ pot	151.9	147.1	145.9	148.3
T3: Aak leaves 20 g/ pot	128.6	124.1	122.3	125.0
T4: Aak leaves 30 g/ pot	143.5	139.5	136.7	139.9
T5: Castor leaves 20 g/ pot	123.0	119.4	116.5	119.6
T6: Castor leaves 30 g/ pot	138.9	134.8	132.2	135.3
T7: Soil treatment with NSKP 20 g/ pot	147.8	143.3	140.5	143.8
T8: Soil treatment with NSKP 30 g/ pot	157.3	153.9	150.9	154.0
T9: Carbofuran 0.1 g/ pot	143.4	121.6	140.1	135.0
T10: Drenching with bavistin 2 g/ l water	119.0	160.3	114.9	131.4
T11: untreated check (inoculated)	90.6	93.3	80.5	88.1
T12: untreated check (uninoculated)	162.5	164.8	165.0	164.1
Mean	136.6	135.9	131.0	

Critical Difference at 5% level, Treatment: 1.7, Sub treatment: 3.5, Treatment X Sub treatment: 6.1

Table 2. Effect of soil treatment with phytotherapeutic substances on dry shoot weight (g) of cucumber infested with *M. incognita* and fungus

Treatments	Dry shoot weight (g)			
	Nematode alone	Fungus alone	Nematode + fungus	Pooled Mean
T1: Neem leaves 20 g/ pot	14.06	13.18	12.74	13.3
T2: Neem leaves 30 g/ pot	21.37	18.98	17.25	19.2
T3: Aak leaves 20 g/ pot	13.25	12.01	11.11	12.1
T4: Aak leaves 30 g/ pot	16.83	15.75	14.75	15.8
T5: Castor leaves 20 g/ pot	12.25	10.97	10.25	11.2
T6: Castor leaves 30 g/ pot	15.33	14.75	13.57	14.5
T7: Soil treatment with NSKP 20 g/ pot	18.15	17.49	16.00	17.2
T8: Soil treatment with NSKP 30 g/ pot	24.24	22.34	21.75	22.8
T9: Carbofuran 0.1 g/ pot	19.50	12.25	18.49	16.7
T10: Drenching with bavistin 2 g/ l water	11.28	24.25	10.76	15.4
T11: untreated check (inoculated)	5.60	5.04	4.67	5.1
T12: untreated check (uninoculated)	25.24	26.01	25.00	25.4
Mean	16.4	16.1	14.7	

Critical Difference at 5% level, Treatment: 1.2, Sub treatment: 2.4, Treatment X Sub treatment: 4.3

The data (Table 3) revealed that dry root weight in all the treatments was significantly better over untreated inoculated checks, nematode alone, fungus alone and nematode + fungus simultaneously. Among the various treatments, maximum dry root weight was observed in NSKP 30 g per kg soil, followed by neem leaves 30 g per kg soil irrespective of whether nematode or fungus inoculated individually or concomitantly. However, in plants inoculated with nematode and fungus concomitantly, dry root weight was maximum in case of NSKP followed by neem leaves as compared to untreated inoculated check.

Effect of different treatments on the nematode reproduction

The data (Table 4) indicated that number of galls per plant in all the treatments was significantly reduced over untreated inoculated checks, nematode alone and nematode + fungus simultaneously. Among the various treatments, minimum number of galls per plant was observed in neem seed kernel powder (NSKP) 30 per kg soil (164), followed by neem leaves 30 g per kg soil irrespective of whether nematode inoculated individually or concomitantly. However, in plants inoculated with nematode and fungus concomitantly, number of galls per plant was minimum in case of NSKP, followed by neem leaves as compared to untreated inoculated check. Among the various treatments, minimum number of egg masses per plant was observed in NSKP 30 per kg soil, followed by neem leaves 30 g per kg soil irrespective of whether nematode inoculated individually or concomitantly. However, in plants inoculated with nematode and fungus concomitantly, number of egg masses per plant was minimum in case of NSKP, followed by neem leaves as compared to untreated inoculated check.

The data (Table 5) revealed that nematode population J_2/200 cc soil in all the treatments was significantly reduced over untreated inoculated checks *viz*., nematode alone and nematode + fungus simultaneously. Among the various treatments, minimum nematode population J_2/200 cc soil was observed in NSKP 30 per kg soil,

Table 3. Effect of soil treatment with phytotherapeutic substances on dry root weight (g) of cucumber infested with *M. incognita* and fungus

Treatments	Dry root weight (g)			
	Nematode alone	Fungus alone	Nematode + fungus	Pooled Mean
T1: Neem leaves 20 g/ pot	3.95	3.47	3.69	3.7
T2: Neem leaves 30 g/ pot	7.06	6.44	6.26	6.5
T3: Aak leaves 20 g/ pot	3.36	3.28	3.13	3.3
T4: Aak leaves 30 g/ pot	5.20	4.04	4.15	4.4
T5: Castor leaves 20 g/ pot	3.24	3.12	2.70	3.1
T6: Castor leaves 30 g/ pot	4.91	3.82	3.75	4.2
T7: Soil treatment with NSKP 20 g/ pot	5.52	4.28	4.44	4.8
T8: Soil treatment with NSKP 30 g/ pot	7.96	7.27	7.22	7.5
T9: Carbofuran 0.1 g/ pot	6.09	3.05	5.36	4.8
T10: Drenching with bavistin 2 g/ l water	3.07	5.75	3.27	4.1
T11: untreated check (inoculated)	1.75	2.24	1.34	1.2
T12: untreated check (uninoculated)	9.00	9.01	8.76	8.9
Mean	5.1	4.7	4.6	

Critical Difference at 5% level, Treatment: 0.37, Sub treatment: 0.75, Treatment X Sub treatment: 1.3

Table 4. Effect of soil treatment with phytotherapeutic substances on number of galls/ plant of cucumber infested with *M. incognita* and fungus

Treatments	Number of galls/plant		
	Nematode alone	Nematode + fungus	Pooled Mean
T1: Neem leaves 20 g/ pot	209 (14.5)	217 (14.7)	213 (14.6)
T2: Neem leaves 30 g/ pot	172 (13.2)	177 (13.3)	174.5 (13.2)
T3: Aak leaves 20 g/ pot	221 (14.9)	228 (15.1)	224.5 (15.0)
T4: Aak leaves 30 g/ pot	186 (13.7)	190 (13.8)	188 (13.7)
T5: Castor leaves 20 g/ pot	227 (15.2)	238 (15.4)	232.5 (15.3)
T6: Castor leaves 30 g/ pot	193 (13.9)	198 (14.1)	195.5 (14.0)
T7: Soil treatment with NSKP 20 g/ pot	177 (13.3)	183 (13.5)	180 (13.4)
T8: Soil treatment with NSKP 30 g/ pot	162 (12.8)	167 (12.9)	164.5 (12.9)
T9: Carbofuran 0.1 g/ pot	153 (12.4)	146 (12.1)	149.5 (12.3)
T10: Drenching with bavistin 2 g/ l water	230 (15.2)	215 (14.7)	222.5 (14.9)
T11: untreated check (inoculated)	328 (18.1)	313 (17.7)	320.5 (17.9)
T12: untreated check (uninoculated)	0 (1.0)	0 (1.0)	0.0 (1.0)
Mean	13.2	13.2	

Figures in parenthesis are the square root ("n+1) transformed values, Critical Difference at 5% level, Treatment: 0.03, Sub treatment: 0.07, Treatment X Sub treatment: 0.10

Table 5. Effect of soil treatment with phytotherapeutic substances on final nematode population/ 200 cc soil of cucumber infested with *M. incognita* and fungus

Treatments	Final nematode population/200 cc soil		
	Nematode alone	Nematode + fungus	Pooled Mean
T1: Neem leaves 20 g/ pot	295 (17.2)	300 (17.3)	297.5 (17.3)
T2: Neem leaves 30 g/ pot	261 (16.2)	267 (16.4)	264 (16.3)
T3: Aak leaves 20 g/ pot	312 (17.7)	319 (17.9)	315.5 (17.8)
T4: Aak leaves 30 g/ pot	276 (16.6)	281 (16.8)	278.5 (16.7)
T5: Castor leaves 20 g/ pot	317 (17.8)	327 (18.1)	322 (18.0)
T6: Castor leaves 30 g/ pot	282 (16.8)	288 (17.0)	285 (16.9)
T7: Soil treatment with NSKP 20 g/ pot	271 (16.5)	276 (16.6)	273.5 (16.6)
T8: Soil treatment with NSKP 30 g/ pot	248 (15.8)	252 (15.9)	250 (15.8)
T9: Carbofuran 0.1 g/ pot	167 (12.9)	235 (15.4)	201 (14.2)
T10: Drenching with bavistin 2 g/ l water	516 (22.7)	498 (22.3)	507 (22.5)
T11: untreated check (inoculated)	694 (26.4)	656 (25.6)	675 (26.0)
T12: untreated check (uninoculated)	0 (1.0)	0 (1.0)	0.0 (1.0)
Mean	16.5	16.7	

Figures in parenthesis are the square root ("n+1) transformed values, CD at 5% level, Treatment: 0.03, Sub treatment: 0.07, Treatment X Sub treatment: 0.1

followed by neem leaves 30 g per kg soil irrespective of whether nematode inoculated individually or concomitantly. However, in plants inoculated with nematode and fungus concomitantly, nematode population J_2/ 200 cc soil were minimum in case of NSKP, followed by neem leaves as compared to untreated inoculated check.

Effect of different treatments on the disease incidence

The data (Table 6) revealed that disease incidence of fungus was reduced significantly in all treatments on cucumber as compared to untreated inoculated checks. Data were recorded 15 and 30 days after sowing. At 30 days after sowing, disease incidence was minimum in case of soil treated with neem seed kernel powder (NSKP) followed by neem leaves as compared to untreated inoculated check while it was maximum in case of carbofuran. Data (Table 7) indicated that disease incidence of nematode and fungus complex was reduced significantly in all treatments on cucumber as compared to untreated inoculated checks. At 30 days after sowing, disease incidence was minimum in case of soil treated with NSKP followed by in case of neem leaves as compared to untreated inoculated check while it was maximum in case of carbofuran.

DISCUSSION

The present investigations showed that the phytotherapeutic substances were effective in reducing *M. incognita* infection, per cent disease incidence and increasing plant growth parameters as compared to untreated inoculated checks.

Shoot length in all the treatments was significantly better over untreated inoculated checks (nematode alone, fungus alone and nematode + fungus simultaneously). Maximum dry shoot weight was observed in treatments of neem seed kernel powder (NSKP) 30 g per kg soil and neem leaves 30 g per kg soil our findings are similar to the

Table 6. Effect of soil treatment with phytotherapeutic substances on fungal incidence (%) of cucumber infested with *F. oxysporum* f. sp. *cucumerinum*

Treatments	% Fungal incidence		
	Pre emergence damping off (after 15 days)	Pre emergence damping off (after 30 days)	Pooled Mean
T1: Neem leaves 20 g/ pot	35 (36.5)	40 (39.5)	37.5 (38.0)
T2: Neem leaves 30 g/ pot	20 (26.9)	25 (30.1)	22.5 (28.5)
T3: Aak leaves 20 g/ pot	35 (36.5)	40 (39.5)	37.5 (38.0)
T4: Aak leaves 30 g/ pot	25 (30.0)	30 (33.2)	27.5 (31.6)
T5: Castor leaves 20 g/ pot	40 (39.5)	45 (42.4)	42.5 (40.9)
T6: Castor leaves 30 g/ pot	30 (33.2)	35 (36.5)	32.5 (34.9)
T7: Soil treatment with NSKP 20 g/ pot	35 (36.5)	35 (36.5)	35 (36.5)
T8: Soil treatment with NSKP 30 g/ pot	15 (23.0)	20 (26.9)	17.5 (25.0)
T9: Carbofuran 0.1 g/ pot	40 (39.5)	45 (42.4)	42.5 (40.9)
T10: Drenching with bavistin 2 g/ l water	5 (13.6)	10 (18.6)	7.5 (16.1)
T11: untreated check (inoculated)	50 (45.3)	60 (51.1)	55 (48.2)
T12: untreated check (uninoculated)	0 (4.1)	0 (4.1)	0.0 (4.1)
Mean	30.4	33.4	

Figures in parenthesis are the angular transformed values, CD at 5% level: Treatment: 1.6, Sub treatment: 4.0, Treatment X Pathogen: N.S.

Table 7. Effect of soil treatment with phytotherapeutic substances on fungal incidence (%) of cucumber infested with *M. incognita* and fungus

Treatments	% Fungal incidence		
	Pre emergence damping off (after 15 days)	Pre emergence damping off (after 30 days)	Pooled Mean
T1: Neem leaves 20 g/ pot	30 (33.3)	40 (39.5)	35.0 (36.4)
T2: Neem leaves 30 g/ pot	20 (26.9)	25 (30.1)	22.5 (28.5)
T3: Aak leaves 20 g/ pot	30 (33.3)	40 (39.5)	35.0 (36.4)
T4: Aak leaves 30 g/ pot	25 (30.2)	30 (33.4)	27.5 (31.8)
T5: Castor leaves 20 g/ pot	35 (36.5)	45 (42.4)	40.0 (39.4)
T6: Castor leaves 30 g/ pot	25 (30.2)	30 (33.4)	27.5 (31.7)
T7: Soil treatment with NSKP 20 g/ pot	30 (33.4)	35 (36.5)	32.5 (35.0)
T8: Soil treatment with NSKP 30 g/ pot	15 (23.0)	20 (26.9)	17.5 (25.0)
T9: Carbofuran 0.1 g/ pot	15 (23.0)	10 (18.6)	12.5 (20.8)
T10: Drenching with bavistin 2 g/ l water	35 (36.5)	45 (42.4)	40.0 (39.4)
T11: untreated check (inoculated)	50 (45.3)	85 (67.7)	67.5 (56.5)
T12: untreated check (uninoculated)	0 (4.1)	0 (4.1)	0.0 (4.1)
Mean	29.6	34.5	

Figures in parenthesis are the angular transformed values, CD at 5% level: Treatment: 1.5, Sub treatment: 3.8, Treatment X Sub treatment: 5.4

results obtained by Agbenin *et al*. (2004) who reported that neem seed powder increased root/ shoot weights and heights but it reduces root knot nematode galls and presence of mycelium on root.

Number of galls per plant in all the treatments was significantly reduced over untreated inoculated checks, nematode alone and nematode + fungus simultaneously. Nematode population in soil in all the treatments was significantly reduced over untreated inoculated checks. Similar finding by Adegbite and Adesiyan (2005) who reported that neem seed reduces nematode population and it is effective in causing larval mortality. Also similar finding were reported by Agbenin and Marley (2004) that number of galls, egg masses and final nematode population in soil decreased significantly in the neem seed powder treated plants as compared to the untreated plants.

At 30 days after sowing, disease incidence was minimum in case of soil treated with neem seed kernel powder (NSKP) followed by neem leaves as compared to untreated inoculated check of fungus alone and these findings confirm that NSKP can be used as natural fungicides to control *F. oxysporum* and to reduce the dependence on the synthetic fungicides. These results of the present investigation are clear indication for potential of neem to control *Fusarium* spp. Joseph *et al.* (2008) showed neem in all concentration (5, 10 and 15%) has fungicide potential. Results showed significant suppression of both *M. incognita* and *F. oxysporum* by Neem seed kernel powder. Paul and Sharma (2002) reported that the leaf aqueous extract of neem inhibited the growth of soil born pathogenic fungi. NSKP was significantly effective against both the pathogens, and it may be due to presence of toxic chemicals in neem seed powder (Singh and Sitaramaiah, 1970).

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Incidence and Population Density of Plant Parasitic Nematodes Infecting Vegetable Crops and Associated Yield Losses in Eastern Uttar Pradesh

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ABSTRACT: Plant parasitic nematodes population densities were determined in 196 root and soil samples collected from vegetables growing areas in Varanasi, Mirzapur, Kushinagar and Deoria districts of eastern U.P. Yield losses linked with nematode incidence were calculated in 19 vegetable crops including two pulse vegetable. The most abundant plant parasitic nematodes detected, in order of decreasing frequency of infestation, were *Meloidogyne incognita, Rotylenchulus reniformis, Hoplolaimus indicus, Tylenchorynchus indicus, T. brassicae, Pratylrnchus spp., Helicotylenchus spp., Xiphenema spp*. and *Longidorous* spp. Kushinagar and Deoria districts were explored first time to determine distribution of nematodes and yield losses caused by them. It is observed that yield losses ranged from 4% to 30.2% depending upon the host and nematode. The main reasons responsible for these losses were unawareness among growers/ farmers about these tiny hidden enemies of crops, lack of resistant varieties, non-availability of effective management tactics including nematicides, farm practices and monoculture crop farming on the same field. This study suggests the need for development of nematode management modules to avoid losses due to nematodes.

Key words: Plant parasitic Nematode, population density, vegetable crops, yield Loss.

Plant parasitic nematodes cause estimated annual crop losses of $ 78 billion worldwide (Barker *et al*., 1998). The estimated overall annual yield loss of world's major crops due to damage by phyto-parasitic nematodes has been reported to the extent of 12.3% (Sasser & Freckman, 1987). In USA, damage caused by these tiny organisms on 24 crops was estimated to be 11 % (Feldmesser *et al*., 1971). Specific estimates of vegetable crop yield losses caused by two important *Meloidogyne* spp. (*M. incognita* and *M. javanica*) ranged from 17 to 20% for eggplant, 18-33% for melon, 24 -38% for tomato and 25% for potato (Kathy, 2000. Jain *et al*., (2007) reported the damage to different crops due to plant parasitic nematodes in term of monetary loss is approximately 21068.73 million rupees. The presence of root-knot nematode, *Meloidogyne* spp. and other ecto-parasitic nematodes together not only increase the damage caused but also predisposes the host plants to attack by fungal and bacterial diseases. Sharma *et al*., (2002) reported that *M. incognita* is most studied nematode species in India and they ranked it first among plant parasitic nematodes recorded from India in terms of damaging economically important agricultural crops. An interaction between *M. incognita* and *Rotylenchulus reniformis* was studied by Singh *et al.*, (2007). They reported that the population density of both the nematodes was mostly competitive in nature, one establishes at the cost of other on tomato. Singh *et al*. 2011 also concluded that *M incognita* is the predominant species of nematode, infesting all vegetable crops grown at Varanasi region.

The area of study was unexplored as per plant parasitic nematodes and losses caused by them is concerned. Nevertheless, the information on the losses caused by the nematodes on vegetable crops in eastern UP is not available in the literature. The objective of this study was to to determine the identity, frequency, prominence value and population density of nematodes associated with vegetable crops and to assess the losses in yield caused by them in eastern part of UP.

MATERIALS AND METHODS

Random soil samples were drawn from root-zones of plants with the help of a shovel at the depth range of 20-25 cm. each sample was mixed thoroughly to make soil uniform in all aspects and placed in an individual plastic bag. A sub sample of 200 cc soil was processed using Cobb's sieving and decanting method followed by Baerman's funnel technique (Southey, 1986) for the extraction of soil nematodes. Aliquot of each processed sample was collected after 48 hrs and nematode were counted by taking 1 ml of this nematode suspension with the help of counting dish under binocular microscope. An average of three counting was considered and multiplied with total volume of the nematode suspension. A total of 196 samples were collected and processed for extraction of plan parasitic nematodes and expressed as the number of individuals per 200 cc of soil. The nematodes were identified on the basis of morphological characteristics of juveniles and adult male and female. *M. incognita* was identified by the perineal pattern of the mature female (Eisenback 1985).

Presence of nematodes in soil was determined at the time of sowing and / or transplanting (initial population). For final population presence of nematodes in vegetable roots and soil was determined at the time of harvest. Root were separated from soil carefully, washed and dried with help of face tissue. Nematodes were extracted from a fresh root composite sub sample of 25 g (McKenry & Roberts, 1985).

The criteria used to assess yield included: Growers interview, visual assessment based on foliage growth (necrotic, chlorotic, stunted and wilted plant); Root symptoms and educated guess to expert opinion; plant mortality, condition of the plant and duration of the crop and most importantly quality and quantity loss in yield based on local market considerations (obtained from nematode affected plants). The yield loss percentage was determined from the relationship between the average highest yield of 10 plant (T) with nil or less nematode infestation (below economic injury level) and the average lowest yield of 10 plants having maximum nematode infestation (t) with above economic injury level. To calculate percent loss, (t) is divided by (T), multiplied by 100. [Yield loss (%) = t/T*100]

The frequency (F) of the nematode genus was determined from the relationship between the numbers of samples in which the nematode was observed (A) divided by the total number of samples (B) taken from that area or crop, multiplied by 100 to express as a percentage (Sawadogo *et al*., 2009). (F=A/B*100). Relative density of the nematode was determined by the total number of nematode from particular area/crop divided by the total number of plant parasitic nematodes, multiplied by 100 to express as percentage.

RESULTS AND DISCUSSION

The present investigation was carried out in four districts (Varanasi, Mirzapur, Kushinagar and Deoria) of eastern UP. Ten plant parasitic nematodes were isolated and identified from the rhizosphere soil and root samples. Among them, the endoparasite/migratory endoparasite *M. incognita* and *R. reniformis* were most abundant frequently encountered nematode species. The frequency of plant parasitic nematodes was recorded in the range of 12.8 to 91.2. The maximum frequency (91.2) was recorded with root-knot nematode, *M. incognita* followed by 81.3 with reniform nematode, *R. reniformis* as evidenced by Table 1. Data showed that root-knot nematode, *M. incognita*, reniform nematode, *R. reniformis* and lesion nematode, *Pratylenchus* sp. were recorded to be infested almost all crops (Table 2). Maximum frequency (91.2%), prominence value (1676.4) and disease incidence was recorded with root-knot nematode, *M. incognita*. This study is in conformity with the previous studies related to vegetable crops in Varanasi region (Singh *et al*., 2011).

The results of the present investigation provide not only the information of major destructive nematode i.e. root–knot nematode, *M. incognita,* which was ranked first in causing disease and yield losses (Sharma *et al*., 2002) associated with vegetable crops grown in eastern UP but also indication of their occurrence, geographical distribution, and possible potential for yield and monetary losses. The yield losses caused by nematodes concomitantly with *M. incognita* is presented in Table-2. Nearly nineteen vegetable crop including pulse vegetables (cowpea and pea) of economic importance were taken into account. The yield loss from 4% to 30.2% was recorded with an average of 14.5%. The

Table 1. Frequency and population densities of plant parasitic nematodes in soil and roots of vegetable crops in eastern Uttar Pradesh.

Nematode species*	Frequency**	Prominence value	Nematode population densities (Max.)	
			200 cc soil	Per g of root
Longidorous spp.	12.8	127.8	118	(-)
Xiphinema spp.	18.2	132.3	98	(-)
Dorylaimus spp.	28.6	96.8	218	(-)
Pratylenchus spp.	30.4	266.4	42	17
Hoplolaimus indicus	37.6	387.6	390	(-)
Tylenchorynchus brassicae	40.0	144.5	64	(-)
Tylenchorynchus vulgaris	42.2	265.3	212	(-)
Helicotylenchus spp.	52.0	325.4	114	(-)
Rotylenchulus reniformis	81.3	1132.2	316	62
Meloidogyne incognita	91.2	1672.4	432	92
Saprophytes ***	100.0	-	3328	(-)

*Nematode species are listed in increasing frequency; ** Frequency (%) of nematode infested samples; ***Non stylet bearing nematode; (-) absence of nematode

ecto-parasites and/or migratory ecto-parasites including sting nematode *Balanolaimus* spp., spiral nematode, *Helicotylenchus* spp., lance nematode, *Hoplolaimus* spp. needle nematode, *longidorous* spp., stubby root nematode, *Paratrichodorus* spp., stunt nematode, *Tylenchorynchus* spp. and dagger nematode, *Xiphenema* spp. have been recorded to be damaging nematode pests of many vegetable crops as they cause destruction of epidermis during feeding (Cooke, 1989, McKenry *et al.* 2001). However, in India, no such studies were conducted which indicate the losses caused by ecto-parasitic and/or migratory ecto-parasitic alone and/or concomitantly with endo-parasitic and migratory endo-parasitic nematode infesting vegetable crops under naturally infested field.

The data presented in Table 2 showed yield losses due to concomitantly association of endo-parasites as well as ecto-prasites present in the same rhizosphere of the same plant under field condition. Ecto-parasitic nematode species damage root tips resulted in growth arrest and reduces the ability to absorb nutrients and water (Anwar & Vangundy, 1989, Carneiro *et al.*, 2002,). Damage due to endo-parasite or migratory endo-parasites are well documented the literature and given preferences by researchers to work on them (Sharma *et al*., 2002). However, under natural infestation on fields, the interaction may be present due to two or more nematode species. Various nematode spp. association seems to be causing synergistic increase in yield loss (Singh *et al*., (2007). Vegetable production is not possible in the tropics and subtropics without considering the nematodes pests (Sikora & Fernandez, 2005) is true in the context of developing countries including India. The methods used to determine some of the information on yield loss relationships in the past suffers from the criticism that nematicides have a range of side effects.

The present study has the benefit of producing information on the relationships between initial population, final population and yield. It provides important information to extension specialists, which can be utilize to create awareness among farmers and a massage to plant protectionists to consider nematodes as major damaging pests of vegetable crops. Further investigations are needed and requires experimental errors to be minimized.

Table 2. Nematode associated with vegetable crops and related yield losses in eastern Uttar Pradesh.

Name of vegetable		Yield loss (%)	Associated nematode based on feeding habit		
Common	**Scientific Name**		**Ectoparasite (epidermal invader)**	**Semi-endoparasite (cortical feeder)**	**Endoparasite (vascular feeder)**
Solanaceous vegetables					
Chilli	*Capsicum annum*	10.8	1,3,5,6,8*	4,9	10
Egg plant	*Solanum melongena*	17.0	1,2,3,5,6,7,8	4,9	10
Tomato	*Lycopersicon esculentum*	28.0	1,2,3,5,6,7,8	4,9	10
Cucurbataceous vegetables					
Bitter gourd	*Momordica charantia*	22.0	1,3,5,6,8	4,9	10
Bottle gourd	*Lagenaria siceraria*	16.0	1,2,3,6,7,8	4,9	10
Cucumber	*Cucumis sativus*	16.2	2,3,5,6,7,	4,9	10
Pointed gourd	*Trichosanthes dioica*	26.0	1,2,5,7,8	4,9	10
Pumpkin	*Cucurbita maxima*	12.8	1,3,5,6,8	4,9	10
Ridge gourd	*Luffa acutangula*	11.0	1,5,6,8	4,9	10
Sponge gourd	*Luffa cylindrica*	9.6	2,3,7,8	4,9	10
Legume vegetables					
Cowpea	*Vigna unguiculata*	30.2	1,2,7,8	4,9	10
Pea	*Pisum sativum*	14.0	1,6,7,8	4,9	10
Cole vegetables					
Brocolli	*Brassica oleracea*	4.0	1,2	4,9	10
Cabbage	*Brassica oleracea var. capitata*	6.0	1,2,6,7,8	4,9	10
Cauliflower	*Brassica oleracea var. botrytis*	8.0	1,2,3,7	4,9	10
Root vegetables					
Carrot	*Daucus carota*	4.5	1,8	4,9	10
Leafy vegetables					
Spinach (Palak)	*Spinacia oleracea*	6.1	1,2,5,6,7,8	4,9	10
Malvaceous vegetables					
Okra	*Ablemoschus esculentus*	18.6	1,2,3,5,6,7,8	4,9	10

Note - *1. *Longidorous* spp., 2. *Xiphinema* spp., 3. *Dorylaimus* spp., 4. *Pratylenchus* spp., 5. *Hoplolaimus indicus*, 6. *Tylenchorynchus brassicae*, 7.*Tylenchorynchus vulgaris*, 8. *Helicotylenchus* spp., 9. *Rotylenchulus reniformis*, 10. *Meloidogyne incognita*.

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Nematicidal Potential of Some Botanical Products Against *Meloidogyne incognita* Infecting Eggplant

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ABSTRACT: Work experiments were designed to verify the efficacy of nine medicinal plant extracts against *Meloidogyne incognita* (root-knot nematode) infecting eggplant (*Solanum melongena* L.). The tested plants were, Turmeric (*Curcuma longa* L.), Ginger (*Zingiber officinale* Roscoe), Peganum (*Peganum harmala* L.), Thyme (*Thymus vulgaris* L.), Bitter Melon (*Citrullus colocynthis* L.), Artemisia (*Artemisia absinthium* L.), Alpinia (*Alpinia officinarum* Hance), Thuja (*Thuja orientalis* L.) and Capsicum (*Capsicum annuum* L.). Results showed that nematode population and eggplant growth parameters were affected when different plant extracts under different concentrations 1, 2 and 3% were applied. Examination of root system showed that egg-masses, galls and females affected markedly. The *P. harmala* at 3% gave the highest reduction with second stage juveniles (J2S)/250 cc soil, galls and egg-masses/root system comparing to other treatments applied and nematode alone. No phytotoxicity to eggplant plants appeared at used application rates.

Keywords: Botanical products, *Meloidogyne incognita*, eggplant, control.

Eggplant (*Solanum melongena* L.) is a member of family *Solanaceae* and widely cultivated vegetable crop in Egypt. It has a huge domestic and export demand. Nowadays it is widely grown in different seasons in open fields and under plastic house conditions especially in newly reclaimed lands. According to the report of the Food and Agriculture Organization (FAO,2016), the total cultivated area of eggplant in Egypt reached 48556 ha which yielded 1194315 tons, with an average of 245967 g/ha. High eggplant yield loss may occur when grown in sandy soils infested with high nematode population, especially in the summer season.

Plant-parasitic nematodes (PPN) are exceedingly notoriously difficult plant pathogens to control. International annual loss in eggplant due to nematodes were 16.9 % (Sasser, 1987). Root-knot nematodes are considered damaging nematode pests of many host plants (Bakr, 2014). Annual world losses occurred because of *Meloidogyne* spp. are about USD$ 100 billion (Brand *et al*., 2010). Heavy infected eggplant, melon and tomato plants give yield losses over 30% (Sikora and Fernandez, 2005). The damage level influenced by a lot of factors for example, nematode specie, level of soil infestation, host cultivar and environmental conditions.

Use of botanical pesticides is a promising method in plant and environment protection from pesticides pollution (Manju and Sankari Meena, 2015; Akhter and Khan 2018). Many plants have been obviously used as an effective means in plant nematode control when used as a soil amendment, plant extracts, grown in rotation, intercropping within susceptible crops (Olaniyi, 2015). These higher medicinal plants have some advantages as antagonistic over synthetic nematicides. They contain active components such as:, isothiocyanates, cyanogenic glycosides, lipids, alkaloids, diterpenoids, polyacetylenes, terpenoids, glucosinolates, polythienyls, quassinoids, steroids, sesquiterpenoids, triterpenoids, phenolics, and other compounds (Bakr *et al*., 2015; Khan *et al*., 2017).

The botanicals may contain no resistance history compounds by the pests, fast biodegradable in cultivated

soil or plant, narrow pre-harvest intervals (PHI) and renewable resources originally (Chitwood, 2002; Nikoletta and Menkissoglu-Spiroudi, 2011). Nowadays, botanicals use is gaining prime importance in the integrated nematode management (INM) methods because these plants produce secondary exudates of volatile or nonvolatile nature by stem, root, flower and leaf (Khalil, 2014; Bakr 2018).

The objective of the study was designed to evaluate some selected plants for ecofriendly controlling of *Meloidogyne incognita* infecting eggplant under greenhouse conditions.

MATERIAL AND METHODS

Dried materials of selected plants based on their traditional usage for medicinal purposes. Turmeric (*Curcuma longa* L.), Ginger (*Zingiber officinale* Roscoe), Peganum (*Peganum harmala* L.), Thyme (*Thymus vulgaris* L.), Bitter Melon (*Citrullus colocynthis* L.), Artemisia (*Artemisia absinthium* L.), Alpinia (*Alpinia officinarum* Hance), Thuja (*Thuja orientalis* L.) and Capsicum (*Capsicum annuum* L.) were obtained from the market and ground into fine powder using a Micro-hammer-mill (KINEMATICA AG, PX-MFC). Then stored in air-dried containers until required for usage.

Multiplication of *M. incognita*

For obtaining a pure culture of *M. incognita*, one egg mass was taken from infected plant and then identified by observation of perineal pattern (Hartman and Sasser, 1985) and was added to infect the black nightshade (*Solanum nigrum*) plants grown in plastic pots 30 cm diameter filled with sterilized sand-clay soil (2:1 v/v) under controlled conditions (25 ± 3 ºC with relative humidity 85%) at the experimental greenhouse, Agricultural Botany Dept., Faculty of Agriculture, Menoufia University, Egypt.

Preparation of *M. incognita* inocula

Two months old, heavily galled black nightshade roots infested by *M. incognita*, used to prepare nematode inoculum. Heavily infested roots washed gently using tap water to take out the attached soil particles. Roots divided into small pieces and then macerated using blender (Monlinex) for 10 seconds each at high-speed two times. Then root solution placed in a jar containing sodium hypochlorite (NaOCl) under concentration 0.5% according to (Hussey and Barker, 1973). The solution in the jar vigorously shaken for 3 min. to allow NaOCl removing gelatin matrix and release the eggs from the egg masses. Root tissues separated by transfer the solution through different size sieves. Eggs were collected on the 20ìm sieve and washed several times to remove residual NaOCl. Eggs then collected in a flask containing tap water. The number of eggs / ml was estimated by counting 4 samples of 1 ml using a counting dish under a stereomicroscope (Bel photonics, Biological Microscope, Bio 1-B) at 100x.

Greenhouse experiment achieved to study the effect of selected plants (Table 1) on *M. incognita*. Eggplant seedlings cv. Balady four weeks old were transplanted into plastic pots 15 cm in diameter. Dried materials of nine medicinal plants were mixed with soil at ratio 1, 2 and 3% (w/w) at time of transplanting. Each plant inoculated with 3000 eggs of *M. incognita* around the young hairy roots. Plants in pots without amendments used as a control treatment. Pots arrange on a bench in the greenhouse in a completely randomized design. Treatments repeated 4 times. Plants removed after 2 months from inoculation, and then roots washed carefully by running tap water. Plant growth and nematode parameters were recorded as follow: Root length (cm), Plant height (cm), Root fresh weight (g), Shoot fresh weight (g), Shoot dry weight (g), Number of galls /root system, number of egg masses /root system. For counting of egg-masses, roots stained as described by (Daykin and Hussey, 1985) by dipping the roots in 0.015% Phloxine-B solution for 20 min., number of females /root system was determined (Mahdy, 2002), number of second stage juveniles (J2s) /250g soil.

Statistical analysis

Data statistically analyzed using Duncan's Multiple Range test ($P=0.05$) using Costat 6.3 version program.

Table 1. Scientific and common names, families and used parts of tested plants.

Scientific name	Common name	Family	Used part
Curcuma longa	Turmeric	*Zingiberaceae*	rhizomes
Zingiber officinale	Ginger	*Zingiberaceae*	rhizomes
Peganum harmala	Peganum	*Nitrariaceae*	seed
Thymus vulgaris	Thyme	*Lamiaceae*	shoots
Citrullus colocynthis	Bitter Melon	*Cucurbitaceae*	fruits
Artemisia absinthium	Artemisia	*Asteraceae*	shoots
Alpinia officinarum	Alpinia	*Zingiberaceae*	rhizomes
Thuja orientalis	Thuja	*Cupressaceae*	fruits
Capsicum annuum	Capsicum	*Solanaceae*	fruits

RESULTS

Treated soil with the selected plant materials, in general, gave a significant suppression of nematodes parameters i.e. galls, egg masses, females and developed stages/root system of eggplant plants infected with *M. incognita* and nematode density in soil compared to non-treated soil under controlled conditions of greenhouse. The reduction in number of J2s/250 g soil varied from treated and non-treated pots as the results cleared that the most effective treatment was *P. harmala* at 3% by 29 J2s/250 g soil followed by *A. absinthium* at 3% by 33 J2s/250 g soil while *A. officinarum* at 1% recorded less effective by 105 J2s/250 g soil. There was a gradual decrease in number of J2s/250 g soil with increase in concentration of each treatment (Fig. 1).

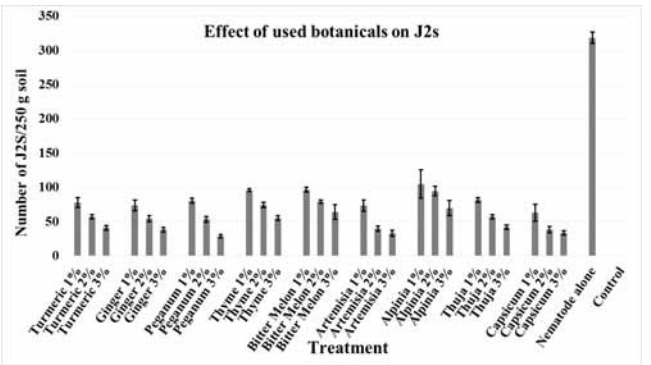

Fig. 1. Effect of used botanicals on number of J2s /250 g soil

Examination of eggplant root system showed that the number of nematode galls/root system was affected markedly by treatments. The results indicated that soils treated with *P. harmala* 3% have a very high reduction of galls /root system (89.3 % reduction) followed by *C. annum* 3% and *A. absinthium* at 3% by 87.6 and 87.1 % respectively while the least reduction was recorded with *A. officinarum* at 1% by 55.4 % compared with the control treatment (Fig. 2).

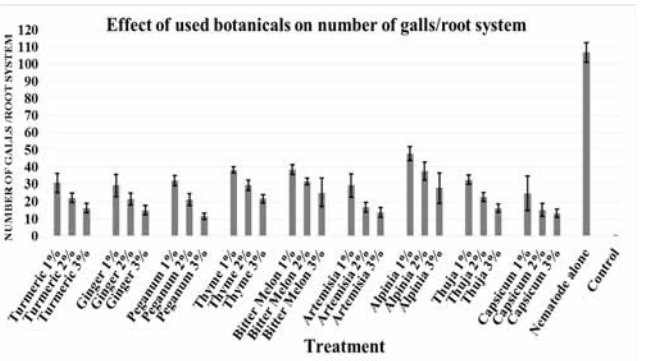

Fig. 2. Effect of used botanicals on number of galls /root system

Results also indicated that application of treatments at the different doses were effective in reducing the number of egg masses/root system compared with plants treated with nematode alone. The highest reduction was also recorded in plants treated by *P. harmala* 3% while *Z. officinale* 3% came in the second but the third one

was *A. absinthium* at 3%, meanwhile *A. officinarum* at 1% came in the last compared with the control treatment (Fig. 3).

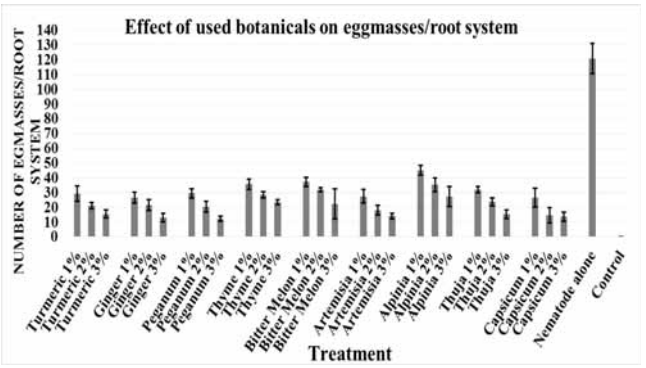

Fig. 3. Effect of used botanicals on number of eggmasses / root system

Observation of root system showed that number of females /root system was markedly affected by using the different plant materials (Fig. 4). *P. harmala* 3% was the effective treatment in reducing number of females followed by *Z. officinale* 3% while the least effective one was *A. officinarum* at 1% compared with the nematode alone.

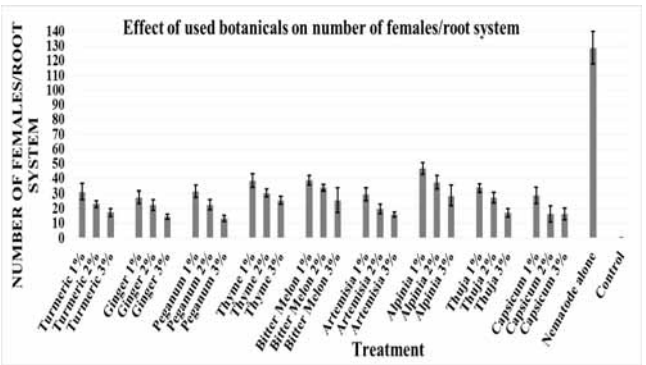

Fig. 4. Effect of used botanicals on number of females /root system

The reduction in nematode criteria participated in enhancement the plant growth parameters in treated plants by botanical materials compared to plants infested by nematode alone. Treatments at different rates generally resulted in an improvement of plant height and root length (cm) more than untreated plants as presented in Fig. (5) and Fig. (6) which revealed that the most effective treatment was *P. harmala* at 3% followed by *A. absinthium* at 3% and *C. annuum* 3%. The least plant height recorded with plants treated with *A. officinarum* at 1% compared to the control treatment (nematode alone).

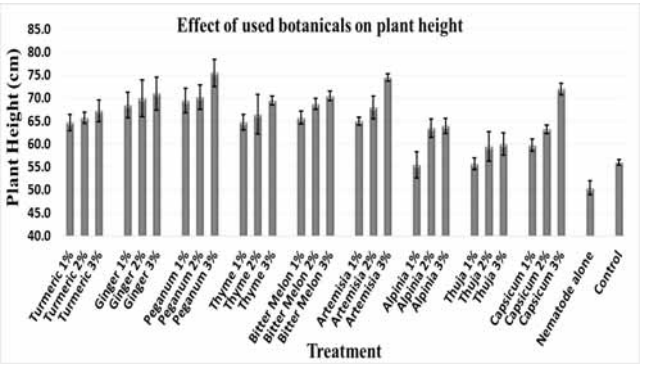

Fig. 5. Effect of used botanicals on plant height of eggplant plants

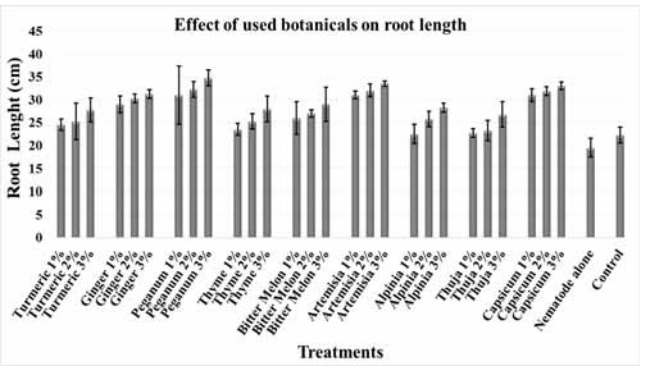

Fig. 6. Effect of different used plants on root length of eggplant plants

Obtained results proved that treated plants with the different treatments affected the root fresh weight of eggplant plants. The *P. harmala* 3% high followed by *C. annuum* 3% and *A. absinthium* at 3% respectively, while the least showed with *A. officinarum* at 1% (Fig. 7).

Results cleared that all treatments affected the eggplant fresh and dry shoot weight. Plants treated *P. harmala* 3% recorded highest fresh and shoot dry weight followed by *C. annuum* 3% and *A. absinthium* at 3% respectively, while plants treated with *A. officinarum* at 1% were the lighter (Figs. 8 and 9). Investigation of eggplant plants general status revealed that there is no phytotoxicity to the eggplant plants due to

use of any treatment at the different concentration especially in shoot system, leaf color and area.

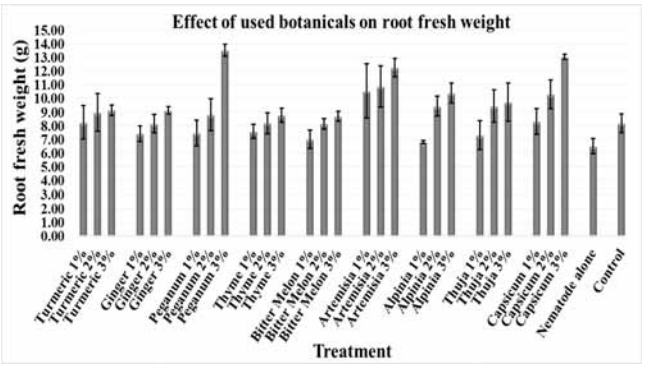

Fig. 7. Effect of used botanicals on root fresh weight of eggplant plant

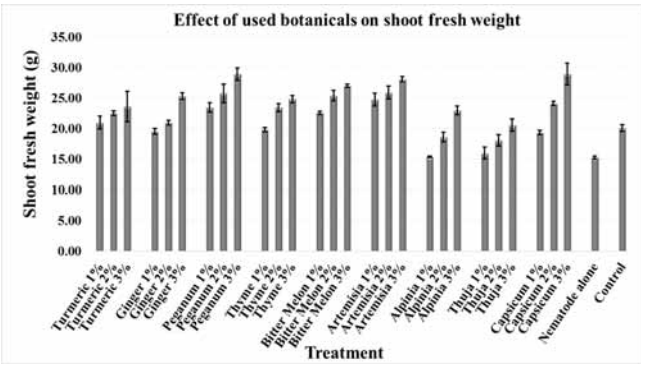

Fig. 8. Effect of used botanicals on shoot fresh weight of eggplant plants

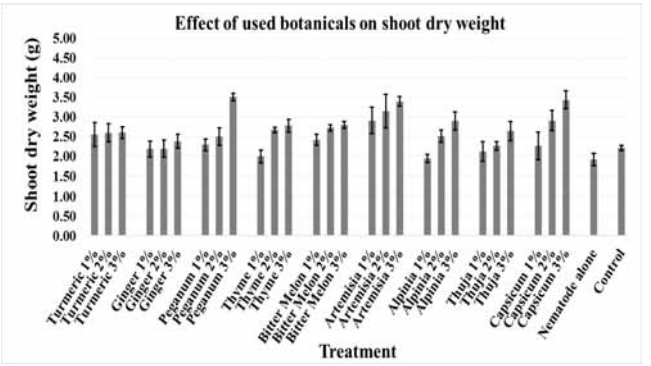

Fig. 9. Effect of used botanicals on shoot dry weight of eggplant plants

DISCUSSION

Results of the current study cleared that all treatments were superior over control. *Curcuma longa* showed satisfactory effect in nematode control. These results were in agreement with those obtained by (Youssef *et al*., 2015), who stated that turmeric showed nematicidal activity against *M. incognita* by decreased number of juveniles in soil, egg masses and galls, hatched juveniles on roots, and enhanced the eggplant plant growth parameters i.e. fresh shoot and root weight, shoots height and dry shoots and roots weights. Abbas *et al*., (2009); Neeraj *et al*., (2017) reported that *C. longa* significantly inhibited egg hatching of *M. javanica* and *M. incognita*. The nematode inhibitory activity may be refer to Curcumin and other derivatives from *Curcuma longa* (diacetyl curcumin sodium curcuminate, triethyl curcumin, tetrahydro curcumin, Bisdemethoxy curcumin, Methylcurcumin, Demethoxy curcumin and Sodium curcuminate) with biological activities as nematicidal compounds (Araujo and Leon, 2001). *Curcuma longa* is cultivated worldwide throughout subtropical and tropical regions. Its rhizomes exhibit some antioxidant effects, nematocidal and anti-bacteria activities (Youssef *et al*., 2015). The curcumin nematicidal potential may revealed to inhibit the activity of glutathione-S-transferase enzyme of *M. incognita*, responsible for survival of nematode in the host plants and the detoxification of *M. incognita* by antioxidant activity (Babu *et al*., 2012).

Zingiber officinale gave satisfied results in reducing nematode parameters. Plant extracts of ginger on the micro plots suppressed the root galling and population of *M. javanica* and *M. incognita* in tomato (Zasada, 2002). Results observed in the current research, have been also substantiated by some researchers (Abbas *et al*., 2009; Salim *et al*.,(2017); Ibrahim and Hussein, 2017). *Zingiber officinale* completely (100%) prevented attack and hatching of *M. incognita* eggs and destroyed juveniles at 1,000ppm concentrations (Bawa *et al*., 2014). *Zingiber officinale* also showed nematicidal activity on *M. incognita* infecting eggplant by decreasing nematode criteria in roots and soil (Youssef *et al*., 2015). The anthelmintic potential of *Z. officinale* may be revealed to the synergetic effect of active phyto constituents i.e. terpenes, alkaloids, steroids, saponins and flavonoids (Singh *et al*., 2011).

Peganum harmala showed the highest effect in reducing number of root galls, juveniles in soil, developmental stages and egg masses of *M. incognita*

at the different dosage rates in comparison to control treatment and these results were similar to the findings of (Radwan *et al*., 2012; Saeed *et al*., 2015; Sholevarfard and Moosavi, 2015). The nematicidal properties could be attributed to some contents like those that harmal's alkaloids including carboline as harmine, harmaline, harmalol, harmol and Harman (Moloudizargari *et al*., 2013) which are considered as strong nematicides (El-Hassan *et al*., 2013). In addition, some of this component dissolve the nematode cell cytoplasmic membrane and the effective functional groups interfering with nematodes enzyme protein structures (Asif *et al*., 2017b).

Thymus vulgaris is a native flowering plant in Mediterranean Region. Thyme oil consists of carvacrol, thymol and other different monoterpenoids (Kubeczka and Formáèek, 2002). *Thymus vulgaris* had good nematicidal activity toward root-knot nematode *M. incognita* Race 2 (Nour El-Deen *et al*., 2016).

Findings cleared that *C. colocynthis* markedly affect plant growth and nematode parameters which is in accordance with (Chaudhary and Kaul, 2013; Nour El-Deen *et al*., 2016) who recorded similar results against *M. incognita* infecting Chilli pepper (*Capsicum annuum* L.), Pomegranate (*Punica granatum*) and *Vigna ungiculata* respectively. Rizvi and Shahina (2014) reported that fruit extract of *C. colocynthis* give 80% of *Meloidogyne* spp. larvae mortality after 72 h of exposure. The nematicidal effect of *C. colocynthis* may be due to its antioxidant activity according to the rich amounts of flavonoids and phenolics (Kumar *et al*., 2008) which may be possessing nematicidal properties such as: Colocynthin and Colocynthetin, Cucurbitacin A, B, C, D, E and α-aelaterin, and other constituents (Tannin-Spitz *et al*., 2007; Torkey *et al*., 2009). Early accumulation of reactive oxygen species and up regulation of plant antioxidants may play an essential act in resistance process to various plant pathogens (Ketta, 2015; Ketta *et al*., 2017).

Results established that *A. absinthium* affected root and shoot fresh weight and shoot dry weight and decreased number of galls, eggmasses and females /root system. This report is similar to those by (Sellami *et al*., 2010) who reported that *A. herba alta* oils suppressed larvae mortality and egg hatching of *M. incognita* from 27.7 to 100% and 18.2-62.8% respectively. The anthelmintic effect of *A. absinthium* may be due to richness of *Artemisia* genus by flavonoids and sesquiterpene lactones such as Artemisinin with nematicidal activity with no toxicity to mammalians (Kerboeuf *et al*., 2008). Artemisinin act by tow mode of action first, by alkylation the parasite specific protein and second by production of free radicals (O'neill *et al*., 2010).

Application of tomato plants by defferent concentrations of *A. officinarum* showed satisfactory effect in plant growth and nematode parameters. These data support previous reports by El-Sherbiny and Al-Yahya, (2011) of nematicidal activity of *A. officinarum* against root-knot nematodes, *M. incognita* on tomato. The nematicical activity of *A. officinarum* may be due to the rhizome main constituents identified such as: α-terpineol , 1,8-cineole, α-pinene β-pinene and terpinen-4-ol, camphor and α-fenchyl acetate (Raina *et al*., 2014). A nematicidal effect against *M. hapla* juveniles and eggs by *A. galanga* rhizome which contain the similar contents was recorded by Jeon *et al*. (2016).

In the current study, also *T. orientalis* effectively reduced the root-knot infection of eggplant and reduce root galling, nematode reproduction and enhancing the plant growth characters. This nematicidal activity of plants against *M. incognita* has been reported by Rather *et al*., (2008), who found that *T. orientalis* reduce root galling and significantly improve the tomato plant growth due to the application of these organic additives. Aqueous extract of *T. orientalis* affected the larvae mortality and egg hatching of *M. incognita* (Kavita *et al*., 2011). The inhibitory effect might be related to the chemical component of *T. orientalis* such as Thujone (Kyo *et al*., 1990). The higher contents of *T. orientalis* of certain oxygenated compounds with lipophilic properties may lysis the nematode cytoplasmic cell membranes and their functional groups connected with structure of enzyme protein (Knoblock *et al*., 1989).

Adding of *C. annuum* powder to eggplant plants undergreen house condition at the three concentrations showed a great reduction of *M. incognita* and inhanced plant growth. This result was in the same line by (Patel *et al*., 1993), who reported that chili pepper suppressed

the population *M. javanica* and *M. incognita* in tomato. *Capsicum annuum* reducing the number of galls and eggs / system of tomato plants infected by *M. javanica* (Neves *et al*., 2009). Ethanol extract of *C. annuum* significantly inhibited egg hatching of *M. javanica* (Abbas *et al*., 2009) and *M. incognita* (Bawa *et al*., 2014). The observed increment in growth in tomato plants may be attributed to the effective nematode control. The larvicidal principles of *C. annuum* may be related to the compound piperolein alkaloid, pipernonaline. Piperamides such as capsaicin which belong to *Capsicum* genus has nematicidal properties (Neves *et al*., 2009).

Therefore, the use of these plants showsed a promise role to suppress root-knot nematode populations and may provide an alternative environmentally safe and economical method for nematode controlling. From current study, the nematicidal effect may be due to one /or more active ingredient so that, further studies under open field conditions is necessary to evaluate the feasibility of insertion these plants as a part of an integrated nematode management strategy. Also, developing formulation, propagation, stimulating mechanism to improve their efficacy and stability is recommended.

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The Study on Life Span of the Nematode, *Teratorhabditis palmarum*

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ABSTRACT: Life span is the length of time for which a person or an animal lives during which various biological processes regulated by several genetic pathways. A lot of genes have been identified in nematodes whose mutations cause several folds increase in lifespan. It was predominantly found that life span of males of nematode, *Teratorhabditis palmarum* was shorter than that of females when compared by taking different parameters. It was also observed that isolated females always lived longer than the solitary males. The average life span of male and female in isolated condition of *T. palmarum* was found to be greater than that of the mated and single-sex groups of males and females. The isolated male and female life span was found to be 11.4 ± 1.34 and 13.2 ± 2.18 days, respectively and when kept together, it was found to be 9.2 ± 1.91 and 10.65 ± 2.23 days for males and females, respectively. Mating affected the life span of both sexes. Lastly in single-sex groups, there was also reduction in life-span of males.

Keywords: *Caenorhabditis elegans*, Lifespan, Mating, Sexes, *Teratorhabditis palmarum*

Aging is a universal process that leads to progressive deterioration of muscular, metabolic, reproductive and cognitive functions which finally affects lifespan.Generally it is a biological process that cannot be easily measured or determined by the organism.However, life span is defined as the number of days an animal remains responsive to external stimuli. It is only a single estimate parameter that define period of time an organism is alive but does not give any actual explanation to the cause of aging.Over the last decade,several different types of genes have been identified that modulate life span. Klass (1983) was the first who reported a method for isolating longevity mutants in *Caenorhabditis elegans* and it was used to isolate two most important mutants, Age-1 and daf-2, for which there is an increased lifespan, are associated with reduced calorie intake. Nematodes exhibit a significant diversity of life histories, which includes considerable variation in rate of ageing and adult life span. Genetically, DAF-16 expression and/or activation are an important mechanism by which species undergoes longer life. Amrit *et al*. (2010) observed that hermaphroditic species are generally shorter lived than the gonochoristic species and show higher levels of daf-16 expression. The overall fitness is primarily contributed by the interactions within and between the two sexes (Andersson, 1994; Arnqvist and Rowe, 2005).If the females capacity is condition dependent in order to permit the damaging effects of males, than selection will delay senescence, favouring robust individuals with longer life spans (Williams and Day, 2003; Maklakov *et al*., 2015).

Life span in nematodes is affected by several factors such as temperature, food availability, stochastic factors (such as lifestyle) and reproductive activity. In *C.elegans*, there is reduction in life span by several parameters, including *E. coli* food availability (Gems and Riddle, 2000; Garigan*et al*., 2002;Garsin*et al*., 2003), higher temperature (Klass, 1977), mating between the sexes (Gems and Riddle, 1996) and attempted mating between males (Gems and Riddle, 2000). Furthermore, lowering of temperature, food availability and reproductive activity can all increase life span. Klass (1977) observed maximum life span of *C. elegans* at 20° C to 19 days, at 25° C to 11 days. Beside *C. elegans*, a lot of work on life span has been done in other nematodes. In the free-living nematode, *Panagrellus redivivus*, maximum life span of males and females at 25° C was 6 days and 20 days, respectively (Duggal, 1978 a&b). In contrast to this, lifespan is also affected by the gender specific differences in nematode

and depend upon whether or not nematode mate. Weadick *et al*. (2016) observed that hermaphrodites were the shortest lived, females the longest with males intermediate life span. Culture conditions also have an important impact in estimation of lifespan. In *C. elegans*, there is 40% increase in life span under axenic liquid culture as compare to culture on *E. coli* agar plates (Croll *et al*., 1977; Mitchell *et al*., 1979). It varies with different culture conditions and methodical problems. In *C. elegans*, *P. Pacificus* and *P. superbus* the survival ratio also affected by anaerobic conditions (Kitazume *et al*., 2018). Lifespan which was measured in NGM medium showed variations in N2 strain of *C.elegans*. Chen *et al*. (2007), Train *et al*. (2008), Klass (1977) and Shook and Johnson (1999) observed lifespan, 14.8, 15, 19 days and 13.3 days in NGM medium, respectively.

In this study, we estimated the average life span of *Teratorhabditis palmarum* by certain parameters. For that, we calculated life span by taking both sexes either separately, combined each other's or single-sex groups of males and females. We also compared it with others nematodes.

MATERIAL AND METHODS

The free living nematode *Teratorhabditis palmarum* was isolated near the fort (27.8° N, 78° E) in Aligarh. Nematodes were extracted from manure by modified (Cobb's, 1918) sieving and decantation and modified Bermann's funnel techniques. *T. palmarum* was cultured on nematode growth medium (NGM) supplemented with *E. coli* OP50 strain as a food source at 23 ± 1° C in 5 mm petridishes. The bacterial strain OP50, maintained in laboratory, was tested. The pure culture of the strain was maintained and stored as slants, stabs and frozen permanents as glycerol cultures at -20°C. The strain was sub-cultured on regular basis on Nutrient broth medium. For preparing the broth culture of the strain, single OP50 colony was inoculated in 100 ml nutrient broth and kept at 37°C for overnight at bacterial rotary shaker. After obtaining the population, nematode were regularly propagated by transferring 5–10 gravid females to fresh *E. coli* plates in order to obtain age-synchronized population and continued the progeny of nematodes. In order to collect J4 stage, handpicked the J4 stage of males and females from the culture by picking needle and placed onto fresh OP50 containing NGM agar plates.

Life Span Analysis

First, the life span of male and female was studied separately. So, followed the age-synchronized population, particularly L4. Most L4 nematodes could be clearly distinguished as male or female according to their genital morphology. Both sexes were segregated at L4 stage and transfered it into 12-well sterile tissue culture agar plates seeded with *E. coli* OP50 as a food source. 20 L4 larvae of each sex were taken and estimated the lifespan separately. Secondly, the life span of male and female was also estimated when placed together. For this, 20 L4 larvae of each sex were taken and placed in same well plates with five replicates for both experiments. Thirdly, measured the life span of single-sex groups of males and females. For this, we took 5, 10 & 15 animals per plates of both sexes and had three replicates for this experiment. The wells were observed daily under a dissecting microscope and the numbers of live and dead worms were counted in each well. Worms that were not moving, did not respond to gentle probing with a needle, or were missing were counted as dead. Transfered the worms daily in new fresh plates. Recorded the date and the number of worms that were alive and dead.

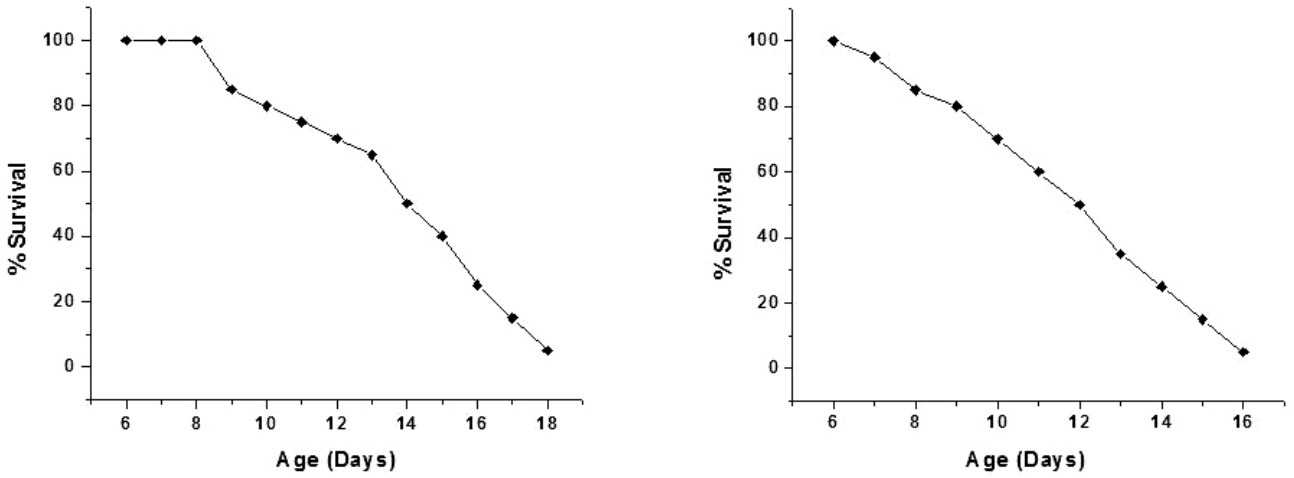
Statistics

All results were expressed as mean ± standard deviation. Graphs were plotted using Origin 6 software. Analysis of variance was performed to identify the significant differences from the control group.

RESULTS

Unmated Female and Male Life Span

Considerable variation was observed in estimated life span of male and female. The life span of solitary unmated females was 13.2 ± 2.18 days. While the solitary males was shortest lived than females i.e., 11.4 ± 1.34 days. Male and female survival curves are drawn in Fig. 1 A &B.



A **B**

Fig. 1. (A) Survival curve for females and (B) Survival curve for males

Mated female and male life span

Generally mating reduced overall lifespan for both mated male and female. Mating with male for their lifetime reduced female lifespan compared with its isolate. The observed life span of mated males and females were 9.2 ± 1.91 and 10.65 ± 2.23 days respectively. Mated survival curves are shown in Fig. 2.

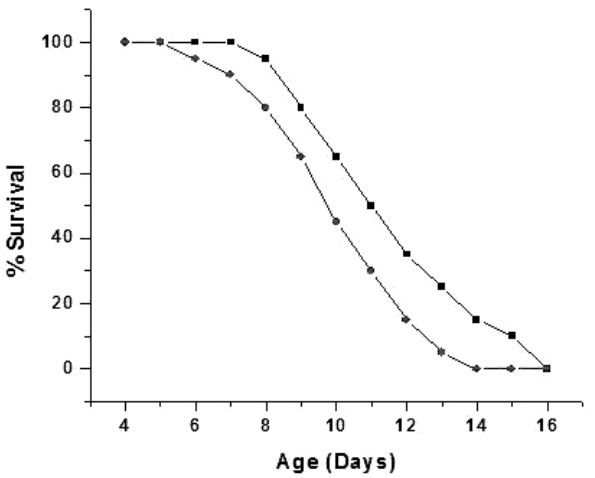


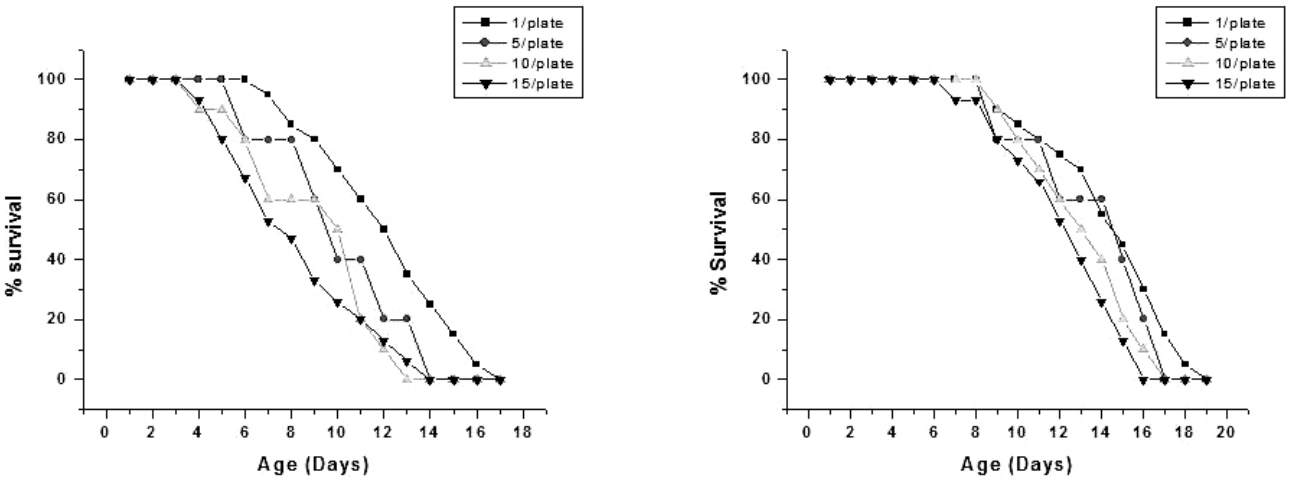
Fig. 2. Combined survival curvesfor males & females

Effect of single-sex groups on life span

It is be common that male attempted mating resulted in reduction of life-span in single-sex groups of males. As the males congregated into clumps, caused decrease in longevity compared with solitary and mated individuals. Since male life span was greatly affected by population density as it nearly was unaffected in female. In three groups of males, animals acquired such a mating plug after 2-3 days. While no mating plugs were observed on solitary males. Finally, observed life span of 5, 10 & 15 animals per plates of male were 9.3 ± 2.7, 8.4 ± 1.3 and 7.6 ± 0.8 days. In contrast to this, slight variations were recorded against the population density in female life span. It was found to be 12.8 ± 2.7, 12.4 ± 1.4 and 11.4 ± 0.7 days of 5, 10 & 15 animals per plates. Survival curves are drawn in Fig. 3 A&B.

DISCUSSION

Generally it has been observed that there is sex differences in life span of animal species, in which male typically shorter lived than female (Comfort, 1979; Smith, 1989). It was also found that virgin male & female live longer than non-virgin. For *Turbatrix aceti*, both virgin male and female had greater life span as compared to their mated counterparts (Kisiel and Zuckerman, 1974). Similarly these finding were reported for *P.redivivus* (Abdulrahman and Samoiloff, 1975) and *Rhabditis tokai* (Suzuki *et al*., 1978). In our nematode, both virgin male and female were lived 17% & 19% longer than mated male and female. This reduced longevity was due to sex-related behaviour or reproductive activity in mated



A **B**

Fig. 3. Effect of population density on male survival (A)and female survival (B)

individuals. The continuous presence of mating competent males significantly shortens the lifespan of the opposite sex (Maures *et al.*, 2014).

However, Gems and Riddle (1996) found that mated males have a greater life span than that of unmated and grouped males. They also concluded that beside reduction in the longevity of mated hermaphrodite by 43%, male remain unaffected by mating. Honda (1925) observed that mating was also found to reduce the mean lifespan of females of the dioecious species *Diplogaster aerivora* from 52 days (range, 33–68 days) to 25days (range, 11–54 days) and in males also reduced mean life span from 43days (range, 15–71 days) to 33 days (range, 10–54days). Possibly one of the reasons in the reduction of male longevity is the capacity to produce mating plugs. If so, this would be an example of a trade-off between fitness traits, in which the deposition of mating plug enhances reproductive success (Barker, 1995) but limits longevity. Similarly in this study, males which attempted to mate and formed the copulation plug caused reduced lifespan in both male and female. While in female it was due to the effect of copulation rather than an increase in egg production (Gems and Riddle, 1996).

However, male have reduce life span either they maintained separately or combined with female & single sex groups. It showed 14% reduction with combined female, when cultured separately reduced by 16% and 27-33% reduced by single sex groups compared with the female. Male life span showed inverse relation with increase in population density, whereas life span of female was not density dependent. This was due to either male clumping or mating that deposited mating plug on each other. Solitary males were more variable than those of grouped males. Its life span was increased by 24%, suggesting that male-male interaction greatly reduced the life span. Male longevity was reduced by the presence of even one other male (Gems and Riddle, 2000b). In *C. elegans*, males showed the homosexual mating clumps formation when they were present in grouping which resulted in halves male lifespan compared with solitary males (Gems and Riddle, 2000b). Recent work had shown in *Caenorhabditis*, the copulation and male harassment can be quite damaging for females and hermaphrodites (Maures *et al*., 2014; Ting *et al*., 2014). In this study, it was found that there is significant variations in estimation of life span under the different forms.

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Morphological and Biochemical Host Response of Fifteen Indian Rice Cultivars to Rice Root-Knot Nematode, *Meloidogyne graminicola*

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ABSTRACT: The relative susceptibility and resistance reactions of fifteen rice cultivars were evaluated against rice root-knot nematode, *Meloidogyne graminicola* under pot conditions. The variation in the synthesis of total phenol (TP) and salicylic acid (SA) as influenced by host reaction and subsequently their impact on the disease etiology was assessed. The cultivars inoculated with 1000 second stage juveniles (J_2s) of *M. graminicola* expressed varied responses. The cv. Shanthi expressed resistance reaction, whereas the cvs. Pusa Basmati-6 and Pusa Sugandh-5 were found highly susceptible to the nematode. The greatest increase in the leaf contents of SA (9-15%) and TP (10-13%) was recorded in the cv. Shanthi, which also exhibited lowest root-knot index (0.8 at 0-10 scale) and decrease in the rice yield. The TP and SA contents of leaves increased up to 15 days of planting, and thereafter, decreased gradually. Increase in the TP and SA was negatively correlated with the root-knot index. Based on the morphological and biochemical host reaction, the relative susceptibility of the 15 cultivars was in the order; Pusa Basmati-6 > Pusa Sugandh-5 > Pusa Sugandh-4 > R-Dhan > Surya > Sharbati > Sadabhar > Virendra > Anjali > Swarna > CR-314 > PA6444 > JKRH401 > Vivekdhan 62 > Shanthi. The study revealed that the cvs. Vivekdhan 62 and Shanthi may be exploited for commercial cultivation of rice in the host zone of rice root-knot nematode, while the cultivation of cvs. Pusa Basmati-6 and Pusa Sugandh-5 should be avoided.

Keywords: Host resistance, salicylic acid, total phenols, rice, *Meloidogyne graminicola*

Rice is the world's most important staple food and globally it occupies 162 mha with an annual production of around 464 mmt (FAOSTAT, 2015). India is the second largest producer of rice after China and produced 104.32 million tonnes of rice during 2015-16 (Anonymous, 2017). Rice is quite susceptible to root-knot nematode and is attacked by *Meloidogyne incognita*, *M. graminicola*, *M. triticoryzae, M. javanica*, *M. oryzae* and *M. arenaria* (Bridge *et al.,* 2005). Amongst these species, *M. graminicola* is a primary pest of rice and unlike other *Meloidogyne* spp., it incites galls on the tip of lateral roots (terminal galls) which are spiral or hook like (Khan *et al*., 2014). *M. graminicola* attacks rice in the nursery as well as in the main field and possesses a substantial threat to rice cultivation in Southeast Asia, where around 90% of the world rice is grown and consumed (Dutta *et al.,* 2012). In India, this nematode is now an established problem and hampers the rice productivity up to 30% (Prasad *et al.,* 2010; Khan *et al.,* 2014); even the yield loss of 20 percent is significant, as it is sufficient to feed 120 million people for one year.

There are various methods available for the management of root-knot nematode in rice viz., flooding, deep plowing, summer fallowing, nematicide application, host resistance etc. Of all the strategies, chemical control has been most widely used by the farmers, but due to the high cost of chemical and its hazardous impact, farmers were compelled to look for more economic and eco-friendly approaches. Use of resistant cultivar is one of the best and safer alternatives for nematode management, without incurring any additional cost. However, in India, the majority of commonly growing rice cultivars have been found to be highly susceptible for this nematode (Devi and Thakur, 2007; Khan *et al.,* 2012; Mhatre *et al.,* 2015). Hence, it is indispensable to identify more resistance/ tolerance cultivars to combat this nematode problem in infested areas.

In plants, phenolic compounds play very vital role in the induction of local and systemic disease resistance (Bari and Jones, 2009). Major players in systemic induced defense are the salicylic acid (SA), jasmonic acid (JA), ethylene (ET) and other phenolic compounds, which are produced in response to plant attack (Biere and Goverse, 2016). Root feeding by sedentary endoparasitic nematodes results in the production and accumulation of SA, JA, and ET at the site of infection (Kammerhofer *et al*., 2015). Consequently, transcriptional reprogramming results in the activation of the core defense system involving SA/JA/ET-mediated pathways in plants including rice (Kyndt *et al*., 2014). The outcome of these highly coordinated signaling responses ultimately determines the host susceptibility and resistance to the nematodes. This indicates that the phenol and SA play important role in host defense mechanisms but their synthesis varies with cultivars and etiology of the associated pathogen. In this context, the present study was carried out to evaluate the relative susceptibility and resistance reaction of fifteen rice cultivars against *M. graminicola*. The variation in the synthesis of total phenol and SA, as influenced by host reaction and subsequently their impact on the disease etiology was assessed under pot conditions.

MATERIAL AND METHODS

Seeds of fifteen cultivars of rice (*Oryzae sativa* L.) viz., Anjali, CR-314, JKRH 401, PA6444, Pusa Basmati-6, Pusa Sugnadh-5, Pusa Sugandh-4, R-Dhan, Sadabhar, Shanthi, Sharbati, Surya, Swarna, Virendra, and Vivekdhan-62 were procured from the International Rice Research Institute Centre, New Delhi, India.

Pure population of *Meloidogyne graminicola* Golden & Birchfield was maintained in a culture bed of 10 × 20 m in the experimental net house. Highly susceptible cv. Pusa 1121 (Kumari *et al.,* 2014) was continuously grown in the bed to support reproduction of the nematode and to maintain a high population of *M. graminicola* (4000-6000 J_2/kg soil). Ten root samples were collected randomly from the bed and association of *M. graminicola* with the galls on rice roots was ascertained by perineal pattern technique (Hunt and Handoo *et al.,* 2009). Juveniles of *M. graminicola* were used as the inoculum of the nematode for this experiment. The nematode juveniles were isolated from the soil using Cobb's decanting and sieving method followed by the modified Baerman funnel technique (Khan, 2008). The identity of the isolated juveniles was confirmed on the basis of morphological characters (Hunt and Handoo *et al.,* 2009). The population of *M. graminicola* obtained using the above process was standardized to 1000 J_2/5 ml of water and was used to inoculate the pots within 48 hrs of isolation.

Earthen pots of 25 cm diameter were filled with 2 kg autoclaved soil (sandy loam) and farmyard manure (FYM) in the ratio of 4:1. The soil and FYM were autoclaved at 15 kg/cm^2 pressure at 121°C for 15 min. Two sets of pots were maintained, one set was inoculated with the 5 ml suspension of *M. graminicola* (1000 J_2) a day before planting. The second set of pots were not inoculated and served as control (un-inoculated). Seedlings of all 15 rice cultivars were transplanted at the center of the pots. Seedlings were watered immediately after planting and the watering continued till harvest. For each cultivar, nine treatments with 3 pots were maintained. Hence, each cultivar had 27 pots. The pots were arranged in a completely randomized block in the net house receiving uniform sunlight. Plants from three pots were evaluated at 2, 5, 10, 15, 20, 30, 60 and 90 days after planting to determine total phenol and salicylic acid contents of the leaf. Additionally, root-knot and egg mass index, yield per plant and soil populations were determined in four months old plants (at harvest).

Leaf samples from each of the 15 rice cultivars were collected and processed separately for total phenol (TP) and salicylic acid (SA) estimation. TP was estimated using the method described by Sharma and Sain (2005). Catechol was used as a standard and the amounts of TP were expressed as µg catechol/g fresh leaf. SA content of leaf of all 15 rice cultivars was estimated using the procedure previously described by Pankaj *et al.* (2005). Standard curve of SA was prepared and the concentration of SA in each sample was calculated using standard procedures (Lowery *et al.,* 1951).

At harvest, roots were gently rinsed under a slow stream of water and stained with acid fuchsin (Byrd *et*

al., 1983). Gall (GI) and egg mass (EMI) indices were measured on 0–10 scales (Bridge *et al.,* 2005). Final soil population of *Meloidogyne graminicola* was determined at harvest.

The plants from a pot, 4 months after planting, were harvested, dried for 2 weeks and thrashed manually to determine the grain yield per plant (with seed husks, without grain milling).

The experiment was conducted during two consecutive years (2013 and 2014). All the data were subjected to the analysis of variance (ANOVA) with the help of MINITAB 11.0 for Windows-7. The year differences in the data for plant yield were significant (*P* d" 0.05); hence the analysis was performed year wise separately. However, the year differences in the data for gall and egg mass indices, soil population and total phenol and salicylic acid assay were non-significant at $P \leq 0.05$, hence, data were pooled and are presented in the graphical form. Bars have been marked with standard errors in all figures. In figure 2 and 3 different alphabets have been assigned according to Tukey's Test at $P \leq 0.05$. The data on plant yield were analyzed using one factor ANOVA at probability levels of $P \leq 0.05$. Tukey's test was also employed to mark significance in the Tables. Correlation analysis was performed to determine the relationship between total phenol/salicylic acid concentration with gall index. The percent variation over the control was also calculated.

RESULTS

The severity of root-knot in terms of the gall index (GI; 0-10 scale) varied considerably with cultivars (Fig. 2). The highest GI was recorded on the cv. Pusa Basmati-6 (5.1) and Pusa Sugandh-5 (4.3). The cv. Sadabhar, Sharbati and Virendra showed GI in the range of 3.5-3.8, which was significantly less than the cv. R-Dhan ($P \leq 0.05$; Fig. 2). Four cultivars viz; CR-314, Swarna, JKRH401 and PA6444 developed 2.8-3.0 GI. The lowest GI was recorded on the cv. Shanthi (0.8). The overall susceptibility of the rice cultivars with regard to galling was Pusa Basmati-6 > Pusa sugandh-5> Pusa Sugandh-4 > R-Dhan > Surya > Sharbati > Sadabhar > Virendra > Anjali > Swarna > CR-314 > JKRH401 > PA6444 > Vivekdhan 62 > Shanthi (Fig. 2). The rice cultivars supported the reproduction of *M. graminicola,* and the order of egg mass production in term of egg mass index on different cultivars was more or less similar to GI (Fig. 2).

Plants grown in nematode inoculated soil exhibited a considerable degree of reduction in yield which varied with cultivars in both years (Table 1). The greatest decrease in the yield was recorded in cvs. Pusa Basmati-6 (44-49%) and Pusa Sugandh-5 (32-38%) over respective controls. The cvs. R-Dhan (20-23%), Surya (16-22%) and Sharbati (13-18%) also exhibited a significant decrease in the plant yield during two years of study (Table 1). The lowest decrease in the yield was recorded in the cv. Shanthi. The cultivars next to Shanthi were JKRH404 and Vivekdhan 62 and did not exhibit a significant decrease in the plant yield (Table 1).

Soil population of *M. graminicola* at the time of harvest (4 months after planting) showed a considerable degree of variation with regard to the cultivars (Fig. 3). Greatest soil population of *M. graminicola* was recorded in the root zone of cv. Pusa Basmati-6 and Pusa Sugandh-5. Nematode population in cvs. Vivekdhan 62 was significantly less than the cv. R-Dhan or Sharbati ($P \leq 0.05$). The lowest population of *M. graminicola* was recorded in the root zone of cv. Shanthi (Fig. 3).

Leaf phenol and salicylic acid

Total phenol content (TPC) of leaves of rice cultivars varied significantly ($P \leq 0.05$; Fig. 4). In general, the phenol contents were significantly higher in the nematode-inoculated plants than un-inoculated plants. In nematode-inoculated plants, TPC increased gradually and reached a maximum concentration on 10 or 15 days after inoculation, thereafter, it decreased and remained unchanged till 30 days after inoculation (Fig. 4). From 30 days onward, the TPC further decreased to a lowest at 90 days. Inoculation of *M. graminicola* resulted in a significantly higher increase in TPC of cvs. Shanthi (15%), Vivekdhan (12%) and JKRH404 (9%) compared to un-inoculated control. The lowest increase in the TPC was recorded in the cvs. Pusa Basmati-5 and Pusa Sugandh-5 (Fig. 4). The correlation analysis revealed higher TPC in the cultivars that developed a low level of GI and vice-versa (Fig. 6).

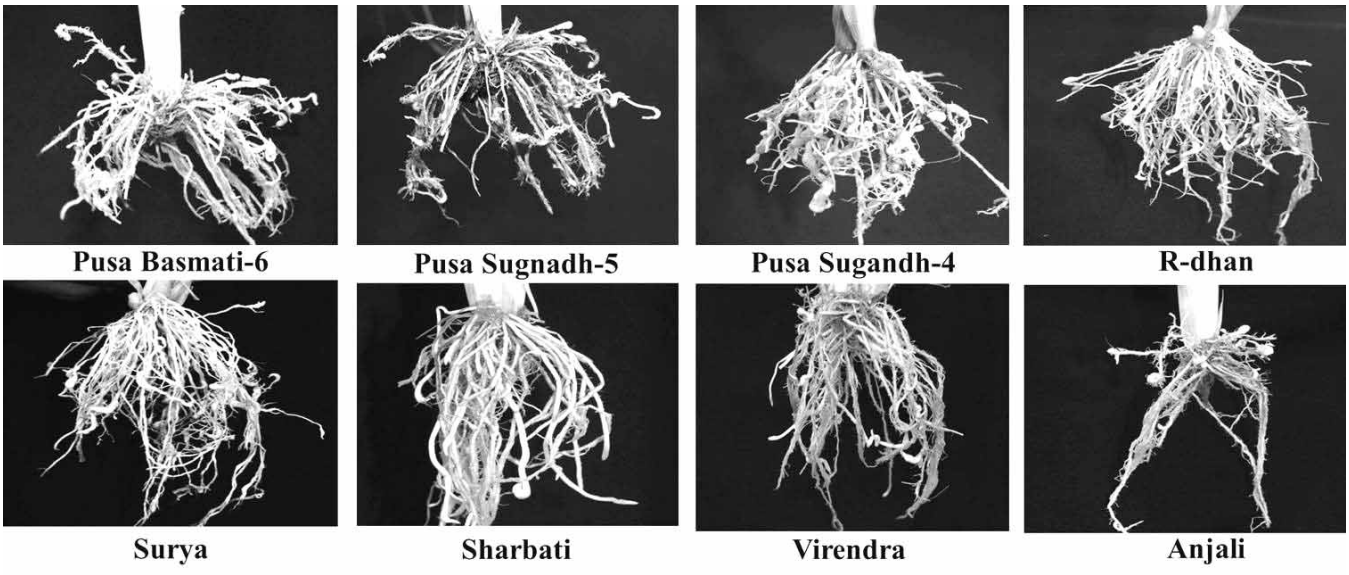

Fig. 1. Characteristic galling on roots caused by *Meloidogyne graminicola* on different rice cultivars

Table 1. Effect of *Meloidogyne graminicola* (1000 juveniles/kg soil) on the grain yield per plant of rice cultivars in inoculated and un-inoculated soil under pot conditions.

Rice cultivars	Grain yield per plant (g)			
	I year		II year	
	Un-inoculated soil	Inoculated soil	Un-inoculated soil	Inoculated soil
Anjali	23.2 ab	21.6 a	23.0 a	21.3 ab
Pusa Sugnadh-4	20.2 abc	18.1 a	20.4 ab	18.8 ab
JKRH 401	19.8 abc	18.7 a	19.6 ab	18.8 ab
PA6444	21.5 ab	19.4 a	21.3 a	19.2 ab
Pusa Sugnadh-5	24.0 ab	16.2 a	24.2 a	15.0 b
R-Dhan	23.7 ab	17.1 a	23.5 a	17.3 ab
Pusa Basmati-6	18.1 abc	9.2 b	18.5 b	10.3 c
Shanthi	16.9 bc	16.6 a	17.2 ab	17.0 ab
Sadabhar	25.3 a	22.8 a	25.1 a	22.7 a
CR-314	21.1 ab	19.3 a	21.6 a	19.7 ab
Sharbati	23.0 ab	20.3 a	23.2 a	20.5 ab
Surya	20.6 ab	16.9 a	20.3 ab	16.9 b
Swarna	23.2 ab	21.4 a	23.4 a	21.1 ab
Virendra	17.8 abc	16.2 a	17.7 ab	16.1 b
Vivekdhan 62	20.5 ab	18.3 a	20.2 ab	17.9 ab
LSD P≤0.05	4.49	3.88	4.69	3.17

Each value is mean of three replicates. Values within a column followed by different alphabets are significantly different at P≤0.05 according to Tukey's test.

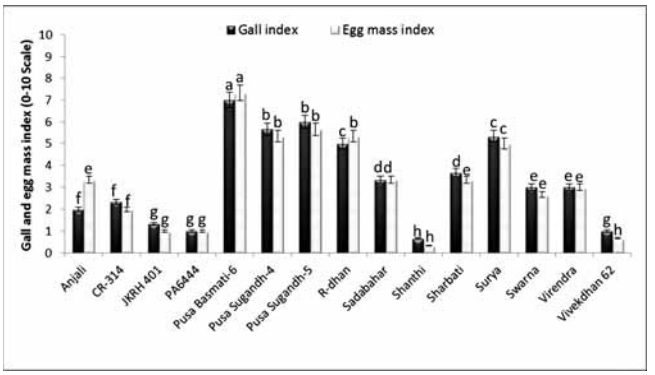

Fig. 2. Gall and egg mass indices (0-10 scale) of *Meloidogyne graminicola* on different cultivars of rice grown in the nematode inoculated soil (1000 J_2/kg soil). Error bars show standard error. Bars with different alphabet are significantly different at P≤ 0.05 according to Tukey's Test

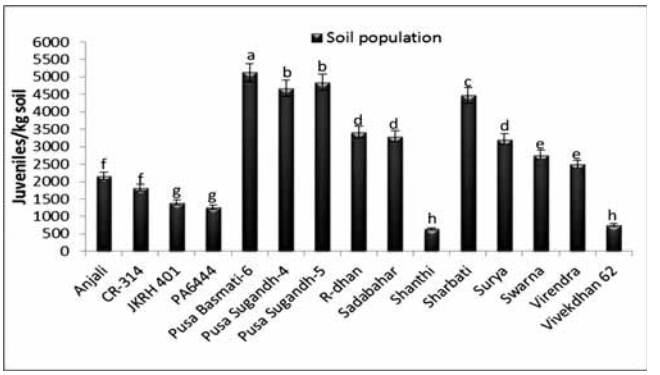

Fig. 3. Soil population of *Meloidogyne graminicola* on different cultivars of rice grown in the nematode inoculated soil (1000 J_2/ kg). Error bars show standard error. Bars with different alphabet are significantly different at P≤0.05 according to Tukey's Test

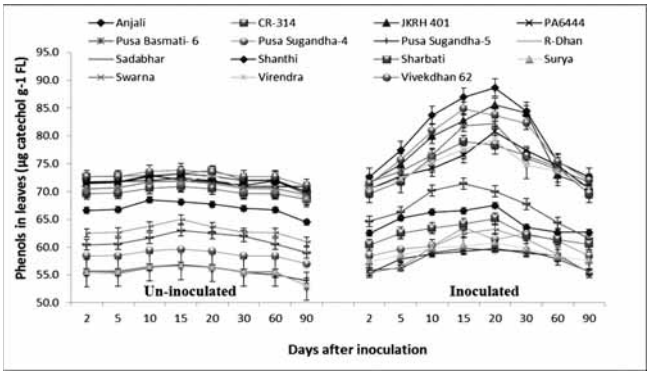

Fig. 4. Total phenols contents of leaves of rice cultivars grown in the soil un-inoculated or inoculated with *Meloidogyne graminicola* (1000 J2/kg) under pot condition. Error bars show standard error. FL= Fresh leaves

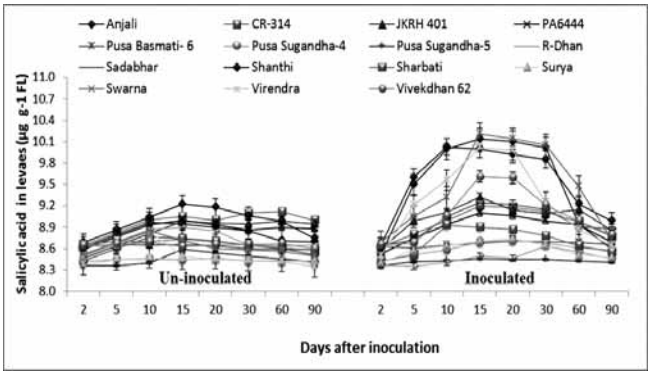

Fig. 5. Total salicylic acid contents of leaves of rice cultivars grown in the soil un-inoculated or inoculated with *Meloidogyne graminicola* (1000 J2/kg) under pot condition. Error bars show standard error. FL= Fresh leaves

The salicylic acid contents (SAC) of rice leaves increased in the nematode inoculated than un-inoculated rice plants (Fig. 5). Significantly greater SAC was recorded in the cvs. Shanthi (13%), Vivekdhan 62 (10%) and JKRH 404 (8%) compared to un-inoculated control (Fig. 5). The SAC in the leaves of cvs. Anjali and CR-314 were recorded highest at 10 days after inoculation, however, the concentration gradually and significantly decreased after 15 days of inoculation and retained a constant level till 30 days (Fig. 5). From 30 days onward, the SAC further decreased to the lowest level at 90 days. The correlation analysis has shown greater SA concentration in the cultivars that developed a lower gall index (Fig. 6).

DISCUSSION

All the tested cultivars express varied degree of susceptibility and resistance to the rice root-knot nematode. The highly susceptible cvs. Pusa Basmati-6 and Pusa Sugandh-5 showed a very high galling and egg mass index with 40-48% reduction in the plant yield. The cvs. Pusa Sugandh-4, R-dhan, Surya, and Sharbati were recorded to be moderately susceptible to the nematode with lower galling indices. However, the cv. Shanthi showed a very mild galling and the reduction in the plant yield was also not significant, hence, on the basis of the disease severity and yield performance, this cultivar can be categorized as moderately resistance/tolerance to *M.*

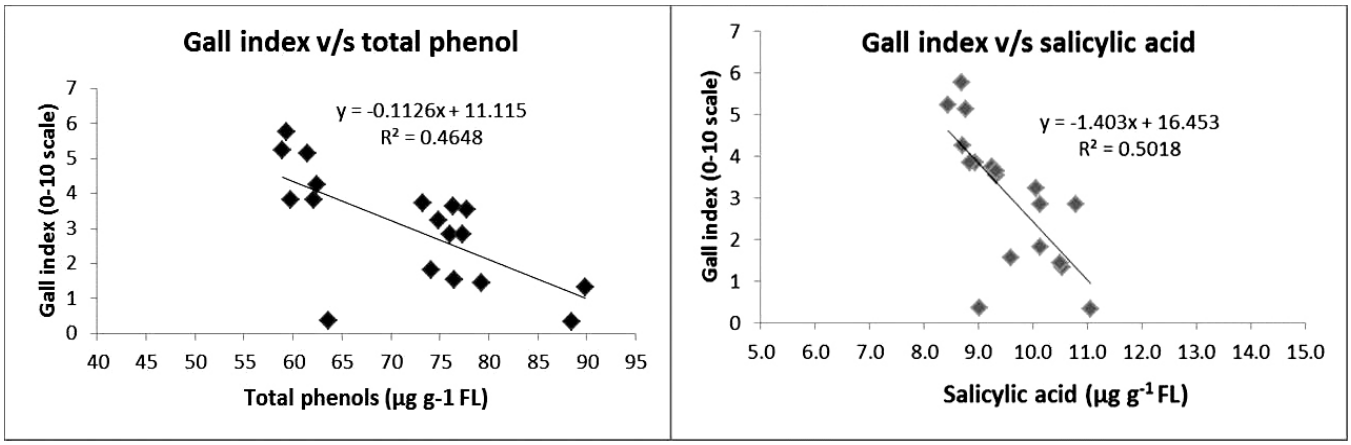

Fig. 6: Correlation between gall index and percent change in total phenol and salicylic acid of rice cultivars subjected to inoculation of *Meloidogyne graminicola* (1000 J_2/kg soil). FL= Fresh leaves

graminicola. The varied reaction of rice cultivars to *M. graminicola* has been reported in other studies also (Devi and Thakur, 2007; Kumari *et al.,* 2016). Many researchers have shown that the nematode may inflict yield loss to rice as high as 30-80% (Dutta *et al*., 2012), depending upon nematode population, soil condition and cultivar (Khan *et al.,* 2014).

Indian rice cultivars have been generally found highly susceptible to *M. graminicola* (Devi and Thakur, 2007; Mhatre *et al*., 2015) and support their reproduction. In the present study the cvs. CR-34, PA6444 and JKRH401 were found to be partially resistance/tolerance to *M. graminicola*, as evidenced by their lower soil population of nematode on these cultivars. Researchers have shown the existence of moderately resistance indigenous rice cultivars to *M. graminicola* (Das *et al.,* 2011; Kumari *et al.,* 2014). In the present study, none of the 15 cultivars were found completely resistant/tolerant to *M. graminicola*. However, the cvs. Vivekdhan 62 and Shanthi supported least proliferation of *M. graminicola* population.

Synthesis of phenols (TPC) and salicylic acid (SA) generally gets accelerated when plants are infected with the pathogens (Khan and Haque, 2013; Kyndt *et al*., 2014). The concentrations of these phenolic are found higher in the plants resistant/tolerant to the nematodes (Kammerhofer *et al*., 2015). In the present study, the increase in the TPC and SA contents of rice leaves was lesser in the cultivars that had a greater gall formation and decrease in the yield of the rice plant. For example in cvs. Pusa Basmati-6 and Pusa Sugandh-5 which exhibited greatest suppression in the plant yield, the lowest increase in the TPC and SA were recorded. Whereas, the highest concentrations of these phenolic were recorded in the cvs. Shanthi and Vivekdhan 62, which expressed moderately resistance/tolerance response. This indicates that the TPC (Meena *et al.,* 2000) and SA (Biere and Goverse, 2016) contribute in the self-defence of plants against nematode attack, including sedentary parasites (Khan and Haque, 2013; Kammerhofer *et al*., 2015). The greater accumulation of SA contributed towards a significant reduction in the infection of roots by *M. graminicola* (Nahar *et al.,* 2011). SA mediated tolerance reaction of cultivars was also evident from the correlation analysis between SA and root-knot index (Fig. 6).

Susceptibility of commonly growing rice cultivars to *M. graminicola* is of a serious concern and necessitates adopting appropriate management strategies in infested areas. Based on the morphological and biochemical host reactions, the cvs. Vivekdhan 62 and Shanthi may be exploited for commercial cultivation of rice in the host zone of rice root-knot nematode, after verification under multi-locational field trials. The cvs. Pusa Basmati-6 and Pusa Sugandh-5 should be avoided in the nematode-infested areas/field because of their higher degree of susceptibility to *M. graminicola*.

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SHORT COMMUNICATION

Bio-Management of Disease–Complex Caused by *Meloidogyne incognita* Race-2 and *Ralstonia solanacearum* in Jute, *Corchoru solitorius* L.

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Jute (*Corchoru solitorius* L.) is extensively grown in eastern India and is most important fibre cash crop where it ranks first in area and contributes about 62% of worlds production (Sinha *et al*. 2004). Like other crops, jute is also prone to attack by many pests and diseases. Root-knot nematode, *Meloidogyne incognita* and wilt causing bacterium, *Ralstonia solanacearum* are often found to attack the crop in the field causing considerable loss in yield as well as that deteriorate the fiber quality. They often form complex where *M. incognita* is often found to predispose the plants to the attack of the bacterium (Bhagawati *et al*., 1996). During survey, it was found that crop was badly infested with root-knot nematode and wilt causing bacteria. After identification root-knot nematode, *Meloidogyne incognita* Race-2 and wilt causing bacterium, *Ralstonia solanacearum* were recorded as major pests involving causing disease-complex on jute.

Various control measures including pesticides were tried to manage various diseases and pests in jute by various researchers (Banerjee *et al*., 2000; Hath and Chakraborty, 2004; Bibha and Bora, 2005; Rahman and Khan, 2010). Biological management offers a scope for ecofriendly approach that can be suitably used in integrated management of the complex disease. Hence, the present investigation was under taken to manage this complex under the project "AICRP on Nematodes in Cropping Systems" from 2008 to 2013 using bio-agents at Assam Agricultural University, Jorhat.

The trials were conducted from 2008 to 2013 during Kharif season at ICR farm, AAU Jorhat, Assam with seven treatments. The soil was almost neutral sandy loam. The experiments were laid out in Completely Randomized Block Design (CRBD) with three replications. The treatments comprised of T_1- Seed treatment with *Pseudomonas fluorescens*@ 20g/kg seed, T_2- Seed treatment with *Trichoderma viride*@ 4 g/kg seed, T_3- Seed treatment with *Paecilomyces lilacinus*@ 10 g/kg seed, T_4- Soil application of *P. fluorescens*@ 2.5kg/ha, T_5- Soil application of *T. viride*@ 2.5kg /ha, T_6- Soil application of *P. lilacinus*@ 2.5kg / ha and T_7- Untreated Control.

The experiment was conducted in an infested plot by root-knot nematode *M. incognita* Race-2 and wilt causing bacterium, *R. solanacearum.* The plot size was 3m x 3m using the susceptible variety JRO 524 (Navin). Seeds were treated with *P. fluorescens*, *T. viride* and *P. lilacinus* by adding CMC as a sticker and dried in shade before sowing. Soil application of *P. fluorescens*, *T. viride* and *P. lilacinus*@ 2.5 kg/ha was done one day ahead of sowing. For this, well rotten and well dried FYM were enriched with the respective bio-gents at 10:1. After through mixing of the bioagents with FYM it was moistened and covered with gunny sheet for 15 days before application. Observation on plant growth parameters and nematode and bacterium multiplication were recorded at the time of harvesting of crop.

Data recorded were analyzed for comparison of means using SAS version 12.0. For analysis data collected from all five trials was subjected to combined analysis after test of homogeneity of error of variance. Since this value was non-significant, the combined analysis was performed. The significance was considered at $P \leq 0.05$.

The result of the experiments presented in Table 1& 2 showed that all the bio-agents were effective increasing plant growth parameters including fibre yield and in reducing the final nematode population, root-knot index (RKI) and wilt incidence.Soil application of the any of the bio-agents was found to be superior as compared to seed treatment.Maximum shoot length (281.75 cm) and shoot girth (6.03cm), and minimum final soil population of *M. incognita* (255/200 cc soil) along with minimum RKI (2.91) were recorded in the treatment where FYM fortified with *T. viride*was applied @ 2.5kg/ha. Maximum fibre yield (15.65q/ha) and ICBR (Incremental Cost Benefit Ratio) were also recorded in the same treatment with an increase of 24.01 % over control. As per bacterium wilt incidence is concerned, a significant ($P \leq 0.05$) reduction was recorded (12.43) as compared to control (22.96) which was reduced by soil application of *T. viride* by up to 45.86%.

Trichoderma species have the ability to use a broad range of compounds and secretes a wide variety of enzymes which in turn are capable of breaking down recalcitrant plant polymers into simple sugars for energy and growth (Kubicek *et al*., 2008; Jaklitsch, 2009). They are used as bio-control agents as they aid in reduction of soil borne diseases of various crops (Lumsden and Locke, 1989) and include more benefits on plants such as promoting plant growth, increased nutrient uptake from the soil, and decreasing the activity of the soil borne pathogens (parasitism, antibiosis, induce resistance and involved in the nematode control process (Harman *et al*., 2004). Murthy *et al.* (2013) tested two isolates viz., T4 and T8 of *Trichoderma asperallum* for their ability to induce the production of defense-related enzymes in plants. They noticed higher accumulation of phenolics in plants pre-treated with T4 and T8 isolates and challenged with *R. solanacearum*. Same mechanism might be operative in the present investigation in reducing bacterium wilt incidence as well as root-knot nematode multiplication resulting in enhanced plant growth and fiber yield.

The present study recorded that maximum reduction of disease-complex and maximum increase in yield was recorded in treatment where *T. viride* was applied as soil

Table 1. Effect of bio-agents on plant growth parameters of jute infected by root-knot nematode, *M. incognita* Race-2 and wilt causing bacterium, *R. solanacearum.*

Treatment	Stem length (cm)	Percent increase over control	Stem girth (cm)	Percent increase over control	Bacterium wilt incidence (%)	Percent decrease over control
*Pseudomonas fluorescens**	266.83	7.36	5.82	25.97	13.70	40.33
*Trichoderma viride***	269.29	8.35	5.44	17.75	15.27	33.49
Paecilomyces lilacinus ***	262.92	5.78	5.17	11.90	17.11	25.48
P. fluorescens#	274.51	10.45	5.66	22.51	12.48	45.64
T. viride#	281.75	13.36	6.03	30.52	12.43	45.86
P. lilacinus#	262.17	5.48	5.26	13.85	15.50	32.49
Control	248.54	-	4.62	-	22.96	-
S.Ed ±	5.28	-	0.19	-	1.15	-
CD (0.05)	11.51	-	0.42	-	2.510	-

**Note – ***ST- Seed treatment at 20g/kg seed; **ST – Seed treatment at 4 g/kg seed; ***ST – seed treatment @ 10 g/kg seed; # SA – Soil application at 2.5kg/ha

Table 2. Effect of bio-agents on nematode multiplication and fibre yield of Jute infected by root-knot nematode, *M. incognita* Race-2 and wilt causing bacterium, *R. solanacearum.*

Treatment	RKI	Percent decrease over control	Final population (200 cc soil + 5g root)	Percent decrease over control	Fibre yield (q/ha)	Percent decrease over control	ICBR
*Pseudomonas fluorescens**	3.42	18.38	303.75	35.46	14.76	16.95	5.57
*Trichoderma viride***	3.30	21.24	295.50	37.22	14.42	14.62	4.77
Paecilomyces lilacinus ****	3.45	17.66	293.67	37.60	14.02	11.09	4.86
P. fluorescens[#]	3.22	23.15	281.33	40.23	14.98	18.70	5.00
T. viride[#]	2.91	30.55	255.00	45.82	15.65	24.01	6.35
P. lilacinus[#]	3.13	25.30	282.33	40.01	15.02	19.02	4.39
Untreated Control	4.19	-	470.67	-	12.62	-	-
S.Ed ±	0.14	-	11.64	-	0.288	-	-
CD(0.05)	0.31	-	25.37	-	0.627	-	-

**Note – ***ST- Seed treatment at20g/kg seed; **ST – Seed treatment at 4 g/kg seed; ***ST – seed treatment @ 10 g/kg seed; # SA – Soil application at 2.5kg/ha

application at 2.5kg/ha. In addition to this, the other two bio-control agents also could reduce the disease-complex significantly as compared to control. It is also concluded that bio-control agents were significantly more effective when they applied as soil application than seed treatment in reducing disease-complex and increase in yield. The root-knot nematode acts as a predisposing factor for attack by the bacterium *R. solanacearum*, thereby causing greater damage than that of either pathogen alone. In the present investigation, the suppression of nematode activity by *T. viride* might be instrumental in minimizing the predisposing effect of the nematode towards the bacterium and challenge with *R. solanacearum* thereby reducing the disease severity and increasing the fiber yield.

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Management of Reniform Nematode, *Rotylenchulus reniformis* Infecting Mung Bean (*Vigna radiata* L.) by Using Bio-Agents

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Reniform nematode is a serious pest of the crop during summer months. Summer mung bean is damaged due to optimal temperatures for nematode multiplication and development. Under water deficit conditions, reniform nematode becomes a serious problem and cause significant yield loss. Management of reniform nematode has been carried out by using bio-agents like *Paecilomyces lilacinus* on brinjal and chickpea (Asraf and Khan, 2008; Vyas *et al*. 2011). An integrated approach with application of potential bioagents (*T. harzianum, Purpureocillium lilacinus* and *P. fluorescens*) as seed treatment was carried out under pot conditions.

The experiment was conducted at Department Of Nematology, Rajasthan Collage of Agriculture, Udaipur in six-inch earthen pots filled with 1 kg infested soil having an initial inoculum of about 3/J2s per g of soil. Talc–based formulations of *T. harzianum, P. lilacinus* and *P. fluorescens* were used as seed treatments @ 5 and 10 g/kg seed. A standard check (*T. viride* at 10 g/kg seed) and untreated check was also maintained for comparison of experimental results. The tratments were: T1-*T. harzianum* @ 5 g/kg seed, T2-*T. harzianum* @ 10 g/kg seed, T3-*P. lilacinus* @ 5 g/kg seed, T4-*P. lilacinus* @ 10 g/kg seed, T5-*P. fluorescens* @ 5 g/kg seed, T6-*P. fluorescens* @ 10 g/kg seed, T7-*T. viride* @ 10 g/kg seed and t8-Unrtreated check. After 10 days of sowing, one healthy plant in each pot was maintained and watered regularly as and when required. Plants were harvested after 45 days of showing.

Observation on shoot length, shoot weight, root length and root weight were taken at harvest. For recording nematodes in the roots, the root were washed carefully in tap water and stained with 0.1% acid fuchsin lacto phenol and kept in lacto phenol for 24 hrs. thereafter, the root were examined thoroughly under a stereoscopic binocular microscope for counting number of female per 5 g root, number of egg masses per plant and number of eggs and larvae per egg mass. After removing the plant from pots, soil was thoroughly mixed and 200cc soil from each pot were taken and processed by Cobb's sieving and decanting technique (Cobb, 1918) followed by Baremann's funnel technique (Christie & Perry, 1951) for estimation of nematode population in soil.

Data presented in table 1 revealed that all fungal and bacterial bioagents applied significantly increased the plant growth and reduced nematode reproduction as compared to untreated check. The maximum shoot length (47.98cm), root length (32.40cm), shoot weight (31.30g) and root weight (9.21g) were recorded with *T. harzianum* @ 10 g/kg seed followed by *P. lilacinus* @ 10 g/kg seed (45.60cm, 31.10cm, 30.20g and 8.10g) and *P. fluorescens* @ 10 g/kg seed (44.20cm, 29.80cm, 29g and 6.75g), respectively. However seed treatments with *T. viride* @ 2.5 kg/ha (standatd check) (9.75 g) and *T. harzianum* @ 10 g/kg (9.15g) seed were found at par with respect to root weight.

The minimum number of egg masses per plant (10.80), no of eggs and larvae per egg mass (131.00), number of females per 5g root (10.60), nematode population per 200cc soil (400.00) and total population (3031) observed with *T. harzianum* @ 10 g/kg followed by *P. Lilacinus* @ 10 g/kg seed (12.20, 142, 13.20, 420.00 and 3426) and *P. fluorescens* @ 10 g/kg seed (13.20, 158, 16.60, 450 and 3871), respectively. However minimum no. of egg masses per plant (9.60), no of eggs and larvae per egg mass (123.00), number of females per 5g root (8.20), nematode population per 200cc soil (380.00) and total population (2716) were observed with

T. viride @ 2.5 g/ha. While maximum of egg masses per plant (19.00), no of eggs and larvae per egg mass (192.00), number of female per 5g root (29.80), nematode population per 200cc soil (775.00) and total population (6795) were observed with untreated check.

These findings are in agreement with Kumar *et al.* (2015) who reported *T. harzianum* as an effective and important fungal bio-agent in controlling reniform nematode, *R. reniformis* in mung bean. Nematode reproduction was significantly reduced when plant treated with fungus *T. harzianum, P. lilacinus* and bacterial bio-agent *P. fluorescens.*

The present investigation revealed that *T. harzianum, P. lilacinus* and bacterial bio-agent *P. fluorescens* at 10 g/kg seed was found to be the best in reducing nematode reproduction.

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Control of Root-Knot Nematode Pest of Okra using *Ocimum gratissimum* Compost

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Okra, *Abelomuscus esculentus,* is one of the highly nutritious vegetable grown in the tropical and subtropical parts of the world (Diouf, 1997). It is susceptible to wide range of pests and pathogens including root-knot nematode, *Meloidogyne incognita* which is responsible for significant yield reduction (Adesiyan *et al.,* 1997 & Katoh *et al.,* 2010).

Different non-chemical control measures are now being considered as a way of reducing the volume of synthetic pesticides hazards in our environment. Different organic materials including manures have been used for managing root-knot nematode (Abolusoro 2012; Abolusoro *et al.* 2013).

The objective of this research work was to examine the effects of *Ocimum gratissimum* compost on the root-knot nematode pest *(M. incognita*).

The experiment was conducted at Landmark University Teaching and Research Farm in the year 2016 and was repeated at the same-time in 2017 on well drained sandy-loamy soil. The experiment design was randomisedcomplete block design comprising of five

Table 1. Effects of *O. grattissimum* compost average number of leaves of *M. incognita* infested okra

Treatment rate	2016			2017		
	4 WAP	6WAP	8WAP	4WAP	6WAP	8WAP
10t/ha	13.80^{b}	25.40^{bc}	26.63^{c}	13.76^{b}	25.60^{b}	36.63^{d}
15t/ha	13.55^{bc}	22.38^{c}	32.38^{b}	13.60^{bc}	22.42^{c}	31.90^{c}
20t/ha	15.00^{a}	28.33^{a}	34.63^{a}	14.90^{a}	28.50^{a}	35.00^{a}
Carbofuran	14.43^{ab}	25.38^{bc}	33.08^{ab}	14.50^{ab}	25.60^{b}	33.25^{b}
Control	12.33^{c}	26.38^{b}	30.88b^{c}	12.39^{c}	26.50^{b}	28.390^{b}

Means followed by the same letter(s) along the same column are not significantly different from each other according to Duncan's multiple range test at p= 0.005

Table 2. Effects of *O. gratissimum* compost on the height (inchs) of *M. incognita* infested okra

Treatment rate	2016			2017		
	4 WAP	6WAP	8WAP	4WAP	6WAP	8WAP
10t/ha	14.10^{a}	25.18^{ab}	25.33^{ab}	14.20^{a}	25.11^{ab}	25.37^{ab}
15t/ha	13.80^{a}	25.39^{a}	25.30^{ab}	13.91^{a}	25.62^{a}	25.79^{a}
20t/ha	13.82^{a}	25.70^{a}	26.33^{a}	13.80^{a}	28.81^{a}	26.00^{a}
Carbofuran	13.90^{a}	25.15^{ab}	25.53^{ab}	13.61^{a}	25.22^{ab}	25.42^{ab}
Control	13.60^{a}	23.92^{b}	24.90^{b}	14.60^{a}	24.90^{b}	25.10^{b}

Means followed by the same letter(s) along the same column are not significantly different from each other according to Duncan's multiple range test at p= 0.005

Table 3. Effects of *O. gratissimum* the yield on *M. incognita* infested okra

Treatment rate	2016		2017	
	Average No of fruits/plot	Average fruit yielded/ plot(g)	Average No of fruits/plot	Average fruit yielded/ plot(g)
10t/ha	22.52^{c}	407^{d}	21.00^{c}	494^{d}
15t/ha	24.50^{c}	617^{d}	22.91^{c}	620^{b}
20t/ha	32.63^{a}	670^{a}	31.90^{a}	679.01^{a}
Carbofuran	25.90^{b}	574^{c}	26.20^{b}	602.00^{c}
Control	14.60^{d}	369.90^{c}	15.63^{d}	403.92^{c}

Means followed by the same letter(s) along the same column are not significantly different from each other according to Duncan's multiple range test at p= 0.005

Table 4. Effects of *O. gratissimum* compost on the soil population and root gall index of *M. incognita* infested Okra

Treatment rate	2016		2017	
	Mean root knot soil population (200ml)	Mean root gall index	Mean root knot soil population (200ml)	Mean root gall index
10t/ha	381[a]	1.80	391[a]	1.69
15t/ha	49[b]	1.69	307[b]	1.50
20t/ha	302[c]	1.42	279[c]	1.39
Carbofuran	290[c]	1.39	275[c]	1.33
Control	1629[d]	4.07	1702[d]	4.00

Means followed by the same letter(s) along the same column are not significantly different from each other according to Duncan's multiple range test at p= 0.005

treatments and each replicated four times. The land was prepared into plots of 4mxlm sites. Okra (NH4e-47-4) seeds were sown at 4 seeds per stand which was later thinned into two healthy plants three weeks after sowing.

The *O. gratissimum* compost was obtained by mixing 10kg of freshly harvested *Ocimum*plant leaves with 2kg of poultry manure in a polytene bag. One kg of wood ash was placed on the materials to neutralize acidity. The materials were thoroughly mixed every month for about four months until there was full decomposition. The decomposed material was air dried and ground into powder form for use.

At three weeks after planting, each seedling on the field was inoculated with approximately 5000 *M. incognita* eggs. The experimental treatments were (*O. gratissimum* compost) a. 10t/ha, b. 15t/ha, c. 20t/ha, d. Carbofuran 3kg, e. Control.

Each treatment except carbofuran was applied as soil amendment and incorporated into the soil at their respective doses two weeks before sowing while carbofuran was applied two weeks after seedling emergence. At week four of the experiment till final harvest, data were collected on number of leaves, height, number of fruits and fruit weights. Final nematodes population, root damage level (gall index) was taken following Taylor and Sasser(1978) on 0-5 rating scale, where o= no gall,1=1-2%,gall; 2=3-10%, 3=11-30 galls; 4= 31-70% galls; and 5= 71-100% galls. All data were subjected to analysis of variance using Duncan's multiple range test at 5% probability level.

Table 1 is the analysis of variance on the number of leaves produced by *M. incognita* infested okra due to treatments. Significant differences were observed effective from week four to eight of the experiment in the two years of investigation. *O. gratissimum* compost at various levels of application (10,15 and 20t/ha) as well as carbofuran significantly increased the number of leaves produced by the root-knot nematode infested okra compared with the untreated control.More leaves were recorded at higher concentration of *O. gratissimum* than the lower concentrations in the two years of experimentation.

There were significant differences in the height of okra due to different levels of *O. gratissimum* application. More height that was significantly different from control and carbofuran as well as other lower levels of application were observed especially at the 8thweek of the experiment in treatments which received the highest dose of *O. gratissimum* (20t/ha) in both years of the experiment.

Higher dosages of application of compost increased the yield (fruit number and fruit weight) more than the lower dosages and were significantlydifferent from the untreated control. The two high dosages (15 and 20t/ha) were significantly better than carbofuran in the two years of experimentation as more yields were recorded in those treatments compared with the control.

The plots that received the highest dose of *O. gratisimum* brought about the highest reduction in *M. incognita* population and significantly different from the control and lower doses of *O. gratissimum*. The root damage was more reduced in higher doses of treatment than the lower doses in the two years of the experiment.

Application of *O. gratissimum* compost brought about a significant decline in the soil population of root knot nematode *M. incognita* on okra. This action subsequently brought about improvement in the growth and yield parameter of okra plant in the experiment. The use of organic manures including compost has been demonstrated by many researchers. The research findings showed that soil amendment with organic manures including compost stimulated the multiplication of micro-organisms like fungi and bacteria, some of these macro-organisms are parasite of nematode. This will bring about suppression of parasitic nematode in the soil hence promoting growth, development and yield of the plants (Kaskavala, 2007; Sheriff 2008; Renco *et al.* 2010; Abolusoro, 2013).

The result from this experiment showed that *O. gratissimum* compost had a nematicidal property that helped in suppressing soil nematode population. In most cases, *O. gratissimum* compost treatments outperformed the carbofuran treatments as observed in the experiment. *O. gratissimum* compost at 20t/ha is therefore recommended for use in the control of root-knot nematode *M. incognita* in endemic soils.

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Evaluation of Ridgegourd Varieties/Cultivars Against Root-Knot Nematode, *Meloidogyne incognita*

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Ridge gourd (*Luffa acutangula*) belonging to family cucurbitaceae popularly known as angled gourd or sponge gourd is a tropical annual climbers, cultivated for its edible young fruits. A ridge gourd also commonly known

Table 1. Reaction of Ridgegourd cultivars / lines against root knot nematode, *Meloidogyne incognita*

Reactions based on Root-knot index	Name of the varieties
Resistant (1.1 to2.0)	Priya, Challenger, Jaipur long, BSS-1009, Tauri, Pallishree (6)
Moderately Resistant (2.1 to 3.0)	Narayana, Aneeta, NHRG-1001, Ridgegourd 12 pata, 12 PataJhinga, Dhenkanal local, Nayagarh local, Yeshvi-38, Indo-US-216 (9)
Susceptible (3.1 to 4.0)	Maharastra16 patajhinga, Hybrid patal tarai, Hybrid jhinga, SS-Ramu, Sumitrs, Machar jhinga, Debsundari, Ramu F1, Cluster, Sevenstar, Athgarh local, Sankarpur local, Mira, Vaishali, Devika-776, MHRG 7, Estilo, SS-Chandrani, SE-19Marwari, Krishna-51, DEB-2404, SC-18, Kaveri, Lavanya, Avanti, BSS-1036, Saniya-4, Arun, Ankur Latika, NS-474, Lumbini (32)
Highly susceptible (4.1 to 5.0)	Aarti, Harsha, F1 Stella, Rohini, Laila (5)

as Turai or Turiya is a native of India and also seen grown as ornamental plant in many parts of the world. It has white pulp spongy flesh containing a gelatinous compound called luffein and has medicinal importance.Ridge gourd is quite lower in saturated fats as well as calories. It really is abundant with dietary fibre, vitamin C, riboflavin, zinc, thiamin, iron, as well as magnesium.

Root knot nematode, *Meloidogyne* species is one of the most wide spread nematode limiting world agriculture productivity, particularly damaging vegetable crops in tropical and subtropical countries (Sikora and Fernandez, 2005). Indiscriminate and injudicious use of chemical pesticide has cause harm to human health. Therefore, economically safe option is the use of resistant varieties an important component of overall integrated nematode management system.

In the present study, 52 varieties of ridgegourd were collected from the locality of Bhubaneswar and nearby villages of Dhenkanal. The experiment was carried out in screen house of the Nematology Department, College of Agriculture, OUAT, Bhubaneswar, Orissa and treatments were arranged in Complete Randomized Block Design with three replications each. Ridgegourd genotypes were transplanted singly in each 15 cm earthern pots containing 1 kg sterilized soil and after establishment of plants they were inoculated with freshly hatched larvae of root knot nematode (*M. incognita*) @ 1000J2/ pot. Ridgegourd plants were uprooted from the pots after 45 days of sowing i.e. 30 days after inoculation and screening of germplasm for resistance and susceptibility against root knot nematode (*Meloidogyne incognita*) was done by adopting 1-5 scales as Highly Resistant (1= no gall/egg mass per plant), Resistant (2=1-10 galls/ egg mass per plant), Moderately resistant (3= 11-30 galls / egg mass per plant). Susceptible (4= 31-100 galls/ egg mass per plant) and Highly Susceptible (5 = more than 100 galls/egg masses per plant) as per Root-knot Index scale suggested by Taylor and Sasser(1978).

Out of fifty two ridgegourd varieties/cultivars screened against root-knot nematode, only six showed resistant reaction with 7-10 number of galls per plant, seven varieties showed moderately resistant reaction with 23-31 number of galls per plant, thirty two showed susceptible reaction with 31-91 number of galls per plants whereas five were highly susceptible with 112-116 number of galls per plants.Maximum gall was recorded in variety HARSHA (116.33) which supported maximum population of 3.27 and least population was recorded as 0.64 in the variety Krishna -51 with gall number 61.33 (Table 1).

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Effect of *Trichoderma* spp. against *Meloidogyne incognita* on Tomato

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"Tomato (*Lycopersicon esculentum* L. MILL) is a major crop of world commerce and one of the most widely grown vegetables. Tomato supplies essential nutrients in human diet; it is a good source of vitamins A and C, potassium, and fiber. Tomato is rich in lycopene DiMascio *et al*. (1989), which is used in the fight against cancer, especially the prostate cancer Giovannucci *et al*. (1995). Tomato is mostly affected by root-knot nematode, *Meloidogyne* spp. (Bhardwaj, 1972). Root-knot infection causes 24-26% loss in tomato (Sasser, 1979).

In order to reduce the population of the nematodes, several control measures are being employed including cultural practices, chemical and biological control methods. As an eco- friendly approach use of bio- control agents like *Trichoderma* spp. for the management of phytonematodes and plant pathogens have received much attention by researchers during recent amendment help in reducing nematode population. Some species of *Trichoderma* have been used widely as biocontrol agents against soil-borne plant diseases (Harman *et al*., 2001). *Trichoderma* isolates have been used successfully to control the damage caused by soil-borne pathogens in greenhouses and under opened-field conditions (Papavizas., 1985). *Trichoderma* species also have been shown to have activity towards root-knot nematode. Some *Trichoderma* isolates were reported to both enhance plant growth and reduce root-knot nematode damage (Windham *et al*., 1989).

The experiment was conducted in the research laboratory and experimentation plot, Department of Plant Pathology and Entomology, Sam Higginbotttom University of Agriculture, Technolgy and Sciences, Allahabad. Three tomato seedlings were transplanted in each pot (15 cm) and 1000 freshly hatched J2 were inoculated after one week of transplanting the seedlings. Untreated and chemical check (carbofuran @ 3 g per pot) was also maintained for comparison.

The *Trichoderma* isolates were being culture in petriplate were grinded well separately with dilution of 100ml of water in it and 10ml of each isolate were applied to the respective pots near the root zone. After 90 DAT (days after transplanting), the plants were uprooted, thoroughly washed and number of galls/ plant were recorded.

The effect of *Trichoderma* isolates on juvenile mortality was studied under in vitro conditions. Approximately one hundred J2 of *M. incognita* were placed in 10 ml of suspension of *Trichoderma* isolates contained in sterilize petri plates and incubated at room temperature. The plates were examined after 24 and 48 hours and the number of dead juveniles were counted. Three replications were maintained. The juveniles inoculated in carbofuran 3G served as check and the juveniles inoculated in sterile distilled water were taken as control.

Table 1 shows that all the treatments significantly reduces the root gall/ root system of tomato when compared with inoculated control. Among the treatments T_4 shows significant decreased of root galls compare to all the other treatments.

Table 2 shows the effect of *Trichoderma* isolates on the larval population of *Meloidogyne incognita*. All the treatments significantly reduces the larval population compare to the inoculated control. Among the treatments T_4 shows maximum reduction of larval population compare to all the other treatments.

Table 3 shows the mortality rate of *Meloidogyne incognita* recorded after 24 and 48 hrs. All the treatments shows significant increase in percentage of mortality compared to the inoculated control. Among the *Trichoderma* isolates maximum larval mortality was observed in T_4.

The fungus provided gave some level of nematode suppression as much as synthetic nematicides. Tomato plants that were treated with *Trichoderma* isolates were less attacked by the root-knot nematodes and also shows significant reduction in root gall/ root system of tomato.

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Incidence of Plant Parasitic Nematodes Associated with Capsicum (*Capsicum annuum* L.) in Himachal Pradesh

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Capsicum (*Capsicum annuum* L.) is one of the most important vegetable crops grown worldwide belonging to the solanaceae family and popularly known as bell pepper, sweet pepper or Shimla mirch. Capsicum is cultivated as commercial cash crop in Himachal Pradesh, Jammu and Kashmir, Uttar Pradesh and Uttrakhand (Kumar and Verma, 2009). In Himachal Pradesh, capsicum crop is grown under an area of 2.50 thousand hectares with annual production 58.29 thousand metric tonnes (NHB, 2017). Several genera of PPNs are associated with capsicum but the genus *Meloidogyne* is most abundant (Sasser, 1977; Khan and Haider, 1991). Capsicum is highly adaptable host for the *M. incognita* responsible for great economic losses annually (Chaudhary and Kaul, 2011).

During the period of 2016-2018, a survey on the incidence of PPNs was conducted in Sirmaur and Solan districts of Himachal Pradesh. Soil and root samples from the capsicum crop were collected and analyzed as per procedure in the Department of Zoology, Eternal University, Baru Sahib, H.P.

In total 72, soil and root samples were collected out of which 58 samples were positive for PPNs. The genera recorded were *Meloidogyne* sp., *Helicotylenchus* spp., *Xiphinema* sp., *Aphelenchus* sp. and *Filenchus* sp. During this survey study 34 samples were collected from Sirmaur and 38 samples were collected from Solan district. The genus *Meloidogyne* was recorded as the most prevalent in capsicum crop. Only one species

Table 1. Frequency of occurrence of various genera of PPNs recovered from soil and root samples of capsicum crop from district Sirmaur during the period of 2017-2018

Genus	Total no. of positive samples	Frequency of occurrence	Relative Frequency of occurrence
District Sirmaur			
Meloidogyne	27	79.41%	36.98%
Helicotylenchus	25	73.53%	34.24%
Xiphinema	10	29.41%	13.69%
Aphelenchus	07	20.59%	09.58%
Filenchus	04	11.76%	05.47%

Total number of samples examined=34

Table 2. Frequency of occurrence of various genera of PPNs recovered from soil and root samples of capsicum crop from district Solan during the period of 2017-2018

Genus	Total no. of positive samples	Frequency of occurrence	Relative Frequency of occurrence
District Solan			
Meloidogyne	28	73.68%	36.84%
Helicotylenchus	31	81.58%	40.79%
Xiphinema	9	23.68%	11.84%
Aphelenchus	6	15.79%	7.89%
Filenchus	2	5.26%	2.63%

Total number of samples examined=38

Table 3. Total population of PPNs associated with capsicum in Simaur and Solan districts of Himachal Pradesh

PPNs	Nematodes Population / 200cc (range)
Meloidogyne incognita	20-1620
Helicotylenchus dihystera	20-820
H. pseudorobustus	20-160
Xiphinema basiri	20-80
Aphelenchus avenae	20-100
Filenchus sheri	20-120

belonging to genus *Meloidogyne* was reported i.e. *M. incognita* with frequency of occurrence 79.41% and relative frequency of occurrence as 36.98% in Sirmaur district (Table 1). Whereas the frequency of occurrence was recorded 73.68% with relative frequency of occurrence 36.84% in Solan district of H.P (Table 2). The total nematode population of *M. incognita* was ranged from 20-1620 juveniles /200 cc of soil (Table 3). The genus *Helicotylenchus* was also predominant in capsicum crop shown frequency of occurrence (81.58%) in Solan district followed by frequency of occurrence as 73.53% in Sirmaur district. During the present survey study, two species *H. dihystera* and *H. pseudorobustus* were collected from various localities of Sirmaur and Solan districts. The total nematode population of *H. dihystera* and *H. pseudorobustus* ranged from 20-820 adults /200 cc of soil and 20-160 adults /200 cc of soil respectively (Table 3). *H. pseudorobustus* was encountered only in 3 soil samples out of 72 examined and was recorded for the first time from capsicum in Sirmaur district of H.P. Present study indicated the

predominance with high population of infective stage juveniles of *M. incognita* and *H. dihystera* among different genera of plant parasitic nematodes associated with capsicum in Sirmaur and Solan district of H. P. Similar observation has been made by Bommalinga (2011) who reported maximum population of *M. incognita* and *Helicotylenchus* around Bangluru district in rhizospheric soil of capsicum. Results are also in agreement with many other researchers (Kim, 1987; Deshmukh *et al*., 1990; Khan *et al*., 1994; Khan *et al*., 2000; Rao, 2004) according to them root-knot nematode (*Meloidogyne*) and spiral nematodes (*Helicotylenchus*) were most abundant species which were responsible to cause significant yield losses to the capsicum, chilli and other vegetables crops in Korea, India (U.P, M.P, Karnataka, Tamil Nadu) and Pakistan. Earlier, Khanna and Chandel (1997) recorded association of *M. incognita, Helicotylenchus vericaudatus* and *Criconemella xenoplax* in capsicum from Solan and Shimla districts of Himachal Pradesh, India. The dagger nematode, *Xiphinema* was recorded infesting capsicum crop in various localities of Sirmaur and Solan district of H.P. Only one species belonging to genus *Xiphinema* was reported i.e. *X. basiri* with frequency of occurrence as 29.41% in Sirmaur district and also reported in Solan with frequency of occurrence 23.68% (Table 1 & 2). The total nematode population of *X. basiri* ranged from 20-80 individuals /200 cc of soil (Table 3). In the present study, *X. basiri* was recorded for the first time from capsicum in Sirmaur district of H.P.

The genus *Aphelenchus* with one species *A. avenae* was recorded in capsicum crop with frequency of occurrence as 20.58% and relative frequency of occurrence 9.58% in Sirmaur district and this genus of PPNs was reported in Solan with frequency of occurrence 15.79% and relative frequency of occurrence 7.89% (Table 1 & 2). The total nematode population of *A. avenae* was ranged from 20-100 adults/200 cc of soil (Table 3). Singh *et al*. (2009) recorded *A. avenae* with frequency of occurrence 13% and relative frequency 3.4% from Uttar Pradesh, Haryana and Punjab. Thakur *et al*. (2015) recorded *Aphelenchus avenae* associated with *Brassica compestris* L. from Patiala locality of Malwa region of Punjab. The genus *Filenchus* with one species *F. sheri* was recorded in capsicum crop with frequency of occurrence as 11.76% with relative frequency 5.47% in Sirmaur district and this genus of PPNs was reported in Solan with frequency of occurrence 5.26% and relative frequency of occurrence 2.63% (Table 1 & 2). The total nematode population of *F. sheri* was ranged from 20-120 adults/200 cc of soil (Table 3). Thakur *et al*. (2015) recorded *F. sheri* associated with *Helianthus annus* L. from Patiala locality of Malwa region of Punjab.

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First Report of Weed-Disease Complex of *Meloidogyne incognita* and *Orobanche cernua* in Brinjal

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The root knot nematodes (*Meloidogyne* spp.) are the most economically damaging group of plant parasitic nematodes distributed globally. The yield loss due to the root-knot nematode, *M. incognita* in brinjal has been estimated from 16 to 41.8 per cent (Anwar and McKenry 2012). Similarly, *Orobanche cernua* Loefl. has been known as a problem to Solanaceous crops, affecting particularly tomato, tobacco, and eggplant (Prasad *et al*., 2009). *O. cernua,* locally known as "Bargawa/ Chargoda" is an achlorophyllous, angiospermic holo-root parasite that depend completely on host to complete its life cycle (Punia *et al.,* 2016). Comprehensive report on crop loss in brinjal due to *O. cernua* is not available in the country, however, the problem in South-East India affects about 50% (40 000 ha) of the crop Parker (1994).

The affected plants exhibited chlorosis of foliage leading to drying of leaves and stunted plant growth, the brinjal plants along with the broomrape, were carefully excavated to confirm the haustorial connection of parasite in root of brinjal and galls (Fig. 1). It was further noticed that there were patches in the field where brinjal plants were badly damaged; it was when the plants were concomitantly infected with root-knot nematode and broomrape as compared to when infected by either root-knot nematode or broomrape alone. The identification of root-knot nematode was done according to the method of Eisenback *et al* (1981) and the identification of *Orobanche* species was done based on the basis of morphological characters as suggested by Parker and Riches (1993). Recently, a disease complex caused by root-knot nematode and *O. aegyptiaca* in brinjal and tobacco was reported by Akhter and Khan (2018(a&b)) and in brinjal and tomato by *O. ramosa* by Kanwar (2017), However, the involvement of weed-disease complex of *M. incognita* and *O. cernua* in brinjal has not been observed. To the best of our knowledge, this is the first ever report of weed-disease complex involving root-knot nematode, *M. incognita* and obligate holo-root parasite, *O. cernua* on brinjal. During the present year, severe economic losses have occurred in brinjal crop (personal communication with local farmers). Severe parasitism of both the pathogens in Banda district was resulted due to the mono-cropping of eggplant. Considering the heavy yield loss being incurred to *S. melongena,* more intensive experimental works and investigations are needed for the management of *O. cernua*. The voucher specimen was deposited in Botany Department, Aligarh Muslim University, Aligarh India.

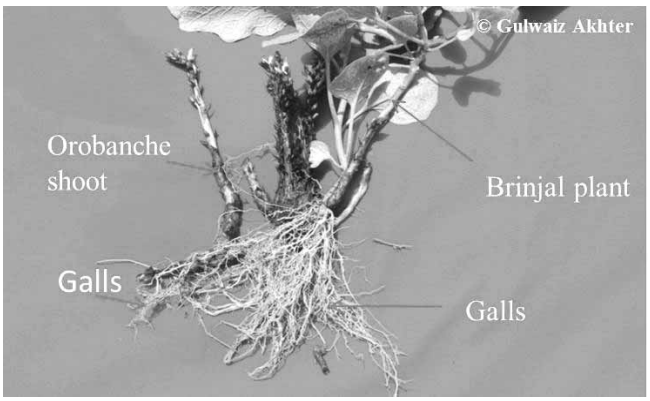

Fig.1. Brinjal roots showing galls and haustorial connection of *O. cernua*

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Effect of Brassicca Cultivar on Biofumigation for Management of Plant-Parasitic Nematodes

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Plants belonging to the families Brassicaeae, Capparidaceae and Caricaceae possess a class of organic compounds containing S and N and derived from glucose and amino acids; called glucosinolates. These water soluble anions are present in cell vacuoles and are separated from the enzyme myrosinase present in the cell cytoplasm. This enzyme hydrolyzes glucosinolates to release methyl isothiocyanate (MIC) which is nematicidal (Fig. 1). If plant biomass is chopped and incorporated in the soil for degradation, the enzyme comes in contact with the substrate to release MIC that is toxic to plant-parasitic nematodes in the soil. The glucosinolate content varies in different plant cultivars. To observe the effect of degradation of *Brassica* leaves on root–knot juveniles in infested soil, a laboratory experiment was conducted wherein fresh leaves of ten cutivars of plants belonging to family Brassicaceae were procured from Division of Genetics, ICAR-IARI. The cultivars were Pusa Karishma, Pusa Mustard 28, Pusa Vijay, Pusa Hira, LOO-2 and E597325 of *Brassica juncea*, RTM 314 of *Eruca sativa*, Pusa Swarnim IGC01 of *B. carinata* and YSH 02 01 of *Brasicca rapa*. The fresh leaf biomass was incorporated @3%w/w in 2kg root-knot (*Meloidogyne incognita*) infested soil in a replicated trial and incubated in sealed polythene bags in a BOD incubator at 25C. An average juvenile density of 25J2/cc soil became undetectable in polythene bags within a week. No significant difference in the effect of 10 varieties were observed. One-two active J2/cc soil were observed in treatment with cultivar LOO-2 of *B. juncea*. There was a natural decline of 8% in juvenile density in untreated control. A significant increase in free-living nematodes was observed in the treated soil, compared to untreated soil.

To substantiate the effect of biofumigation under field conditions, another experimental trial was conducted in microplots, the effect of biofumigation using five different cultivars of *B. juncea* (PM21, PM24, 9MST(00)12-11, MST (00)12-12 and Pusa Vijay. The microplots were infested with several plant-parasitic nematodes predominantly reniform nematode, *Rotylenchulus reniformis*. The other plant-parasitic nematodes were *Meloidogyne incognita*, *Tylenchorhynchus* spp., *Hoplolaimus indicus* and *Helicotylenchus* sp.. The saprozoic nematodes included rhabditids, cephalobids and dorylaimids. The field was divided into small microplots of size 2m X 2m and

Table 1. Effect of biofumigation on plant-parasitic and saprozoic nematodes in soil after 2 weeks.

Brassica Cultivar	Covered soil			Uncovered soil		
	PPN	Saprozoic nematodes	SI	PPN	Saprozoic nematodes	SI
T1(PM21)	300	16750	0.98	1913	1320	0.40
T2 (PM24)	1240	14620	0.92	1507	2160	0.58
T3 (9MST(00) 12-11	620	1790	0.74	1320	2910	0.68
T4 (MST(00) 12-12	960	11220	0.92	1080	3600	0.77
T5 Pusa Vijay	1100	2495	0.69	1460	1980	0.57
Untreated Control	1250	3050	0.70	1600	1550	0.49

PPN=Plant parasitic nematodes,/250cc soil ; SI=Saprozoic index =No. of saprozoic nematodes/No. of PPN + Saprozoic nematodes

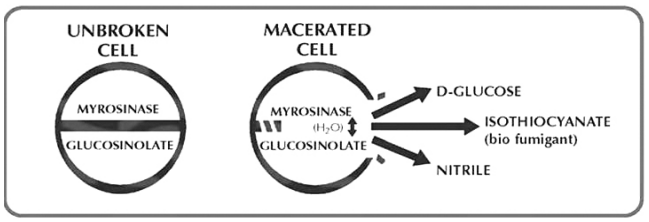

Fig. 1. The process of biofumigation

chopped leaves from each cultivar were incorporated @ 3tonnes/ha and covered with 60 microns transparent polythene sheet for 2 weeks and nematode densities in soil were observed. The plots where cultivar PM21 was incorporated and covered with polythene showed the least number of plant-parasitic nematodes (300/250cc soil) and the highest saprozoic index(0.98), compared to 1250/250cc soil and a SI of 0.70 in untreated control . The plots where biomass was incorporated but not covered with polythene did not show a significant decline in plant-parasitic nematodes or an increase in saprozoic nematodes. The factors which determine the biofumigation potential (BP) of a particular Brassica genotype for a given target organism are the total biomass, glucosinolate content in plant parts and toxicity of hydrolysis products of glucosinolate to target organism (Kirkegaard, 1998).

With non availability of chemical pesticides in the market the use of biofumigation can be exploited for nematode management especially in places where biofumigant plant biomass is available.

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Management of Root-Knot Nematodes (*Meloidogyne* spp.) Using Different Chemicals in Tomato Nursery

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Tomato is often severely attacked by root-knot nematode, *Meloidogyne* spp. a predominant and widely prevalent species inflicting serious loss in tomato (Sasser, 1989; Reddy, 1986; Bhatti and Jain, 1977; Kamran *et al*., 2011; Grace *et al*., 2009; Cetintas and Yarba, 2010). Forty per cent yield loss in tomato due to *M. incognita*, was reported by Singh and Kumar (2015). Various centers of 'All India Co-ordinated Research Project on Nematodes in Agriculture' estimated yield losses in different cultivars of tomato which ranged between 5 – 37 per cent (Anon., 2017). Therefore, present investigation was carried out to manage root-knot disease in tomato nursery.

The present investigation was carried out in root-knot (mix population of *M. incognita* and *M. javanica*) nematode sick nursery of Department of Nematology, B. A. College of Agriculture, AAU, Anand during *kharif* 2016-17. There were total six treatments with four replications. (T_1: Carbosulfan @ 2.5 l/ha, T_2: Cartap hydrochloride @ 3 kg/ha, T_3: Carbofuran @ 3 kg /ha, T_4: Phorate @ 3 kg /ha, T_5: Dazomet @ 300 kg /ha and T_6: Control (Untreated check). In nursery, 1.2 x 1.2 m sized 24 nursery beds were prepared. Seeds of tomato (Gujarat Tomato-2) were broadcasted in prepared beds @ 3 g/ bed. Bed without any chemical application was kept as an untreated check. Germination count per 225 cm^2 was recorded at four different spot in each bed. Number of transplanted seedlings were recorded. After 5 weeks of sowing, roots were washed gently under running tap water. Observations on seedling height, weight and root-knot index (0-5 scale) were recorded. Roots were cut in to 2-3 cm length and 3g roots were stained in 0.05 per cent acid fuchsin in lactophenol and were examined for

Table 1. Effect of different chemicals on plant growth characters of tomato

Treatment	Germination count/225 cm^2	Seedling height (cm)	Freshweight (g)		Transplantable seedlings/ bed (1.44 m^2)
			Shoot	Root	
T_1 (CAS)	12.75	15.7	46.00	5.63	187.75 (6.07)*
T_2 (CH)	6.50	12.9	42.57	4.12	32.75 (-81.50)
T_3 (CAR)	13.00	16.3	54.50	6.08	182.25 (2.97)
T_4 (PHO)	13.50	18.4	65.62	6.70	202.00 (14.12)
T_5 (DAZ)	17.15	22.6	82.92	7.03	346.50 (95.76)
T_6 (CON)	12.60	12.5	42.96	6.66	177.00
SEm±	0.91	1.0	5.13	0.51	—
CD (0.05)	2.67	3.0	15.46	1.53	—
CV %	14.52	12.3	18.40	16.83	—

*****per cent increase/decrease over control

nematode population. At the time of termination of experiment final nematode population per 200 cm^3 soil was recorded.

Initial root-knot nematode population was 210 J_2 per 200 cm^3 of soil in nursery. Germination count was maximum (17.15) in dazomet (T_5) and it differed significantly with rest of the treatments. Results obtained in the treatment of carbosulfan (**(**T_1), carbofuran (T_3), phorate (T_4) and control (T_6) were stastically at par with each other. Germination count was minimum (6.50) in cartap hydrochloride (T_2) which may be due to toxic effect (Table 1). Babu and Umarjan (2015) also observed significant inhibition in germination and shoot-root growth due to toxic effect of cartap hydrochloride in barley. Significantly highest number of transplantable seedlings *i.e.* 271.50 were found in dazomet (T_5) treatment. Next best treatment was phorate (T_4) which did not differ significantly with the treatment of carbosulfan (T_1), carbofuran (T_3) and control (T_6). Lowest number of transplantable seedlings were noticed in the treatment of cartap hydrochloride (T_2) at 1st and 2nd pulling (Table 1). Seedlings raised in the beds treated with dazomet had maximum seedling height (22.6) and stastically differed with rest of the treatments. Next effective treatment was phorate (T_4) which remained at par with carbofuran (T_3) and carbosulfan (T_1). Seedling height was lowest in the control (T_6). However, it was stastically at par with cartap hydrochloride (T_2) (Table 1). Maximum Fresh shoot weight (82.92) was recorded with dazomet (T_5) and it significantly differed from rest of the treatments. Phorate (T_4) was second highest. Treatment of cartap hydrochloride (T_2) had lowest shoot weight and it remained at par with control (T_6), carbosulfan (T_1) and carbofuran (T_3) (Table 1). Maximum fresh root weight (7.03) was registered in dazomet (T_5) followed by phorate (6.03) (T_4), control (6.66) (T_6), carbofuran (6.07) (T_3) and carbosulfan (5.63) (T_1). They were statistically at par with each other. It was minimum in cartap hydrochloride (4.11) (T_2) followed by carbosulfan (5.63).

Root-knot index was significantly less (0.24) in the treatment of dazomet (T_5) as compared to rest of the treatments. Control (T_6) had maximum root-knot index which significantly differed with carbosulfan (T_1), cartap hydrochloride (T_2) and carbofuran (T_3) (Table 2). Significantly less number of females (1.40) were recorded in the dazomet (T_5) treatment followed by phorate (T_4). control (T_6) had maximum number of females. Nematode population, J_2 in soil was also lowest in dazomet (T_5) and differed significantly with other treatments including control (T_6). Control (T_6) had maximum (3.12) juveniles/ 200 cm^3 of soil. Total nematode population was significantly

Table 2. Effect of different chemicals on multiplication of *Meloidogyne* spp. on tomato

Treatment	RKI(0-5)*	Nematode population		
		No. of females/3 g root	No. of juveniles/200 cm^3 soil	Total
T_1 (CAS)	2.35 (30.47)**	2.31 (203)	2.80 (630)	2.93 (850)
T_2 (CH)	2.50 (26.03)	2.37 (233)	2.86 (723)	2.99 (976)
T_3 (CAR)	2.40 (29.00)	2.35 (223)	2.81 (645)	2.95 (890)
T_4 (PHO)	1.30 (61.54)	2.13 (134)	2.63 (426)	2.76 (574)
T_5 (DAZ)	0.24 (92.90)	1.40 (24)	2.22 (165)	2.29 (194)
T_6 (CON)	3.38	2.61 (406)	3.12 (1317)	3.24 (1737)
SEm $\pm$	0.15	0.06	0.08	0.07
CD (0.05)	0.43	0.17	0.24	0.19
CV %	14.65	5.17	6.11	4.62

*0 = Free; 5 = Maximum disease intensity. Figures in parentheses are retransformed values of Log X+1, ** per cent reduction over control.

lower (2.29) in dazomet treatment (T_5) as compared to rest of the treatments. Phorate (T_4) was next to dazomet but stastically non significant with carbosulfan (T_1) and carbofuran (T_3). Total nematode population was significantly higher in control (T_6). Overall results showed that dazomet is most effective in reducing nematode population and root-knot nematode index and thereby increase plant growth followed by phorate and carbofuran (Table 2). Results obtained in this study are also conforming results of Patel and Patel (2009), Anon. (2014), Dhillon and Kaur (2016) and Nie *et al*., (2016).

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Nematodes of Protected areas of Uttarakhand, India: New Records

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The knowledge of biodiversity of any conservation or protected area is important for fundamental and applied purposes. In soil fauna, terrestrial nematodes plays an important role in maintaining eco-balance. Nematodes remained explored in a scatter way across the India in any conservation or protected areas. An effort has been made to present the database of conservation areas of Uttarakhand. During the study, 5 species terrestrial nematodes are recorded first time from Uttarakhand state, of which *Metaporcelaimellus littoralis* (Orselli & Vinciguerra, 1999) Sergio Álvarez-Ortega, Sergei A. Subbotin and Reyes Pena-Santiago, 2013 is being reported first time from India.

Field surveys were carried out during 2010-17 of protected areas of Uttarakhad. Soil samples were collected around the roots of forest trees and medicinal plants from Sonanadi WLS, Kotdwar district; Jhilmil Jheel Conservation Reserve, Haridwar district, Valley of Flower National Park, Chamoli and Govind Wildlife Sanctuary and National Park, Uttarakhand.

Extraction of plant and soil nematodes from soil samples was made through modified Baermann funnel technique. The nematodes were fixed in hot FAA and dehydrated in glycerin-alcohol (5:95 parts) by slow method (Seinhorst, 1959). The dehydrated nematodes were

mounted in anhydrous glycerin. The slides were sealed with wax. Identification of nematodes were taken under Compound Microscope (Olympus 51X). All the specimens have been registered and deposited in National Zoological Collection, NRC, ZSI, Dehradun.

Abbreviations: L= Total length; a= Total length/ Maximum body-width; b= Total length/pharynx-length; c= Total length/tail-length; c'= Tail-length/Anal body-width; V= Anterior-Body-Length/ Total length x 100.

During the study of nematode fauna of Sonanadi WLS, Kotdwar district; Jhilmil Jheel CR, Haridwar district, Govind WLS and NP, Uttarkashi and Valley of Flower National Park, Chamoli, Uttarakhand. One species *Metaporcelaimellus littoralis* (Orselli & Vinciguerra, 1999) Sergio Álvarez-Ortega, Sergei A. Subbotin and Reyes Pena-Santiago, 2013 recorded first time from India and 4 species *Mesdorylaimus novus* (Dey & Baqri, 1984) Ahmad, 1993; *Metaporcelaimellus indicus* (Baqri & Jairajpuri, 1968) Sergio Álvarez-Ortega, Sergei A. Subbotin and Reyes Pena-Santiago, 2012; *Nygolaimus shamimi* Bohra and Sultana, 2008 and *Alaimus prihamus* Choudhary and Jairajpuri, 1984 first time from Uttarakhand.

Mesdorylaimus novus (Dey & Baqri, 1984) Ahmad, 1993
(Plate-1: E-F)

Measurements: Female (1): L=1.5 mm; a=40; b=5.2; c=7; c'=8.7; V=46; Odontostyle=11 µm; Odontophore= 16 µm.

Description: Female: Body slightly ventrally curved upon fixation. Lip region marked off from the body by a slight depression, lips amalgated. Odontostyle 1.0 times the lip region width. Odontophore 1.5 times the lip region width. Reproductive system amphidelphic. Prerectum 1.8 times anal body width. Tail long, filiform,

Habitat and Locality: Collected from soil around the roots teak at Pakhro beat, Sonanadi WLS.

Distribution: Darjeeling, West Bengal

Remarks: This species is recorded first time from Uttarakhand.

Metaporcelaimellus indicus (Baqri & Jairajpuri, 1968) Sergio Álvarez-Ortega, Sergei A. Subbotin and Reyes Pena-Santiago, 2012
(Plate-1: C-D)

Measurements: Female (1): L=2.2 mm; a=46; b=4.2; c=38; c'=1.8; V=52; Odontostyle=14 µm; Odontophore= 30 µm.

Description: Female: Body ventrally curved upon fixation. Lip region marked off from the body by a depression, wider than the adjoining. Lips low and somewhat angular. Labial papillae present. Amphids broad and shallow. Reproductive system amphidelphic. Prerectum 4.1 anal body width. Tail conoid, ventrally arcuate with rounded tip

Habitat and Locality: Collected from soil around the roots of teak at Pakhro beat, Sonanadi WLS , Kotdwar.

Distribution: Mainpuri, Uttar Pradesh.

Remarks: This species is recorded first time from Uttarakhand

Metaporcelaimellus littoralis (Orselli & Vinciguerra, 1999) Sergio Álvarez-Ortega, Sergei A. Subbotin and Reyes Pena-Santiago, 2013
(Plate-1: A-B)

Measurements: Female (1): L=1.2 mm; a=18; b=4.2; c=29; c'=1.6; V=55; Odontostyle=12.5 µm; Odontophore=40 µm.

Description: Female: Body cylindroid, ventrally curved upon fixation. Amphid cap like. Lip region marked off from the body by a depression, wider than the adjoining body. Lips low and somewhat angular. Labial papillae present. Cardia hemispheroid. Reproductive system amphidelphic. Vulva pore like Vagina with sclerotized piece. Prerectum 2.2 anal body width. Tail convex-conoid, slightly ventrally arcuate.

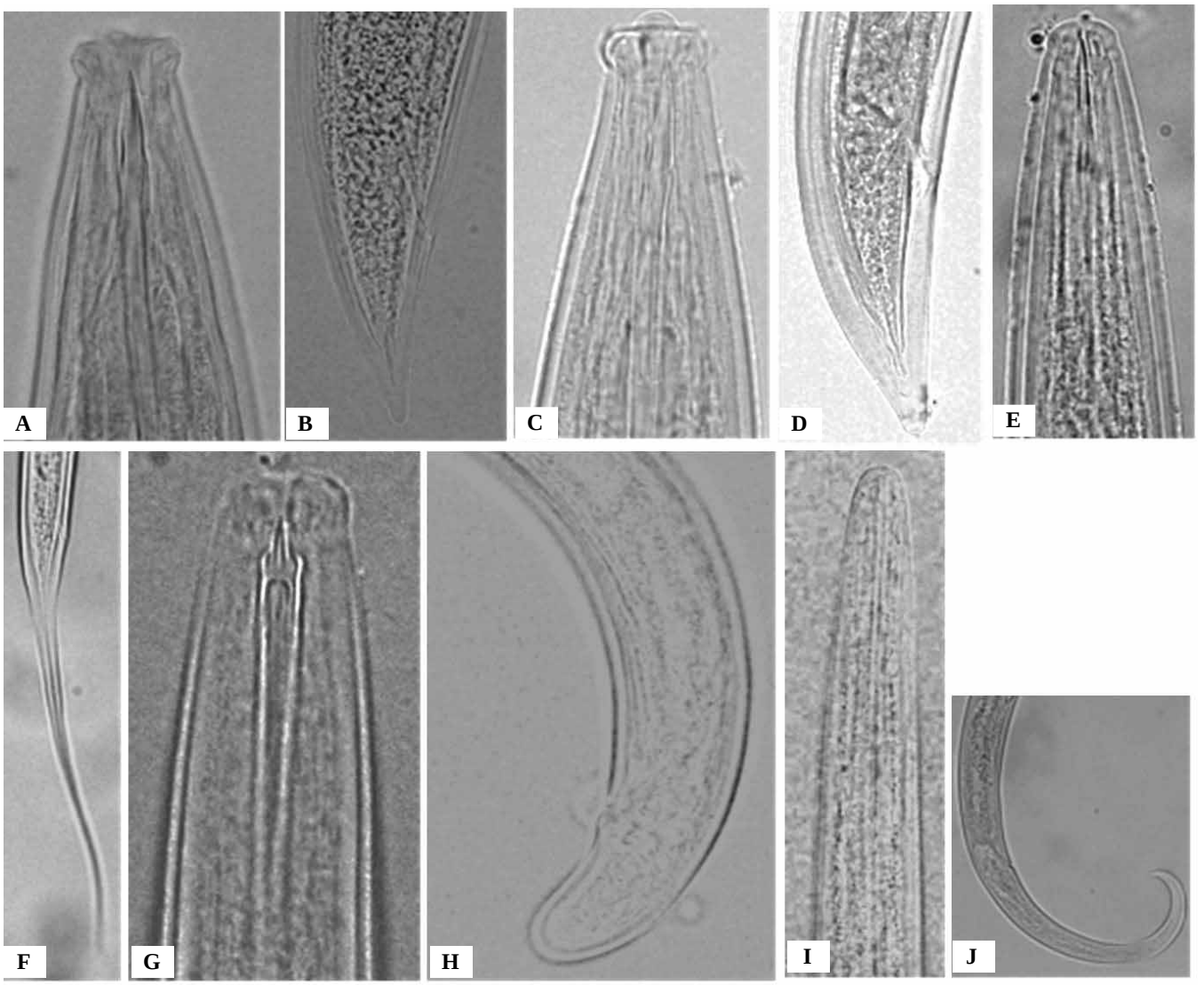

Plate-1. ***Metaporcelaimellus littoralis*** **Female: A. Anterior end, B.Tail:** ***Metaporcelaimellus indicus*** **Female. C. Anterior end, D. Tail;** ***Mesdorylaimus novus*** **Female. E. Anterior end; F. Tail** ***Nygolaimus shamimi*** **Female. G. Anterior end; H. Tail;** ***Alaimus prihamus*** **Female: I. Anterior end, J. Tail**

Habitat and Locality: Soil around the roots of grasses, Dewari Pul, Valley of Flower National Park, Chamoli.

Elsewhere: Italy

Remarks: This is new record from India.

Nygolaimus shamimi Bohra and Sultana, 2008
(Plate-1: G-H)

Measurements: Female (1): L=1.0 mm; a=40; b=3.9; c=36; c'=1.6; V=47; Tooth=4 µm

Description: Female: Body sright upon fixation. Lip region marked off from the body. Lips rounded. Tooth deltoid type. Labial papillae present. Amphids broad and shallow. Reproductive system amphidelphic. Prerectum 1.4 anal body width. Tail conoid with rounded tip.

Habitat and Locality: Jhilmil Jheel Conservation Reserve, Haridwar district, Uttarakhand .

Distribution: Alwar and Jodhpur, Rajasthan.

Remarks: New Record from Uttarakhand.

Alaimus prihamus Choudhary and Jairajpuri, 1984
(Plate-1: I-J)

Measurements: Females: L(2)=1.1-1.3 mm; a=53-55; b=4.4-4.7; c=9-10; c'=9-11; V=43-45;

Description: Female: Body ventrally curved upon fixation. Lip region rounded and continuous with body. Basal expanded part occupying 14-18% of oesophagus length. Cardia discoid. Reproductive system mono-opisthodelphic. Vulva transverse. Tail elongate conoid.

Habitat and Locality: Soil around roots of *Thalictrum foliolosum* from Mariothi Sopam Van, Govind WLS, Uttarkashi.

Distribution: Gauhati, Assam.

Remarks: New Record from Uttarakhand.

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