ANALYSIS OF ANTI-FUNGAL ACTIVITY OF SELECTED MEDICINAL PLANT EXTRACTS AGAINST ALTERNARIA LEAF BLIGHT OF BOTTLE GOURD

Thesis · October 2020

DOI: 10.13140/RG.2.2.14654.10564

4 authors, including:

Sonia Yaseen
University of Agriculture Faisalabad

Muhammad Aslam Khan
University of Agriculture Faisalabad

Safdar Ali
University of Agriculture Faisalabad

ANALYSIS OF ANTI-FUNGAL ACTIVITY OF SELECTED MEDICINAL PLANT EXTRACTS AGAINST *ALTERNARIA* LEAF BLIGHT OF BOTTLE GOURD

Sonia Yaseen

(Regd. No. 2018-ag-3658)

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF PHILOSOPHY
BOTANY

DEPARTMENT OF BOTANY

FACULTY OF SCIENCES

UNIVERSITY OF AGRICULTURE, FAISALABAD, PAKISTAN

2020

DECLARATION

I hereby declare that the contents of the thesis **"Analysis of anti-fungal activity of selected medicinal plant extracts against *Alternaria* leaf blight of bottle gourd''**product of my own research and no part of it has been copied from any published source (except the references, standard mathematical or biochemical models/ equations/ formulae/ protocols etc.). I further declare that this work has not been submitted for award of any other diploma/degree. The university may take action if the information provided is found inaccurate at any stage.

Sonia Yaseen
2018-ag-3658

To,

The Controller of Examinations,
University of Agriculture,
Faisalabad.

We, the supervisory committee, certify that contents and form of the thesis submitted by **Sonia Yaseen** (Regd. No. **2018-Ag-3658**) have been found satisfactory and recommend that it be processed for evaluation by the external examiner(s) for the award of degree.

SUPERVISORY COMMITTEE:

Chairman: ______________________________

(Prof. Dr. M. Aslam Khan)

Member: ______________________________

(Dr. Muhammad Shahbaz)

Member: ______________________________

(Dr. Safdar Ali)

O! GOD OPEN OUR EYES,
TO SEE WHAT IS BEAUTIFUL,
OUR MINDS TO KNOW WHAT IS TRUE
OUR HEARTS TO LOVE WHAT IS GOOD

Dedicated to

HOLY PROFHET (PBUH)
THE GREATEST SOCIAL REFORMER

MY WORTHY PARENTS
THE TOIL AND SWEAT OF AFFECTIONATE PARENTS AS MORAL SUPPORT ENSHRINED AND GRAFTED IN ME UNTIRING ZEAL TO GET ON THE HIGHER IDEALS OF LIFE

MY BROTHERS AND SISTERS
FOR THEIR LOVE, PATIENCE, ENCOURAGEMENT AND UNDERSTANDING THAT INSPIRED ME TO ACCOMPLISH THIS HUMBLE EFFORT

ACKNOWLEDGEMENTS

All thanks to almighty "Allah'' who is entire source of knowledge and wisdom endowed to mankind and for equipping his humble creatures with mental faculty. I firmly believe that God will never spoil my efforts, every piece of work is reward according to nature and degree of devotion for it. All praises after Almighty Allah are due to His **Holy Prophet Hazrat Muhammad (P.B.U.H),** the most perfect and exalted among us and who is forever a source of guidance and knowledge for humanity as a whole.

I wish to acknowledge my supervisor **Dr. Muhammad Aslam Khan**, Department of Plant Pathology, University of Agriculture, Faisalabad for providing all the necessary facilities in accomplishment of the research work. I am also grateful to him for his constructive criticism, valuable suggestion and constant encouragement throughout my work.

I extend my deep gratitude to **Prof. Dr. Safdar Ali**, Department of Plant Pathology, University of Agriculture Faisalabad for his skilled guidance, suggestions, inspiration and comments without whose assistance; this mammoth task would not have been possible. Very special thanks to **Dr. Muhammad Shahbaz,** Department of Agricultural Botany, University of Agriculture, Faisalabad and member of my supervisory committee on having made commendable suggestions during research work.

Words are lacking to express my humble obligations to my parents whose inspiration had ever been a house light for me. I am also be-holden to my sister Sana Yaseen, my sincere friends, and all other well-wisher for their consistent support and encouragement during my studies.

Sonia Yaseen

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURS

ABSTRACT

Bottle gourd (*Lagenaria siceraria* L.), first domesticated vegetable crop is one of the most important nutritional and marketable crop in the world. A number of factors are responsible for the deterioration of bottle gourd crop. Among these factors, *Alternaria* blight is one of the most devastating one, which is caused by *Alternaria cucumerina* and has great economic importance all over the world. It is commonly associated with gourd crop and is prevalent throughout Pakistan. Field trials were conducted at the research area of Department of Plant Pathology to analyze the anti-fungal activity of plant extracts against the *Alternaria cucumerina*. Identification of resistance source against the *Alternaria* leaf blight is very important. Ten varieties of bottle gourd were collected and evaluated in field. Three varieties of bottle gourd were showed moderately resistant response (Anmol F_1, Bemisal, and Green gold). Plants extracts were evaluated for the control of disease. Eight treatments which consisted of extracts of fresh leaves of Neem, Ginger cloves, Onion bulb, combination of treatments and one as control with distill water. Data of disease severity and environmental factors were recorded with 7 days' interval and statistically analyzed. Plant extracts were assessed under field conditions. The combination of treatments (*Allium cepa* + *Azadirachta indica* + *Zingiber officinale)* showed minimum disease severity as compared to control.

CHAPTER # 1 INTRODUCTION

Bottle gourd (*Lagenaria siceraria* L.) a gourd with white colored flowers, also known as Cucurbitaceous family climbing plant. Cucurbits are vegetable crops that belong to the *Cucurbitaceae* family, comprising 118 genera and 825 species. Most of those species are eaten worldwide as food. It is the largest group of vegetables in summer season, of which more than 20 are commercially grown in India (Choudhary *et a1*., 2002). Cucurbits are an excellent fruit in nature with composition of all the important components required for human health. The genus *Lagenaria* consists of five other species, namely L. *brevifilora* (Benth) Roberty, L. rufa (Gilg) C Jeffrey, L. *sphaerica* E Mey, L. *abyssinia* (Hook. F.) C Jeffrey and L. *guineensis* (G Den) C Jeffrey, of which L. *siceraria* is the most cultivated (Erickson *et al*. 2005).

It is an important vegetable that can be grown in tropical and subtropical countries (Harika *et al*., 2012). It is one of the first plant species to be domesticated for human use, providing food, medicine, and a wide variety of utensils and instruments made from the large, hard-shelled fruit. It is usually grown in open fields or in the beds of rivers. It is grown in dry climate zones. Its fruit can also be used as a vegetable and for sweet making. It is easily digestible in vegetable form, especially to the patients (Thamburaj and Singh, 2000). It is cultivated in Pakistan on an area of 5492 ha with an annual production of 55640 tons while its production in Punjab is 44304 tones (FAO, 2018). The National average yield is 10.6 tons per ha. The low productivity of bottle gourd is due to lack of proper disease management so its yield can be increased by using the proper management system. Bottle gourds are grown and consumed regularly in different parts of the world, especially Asia. It appears to have first arisen in Africa. It is now widely grown throughout the tropics, notably in India, Indonesia, Malaysia, China and South America. Bottle gourd is a significant vegetable from home garden. Bottle gourd has been stated to be the only most widely spread cultivator and popular to both the old world and the new world since ancient times. It is also called kaddu, lauki, and tumri in Hindi, sorakaya in Telugu, sorekayi and lau in Bengali and Assamese, lauka in Nepali and ghiya in Punjabi.

The fruits are medicinal in nature and are used as cardio-tonic, aphrodisiac, hepatoprotective, analgesic, anti-inflammatory, expectorant, diuretic, and antioxidant (Mohan *et* *al*., 2012). The mature dried fruit is used to make jars, utensils, and musical instruments for storage (Decker-Walters *et al*., 2001).

It is gaining importance due to its high yield potential, steady market price throughout the season. Its fruit contain high nutritional value like (0.2g fats, 1.2g proteins, 3.75g carbohydrates, o.7g fiber, 15 Cal per 100 g dry weight energy) (Hanif *et al*.,2006). It also used as a diuretic, cooling, analgesic, anti-ulcer (Milind and Satbir, 2011). Its seed also contain high quality of proteins, fatty acids, vitamins such as (vitamin A, E and C) (Hegazy and El kinawy, 2011). Traditional healers advocate bottle gourd fruit juice for flatulence, diabetes mellitus, hypertension, liver diseases and as a diuretic (Ghule *et al*., 2007). It is part and parcel of complementary and alternative therapies and there is a prevalence in India of drinking a glass or two of freshly prepared juice on an empty stomach. Although bottle gourd extracts have a few beneficial effects in animal models (Deshpande *et al*., 2008), there are few human studies.

The bottle gourd varies widely for different morphological characteristics such as fruit bearing capacity, fruit girth, fruit length, main shoot length, number of branches per plant, number of seeds per fruit, seed weight, seed length, seed width, seed shape and seed color (Yetisir *et al*., 2008). Tender fruits are used as vegetables, for the preparation of sweets and pickles particularly in the hills. It has a cooling effect and prevents constipation, and cardiac and diuretic effects. Contains pulp on fruit. Outside, the pulp is applied as a poultice and cooling solution to the saved head delirium, and rubbed on the palm of the feet and hands to reduce the heat effect. The bitter fruit is poisonous and is used as a potent purgative. Once the bitter fruit ash is combined with honey it is a useful treatment for night blindness. The leaves are used in the form of decoction using sugar to treat the jaundice. In the manufacture of various types of musical instruments, jugs, domestic utensils for storing liquid and food items, and fishing net floats, the hard shells of mature fruit are used.

Lagenaria seeds contain a high linoleic acid content and sterolic compounds. Seed has potential for protein replacement in alternative diets focused on cereals or in replacing animal proteins in traditional foods (Ogunbusola *et al*., 2010). Its fruits are monetic in nature, the male flower first appeared during the start of growth, and the female flower later formed (Sarkar, 2004). Its fruit has many different forms, such as elongated, circular, small and large, and also contains plumps and multiple seeds (Milind and Satbir, 2011). Bottle gourd has had a major presence in rural life in Serbia, mainly for household and agricultural uses. More recently, its function as a rootstock for watermelon grafting in vegetable cultivation, and as a decorative decoration in art, is becoming more prominent. Although large fields under bottle gourd crop are rarely found in Serbia, this species is one of the most popular in India and some other parts of the world (Sivaraj and Pandravada, 2005).

Bottle gourd requires a warm growing season and is frost-sensitive. The germination is possible between 25–30 ° C and under 15 ° C has been adversely affected. It can be grown in various soil types, but ideal for cultivation are sandy loams to loamy with good drainage. The pH range between 6–7 is conducive to growth, while adverse effects are seen in acidic and alkaline conditions. The light reduction of up to 50 per cent PAR also gave good results in bottle gourd growth (Haque *et al*., 2009). In hot and humid weather conditions it can grow better. For their propagation it requires high temperature and long day duration. Sandy loam soil having pH 6.5 is needed for better growth. Seed sowing depends on climatic conditions in pots and fields (Tan *et al*., 2009).

Irrigation promotes excessive vegetative growth at frequent intervals, particularly in heavy soils. The irrigation water should not humidify the vegetative parts, especially when flowering, developments in fruit set and fruit are in progress. High amounts of irrigation have been observed to reduce bottle gourd productivity (Tan *et al*., 2009). Sewage irrigated bottle gourd has been found to have a higher absorption of nutrients than tube well irrigated crops (Paul *et al*., 2006). *Alternaria* leaf blight develop rapidly in warm and humid weather, so the whole area may have been affected by chemicals and frost. The severity of crop loss would be further if plants get infected early in the season. *Alternaria* leaf blight is found mainly on watermelon and muskmelon, but on squash cucumber, gourds, and pumpkin may occur. The disease has an effect on foliage and rarely on fruit. *Alternaria* leaf blight caused by the *Alternaria cucumerina* fungus affects mostly older plants, while seedlings may also be infected.

Many diseases such as fruit rot, root rot, wilt, leaf spot, powdery mildew, bottle gourd mosaic virus and so on affect bottle gourd. Among all diseases, *Alternaria cucumerina* leaf blight is a major problem in India and has now become a limiting factor in boosting bottle gourd yield. *Alternaria cucumerina* is a worldwide occurrence fungus that can infect most cultivated cucurbits. The disease originally appeared in the field just after the emergence of the seedling. On the leaves there are small, scattered, water-soaked, yellowish and circular to oval shaped spots. Subsequently, these spots are converted into brownish in color surrounded by chlorotic area with usual clustered circles, which coalesced to form broad irregular patches on leaves showing blighted appearance that may result in leaves becoming withered and shed. Disease-induced economic yield losses are 20 to 60 per cent (Bruce 2013). *Alternaria* causing a global economic loss by contamination of several crops (Kirk, 2008). *Alternari*a *alternative* is the cause of early blight of potato, leaf spot diseases in *Withania smnifera* (Pati *et al*., 2008). *Alternaria* leaf spot, and *alternaria* spp leaf blight. These are among the most damaging of fungal diseases (Koike *et al*., 2007).

Symptoms of *Alternaria* disease are irregular, circular dark brown leaf spot colors on the leaves, and contain concentrated rings in the leaf spot as well. The microscopic view of *A. cucumerina* culture suggests that the width of conidiophores varies from obclarate to pyriform (Wagh *et al*., 2013). Favorable temperatures range from 16 to 24 ºC where the time for sporulation varies between 12 and 14 hours. The presence of moisture in the form of rain, dew or high humidity is important for infection and a minimum of 9-18 h is required of most species. Continuous moisture of 24 h or more essentially ensures infection. Large numbers of mature spores are produced within 24 hours if relative humidity is greater than 91.5 percent (at 20 ºC). The pathogenic plant species *Alternaria* live as spores and mycelium between crops in infected plant residues, or in and on seeds.

Seed borne fungus can attack seedlings causing damping-off, stem lesions, or collar rot. Most often, how-ever the fungus grows and sporulates on plant residues during rain, heavy dew, or under conditions of good soil moisture. Spores are splashed or wind-blown onto plant surfaces where contamination takes place. Spores need enough moisture to germinate and become infected. The host either penetrates directly through wounds, or through stomata. Stressed, frail, aged, or injured tissues are more likely to be invasive than powerful, vigorous tissues. *Alternaria cucumerina* is a saprophyte on rotting crop waste allowing leaf blight to winter in the soil. This spreads from plant to plant, as conidia is transferred by wind and splashing water from diseased plants to susceptible tissues. Germinating spores, which grow, penetrate the host directly as well as through wounds and natural openings. Wet conditions and warm temperatures are best suited for disease growth. *Alternaria* has been found to influence all the plant aerial sections i.e. stem, leaves, fruits, seeds and heads.

Its improvement in production through the use of organic and biochemical fertilizers to reduce the nutrient deficiency. The Vermi compost (50 per cent) of biochemical fertilizers can increase its growth and yield (Singh *et al*., 2012). Excessive, continuous use of inorganic fertilizers on vegetable crops can damage the soil and the environment. Ongoing use of inorganic fertilizers alone cannot sustain high levels of productivity in vegetable crops (Dass *et al*., 2008). Organic fertilizers can result in low productivity of several vegetable crops with high nutrient requirements because of their low levels of nutrients available for planting (Cavigelli *et al*., 2008). Chemicals and fungicides are used to combat *Alternaria* leaf blight diseases. Synthetic fungicides have severe effects on the aquatic and terrestrial ecosystems while copper accumulation is caused by severe use of copper fungicides that adversely affect micro-organisms (Wightwick *et al*., 2010). Many researchers are finding many successes but creating many environmental and human health issues. Now plant extracts are the latest solutions to pathogen control, and they have no side effects on human health and the climate. (Wightwick, *et al*., 2010). Phyto-extracts are biologically antifungal agents with the potential to surmount fungal diseases without risks to human health (Shuping and Ekoff, 2017). Secondary plant molecules have antimicrobial, antifungal competencies (Bourgaud *et al*., 2001).

Scientific work which is now focused on naturally derived plants has a therapeutic and anti-carcinogenic agent for human health. Phyto-extracts have been shown to be better for natural pesticides because they are environmentally friendly and have the best results in controlling fungal growth (Parajapati *et al*., 2017). The extracts from garlic bulbs also have an inhibitory effect on *Alternaria tenuis* mycelial growth (Datar *et al*., 1996). The neem leaf extract also has a high efficacy to control the *Alternaria solani* 's radial growth (Sharma *et al*., 2007). Plant extracts also control the growth of myceliums and inhibit spore production in many species of fungi (Malkhan *et al*., 2012). That is why phyto-extracts were evaluated in-vivo management of *alternaria* leaf spot.

Objectives

Following objectives were followed:

- To check the efficacy of different plants extracts against *Alternaria cucumerina* in bottle gourd

- To identify source of resistance through screening against *Alternaria* leaf blight under field conditions.
- To determine relationships of epidemiological factors with *Alternaria* leaf blight disease of bottle gourd

Lines of Work

- Collection of seed
- Establishment of field experiment
- Preparations of plants extracts
- Data collection
- Data were analyzed by statistical methods

CHAPTER 2 REVIEW OF LITERATURE

2.1. Nutritional value

Punjab Agriculture Department (2014) stated that because of agriculture, the various countries in the world that are popular due to agriculture and Pakistan also included in those countries. It is an agricultural country whose populations are dependent on agriculture. It is an agrarian state whose citizens and financial system rely on agriculture either indirectly or directly (16 per cent indirectly and 70 per cent directly). Growing crops is the basis of Pakistan's economy. Financial statement for 21 percent of GDP with yield dependent on agriculture gets 80 percent of the country's total export profits. This zone occupies more than 43.7 per cent of manual labor force.

Punjab Agriculture Department (2014) reported the crops are the liveliest agricultural sub-sector comprising 39.6 per cent of crop production and 8.3 per cent of GDP. Productivity produces a ripple effect in Pakistan's rural population as a result of any change in farming. The growth through rapid agriculture can inspire and maintain the pace of industrial growth. Rapid growth also maintains a mutually reinforcing method of persistent economic growth setting into action. Punjab contributes 76 per cent to the country's annual food grain production. "According to the Punjab Department of Agriculture, Punjab has fifty-seven percent of the entire refined area and sixty-nine percent of the entire cropped area of Pakistan. It also supplies 34% of the dates, citrus 95%, mango 66% and guava 82% of the total national yield of these fruits.

Garcia-Mas *et al*. (2012) reported the economic importance among vegetable crops worldwide *Cucurbitaceae* ranks second only to *Solanaceae.* Bottle gourd (*Lagenaria siceraria*) plant is a cosmopolitan legume that is underused in most tropical and subtropical nations. On sandy or loamy soil, the plant can be grown from seeds. It can be treated as a prostrate ground cover or as a climber; in which case some support may be needed for the maturing fruits. The plant is commonly grown in sudan savanna in Nigeria, especially in Zamfara State, where it is planted as a cash crop. Its fruits are used as containers and musical instruments, while the seeds are used as a "egusi"-like soup thickener and as a source of edible oil.

Maigandi and Ngang, (2002) reported the purpose of this study is to evaluate the proximate composition, profile of amino acids, and mineral content of *Lagenaria siceraria's* whole seed, dehulled seed and seed coat to provide information on its nutritional values.

(Ghule *et al*. 2009) reported that bottle gourd prevents excess sodium loss and reduces tiredness especially during summertime. It is low-calorie diet, perfect for diabetes and jaundice-stricken men. The fruits have medicinal values and are used as cardiotonic, aphrodisiac, hepatoprotective, analgesic, anti-inflammatory, expectorant, diuretic and antioxidant agents.

Decker-Walters *et al*. (2001) reported the mature dried fruit is used to make jars, utensils and musical instruments for storage. It is commonly grown in India, the Philippines, Malaysia, China, Hong Kong, Tropical Africa and Brazil. Ghule *et al*. (2009) said, it is an herb widely cultivated in tropical countries such as India. The unripe fruits are commonly consumed as vegetables. Traditional medicine in India is widely practiced.

Ghule *et al*. (2007) reported that orthodox healers recommend bottle gourd fruit juice for flatulence, diabetes mellitus, hypertension, liver disorders and as a diuretic. Deshpande *et al*. (2008) examined that it is part and parcel of complementary and alternative therapies and there is a prevalence in India of drinking a glass or two of freshly prepared juice on an empty stomach. While bottle gourd extracts have a few beneficial effects in animal models, there are few human studies. On the contrary, Puri *et al*. (2011) observed some serious ill effects of taking bottle gourd juice particularly if it is bitter.

The inadequate supply of animal protein sources and the high cost of the few usable sources of plant protein have prompted extensive work into harnessing the nutritional potential of lesser known underused legumes and oil crops (Enujigha and Akanbi, 2005).

2.2. Production in Pakistan:

In Pakistan, it is cultivated on an area of 5492 ha with annual production of 55640 tones while in Punjab its production is 44304 tones (FAO, 2018). The low productivity of bottle gourd is due to lack of high yielding varieties so its yield can be increased by using the proper management system.

2.3. Origin and history

Emina *et al*. (2012) reported the morphological analysis and archeological evidence suggested ocean dispersal from Africa to other parts of the world of wild bottle gourd fruits followed by independent domestications. The Asian bottle gourd type had long been present in

Europe. His arrival in the Americas from Asia was believed to be Africa's (Erickson *et al*., 2005). It was found that the Polynesian bottle gourd is of dual origin.

Clark *et al*. (2006) reported the molecular markers have shown that chloroplast alleles are predominantly of Asian origin and of American and European nuclear origin. In India, bottle gourd was mentioned as 'alabu' by prehistoric Sanskrit scriptures, and its use as 'tambura,' 'veena' and 'kamandalu'. (Smith, 2005) reported that, Bottle gourd was present in East Asia 7000 years ago but its spread to the far south is not evident in prehistory as shown by dashed line. The bottle gourd from Southeast Asia may be a recent arrival from India, about 200 B.C. (Green, 2000), which extends into the Vanuatu to the far East. Bottle gourd gap in western Polynesia indicates that it was not introduced via human-mediated dispersal from Asia into Polynesia (Whistler, 1990). Natural dispersal is still possible, however, since Lapita pottery was widely available as an alternative for containers there. In eastern Polynesia, before 1200 A.D., the bottle gourd was present. Also (Green, 2000), and may have been introduced either by natural floating or human-mediated dispersal from the Americas.

(Green, 2005) documented that human mediated arrival from South America Easter Island may have been made by Polynesian travelers around 1000 A.D. To the Peruvian coast and back to the Archipelago of Tuamotu together with sweet potatoes. Similarly, Polynesian explorers may have introduced the North American bottle gourd on a return sailing trip from Hawaii to the Californian Channel Islands around 400–800 A.D.

Jones and Klar, (2005), stated though this theory has yet to be put to the test. The bottle gourd has a lot of variation for various morphological characters such as fruit bearing capacity, fruit girth, fruit length, main shoot length, number of branches per plant, number of seeds per fruit, seed weight, seed length, seed breadth, seed shape and seed color. Emina *et al*. (2012) reported the principal analysis of components L. *Siceraria* suggests that fruit size, seed size, fruit shape and seed shape are major components of the bottle gourd variability.

Kumar *et al*. (2011) stated that *L. siceraria* germplasm is more diverse than its wild relatives viz. *L. sphaerica*, *L. abyssinica* and *L. breviflora* for qualitative and quantitative traits. The variability for various characters was found wide in Europe, India and Japan.

2.4.1. Cytology

Enonch *et al*. (2008) reported the *Lagenaria's* chromosome number is 2n = 22 with normal 11 bivalent meiosis, median centromere, and stable taxon cytology. However, in 117 bottle gourd accessions an average DNA content was 0.734 ± 0.017 pg/2C. This value is considered more accurate since flow cytometry was used to test the samples. A major difference was found between the genome size of the types gourd and Egusi. Gourd types had a smaller genome size than the Egusi types (2C = 0.747pg). But, polyploidy was not detectable in L. *Siceraria* but variations in DNA content were observed within the accessions. The association of the size of the genome with the seed forms and that regions was also found. The variation in seed types indicates the role of evolutionary differences and geographic regions as genome adaptation to unknown ecological parameters linked to the altitude.

2.4.2. Sex Expression, Floral Biology and Cross ability

Singh *et al*. (1996) reported that in bottle gourd, the flowering starts 40–50 days of sowing, but is also influenced by cultivar conditions and environment. In general, in the axis of nodes on main and secondary branches staminate flowers appeared first than the pistillate. First female flower node is regarded as an indicator of earliness. In bottle gourd and andro-monoecious genetic stock (Andromon 6), monoecious sex expression predominates as being recessive to monoecious by single gene. The flowers of bottle gourd are white with five sepals (1.0–1.5 cm long and 0.2–0.3 cm wide) and five petals (2.5–4.0 cm). In South India, anthesis occurs between 9.00 A.M. to 2.00 PM and stigma remain receptive for 24 hours before and after anthesis (Joshi and Gaur, 1971). It shows the effect of that climate on bottle gourd's receptivity to anthesis and stigma. It was found that cultivated bottle gourd cannot be crossed with five other relatives of the genus *Lagenaria* i.e. L. *breviflora*, L. *abyssinica*, L. *Shaerica* & L. *Guineensis* (2007 and Anonymous, 2012).

2.4.3. Molecular markers

Decker-Walters *et al*. (2001) examined that there are few molecular markers which were used in bottle gourd for various studies. The RAPD (Random Amplified Polymorphic DNA) markers were initially used to study genetic resources in bottle gourd. 64 RAPD markers are used on 74 L accessions. *Siceraria* from large geographical regions showed that African accessions differed from the new world, but some degree of affinity was shared with the Asians.

Decker-Walters, *et al*. (2004) exaimed once again by placing germplasm in Zimbabwe far apart from the new world. The RAPDs markers also distinguished various species of *Lagenaria* (Morimoto *et al*., 2006). Singh *et al*. (2010) also pointed to the potential of RAPD markers for evaluating hybrid seed purity. It was classified by using the sequence-related amplified polymorphism markers. US PIs *L. siceraria* into two major clusters. One cluster includes accessions mostly from South Asia (India) and a few from the Mediterranean and Northeastern Africa region.

Levi *et al*. (2009) reported the second cluster comprises PIs from South Africa and North, Central and South America, as well as from China, Indonesia and Cyprus. Xu *et al.* (2011) developed the first set of bottle gourd SSR markers and was used to estimate the phylogenetic relationships in Chinese germplasm. This group of SSR markers demonstrate high transferability in watermelon (41.00%) followed by loofah (20.00%), pumpkin (11.00%) and bitter gourd (4.00%) respectively.

Levi *et al*. (2008) stated that polymorphism between L. *Siceraria* accessions obtained worldwide and elucidated its phylogenetic relationship with other Cucurbit species using water melon related SSR markers based on EST. A high amount of conserved sequences was also detected within bottle gourd and cucumber. Developing SSR markers will enable marker-assisted breeding to begin in bottle gourd, and sequence information will enable comparative genomics studies across various cucurbit species. The expressed sequence tags (ESTs) facilitated the development of single sequence repeat (SSR) markers in various plant species, called EST-SSRs. EST-SSRs are more conserved coding regions than the genomic regions identified by SSR markers. Thus, EST-SSRs might be more transferable in related species than SSR markers by cross-amplification. Seventy-four EST-SSR watermelons tested for transferability in various cucurbit species and showed adequate polymorphism among L.

(Ping *et al*. 2011) reported that accessions of *siceraria* gathered all over the world. This study clarified the potential of EST-SSR watermelon in genetic analysis of other cucurbit species. As large numbers of SSR markers were developed in cucumber and other cucurbits, which can be used for bottle gourd transferability studies to better understand the genomics of bottle gourds. There is one report with AFLP markers on the mapping of powdery mildew, and out of 100 markers, E-ATG / M-CTC was linked to powdery mildew. AFLP fragment was subsequently converted to a sequence-characterized amplified region (SCAR) marker called GPDSATG / CTC75. This SCAR marker can be used in bottle gourd for MAS resistance to powdery mildew.

Han *et al*. (2005) developed Agrobacterium mediated transformation protocol in Bottle Gourd. Cotyledon inoculation with binary vector AGL-1 strain of bacterium (pCAMBIA3301) with glufosinate ammonium resistance (bar) gene and the — D-glucuronidase (GUS) reporter gene gave transgenic plants 1.90 percent frequency. Further improvements to the genetic engineering transformation protocol and RNAi studies in bottle gourd are needed.

2.4.4. Taxonomy

(Crous *et al*. 2009) reported for isolates grown on synthetic nutrient-poor agar plates (SNA, Nirenberg 1976) with a small piece of autoclaved filter paper placed on the agar surface, morphological descriptions have been made. Cultures were incubated for 7 days under Cool White fluorescent light at moderate temperatures (~22ºC), with an 8 h photoperiod. The sellotape technique was used to make slide preparations with Shear 's medium as mounting fluid.

(Crous *et al*. 2004) reported the working on photographs of the characteristic structures were taken using Differential Interference Contrast (DIC) illumination with a Nikon Eclipse 80i microscope. They measured growth rates after 5 and 7 days. Colony characters were noted after 7 d, Rayner (1970) rated colonial colours. Deposited nomenclatural data in MycoBank.

2.5.1. Symptomology of *Alternaria*

(Thomma, 2003) stated, they are cosmopolitan moulds, and can be found in soils, plants, fruit, feed and indoor air. The *Alternaria* genus includes both saprobes and plant pathogens that have been reported to have infected crops in the field worldwide and caused many plant products to decay after harvest. Species of *Alternaria* are commonly found on small grains, causing production and processing yield losses.

(Ostry, 2008) reported *Alternaria* spp, thanks to their growth even at low temperatures. They are well known post-harvest pathogens, responsible for food spoilage during refrigerated transportation and storage. Kumar *et al*. (2011) reported, that Bottle gourd is a stand point between the greatest frequently vegetable among the cucurbitaceous family. Bottle gourd grow best in the warm areas of the world. Bottle gourd have a many medicinal value. Latin *et al*. (1994) reported that *Alternaria* leaf blight can causing, 30% yield losses and also negative effects on the dissolved contents of the fruits.

Neeraj and Verma, (2010) reported, by survey that bottle gourd, bitter gourd, ridge gourd and ash gourd all these pre dominant by the *Alternaria* leaf spot. *Alternaria cucumerina* and *Alternaria alternate* effect the cucumbers all over the world. There spore can be conveyed by air through a long distance, also by rain and warm and humid weather conditions (60-80%) that is best for disease development. In these weather conditions plants yield also be decreases.

(Verma and Verma, 2010) stated that majority of the *Alternaria spp*. threat to different crops in all over the world causing different types of diseases. Among all the diseases *Alternaria* leaf spot of bottle gourd is more drastic that is caused by *Alternaria cucumerina*. This pathogen had been isolated from the diseased leaves of the bottle gourd and from the infected and shriveled seeds that were small with brown discoloration on the surface and with low germination potential.

Meena *et al*. (2010) reported that *Alternaria* causes a high yield loss in crops, after indication by symptomology, the spots are dull dark colored. The spots formed on the leaves and also contain concentric rings on the leaves. After some times, the smaller spots get attached to the larger spots and leaves curves are formed as a result. *Alternaria* leaf blight established then seed should be planted after the proper medications and if early sowing done than deep wrinkling can be done.

(Marraiki *et al*. 2012) reported that leaf spot diseases, *Alternaria spp*. Is more problematic for the spinach crop, causing *Alternaria* leaf spot of spinach. In Saudi Arabia *Alternaria* leaf spot of spinach was firstly reported in 2009. The disease mortality rate was found to be 80%. The leaf spot of spinach started in October and remained at high from November to January.

Sarbhoy *et al*. (2006) reported the *Cercospora* leaf spot on cucurbits. It is caused by *C. citrulina*. The *cercospora* is a main stand out among all the sicknesses and it is difficult to harvesting the jug gourd due to the *cercospora* leaf spot. It is first time recorded by Mukhtar *et al*., (2013) in Pakistan.

Zitter *et al*. (2007) stated that *Alternaria* leaf spot and *Alternaria* leaf blight are the disparaging fungal disease that effecting the cucurbit crops. A high degree of seed invasion, moisture content of the seed, storage period and prevailing range of temperature effects the development of the seed born fungi. The pathogen always present externally, internally and causing the seed abortion, necrosis or loss of the seedling and also harmful effects on plant growth. For successful seedling growth a seed treatment can be done by priming and hot water soaking process.

Ellis *et al*. (1971) reported the identification of *Alternaria spp* become easily due to their morphological features and also by conidia arrival. The shape of conidia is single, ellipsoidal, catenae and multi-celled and light brown in color. The species can be separated due to their diagonal and longitudinal septations.

Meena *et al*. (2002) reported that, blight disease is one of the most dominant diseases caused by the genus *Alternaria* which causes average yield loss. Symptoms of this disease include irregular gray to dark brown leaf spots with deep lines within the spots on the leaves. The circular spots often coalesce to form large patches which lead to the blight of the leaves. Small dark colored spots on pods and tender twigs are also formed in several cases (Valkonen and Koponen, 1990). For *Alternaria* blight management, early sowing of properly stored clean certified seeds after deep ploughing along with clean cultivation, timely weeding and maintenance of optimum plant population, avoidance of irrigation at flowering stages and pod formation are some of the steps to be followed to effectively manage the illness.

Saharan and Verma (1994) also documented that they are responsible for harmful infection of leafy crops. This disease has been infected in all parts of the plant, and many green leafy crops. After infection, it has various symptoms on crops. The conidia are produced as a result of this disease. Such conidia, in colour, inflorescence and on seeds and petioles, are blackish to brown. Conidia development is much like mosaic patches beneath the germinating spores on tissues.

2.5.2. *Alternaria* toxins

Wolpert *et al*. (2002) reported that a number of pathogenic plant fungi including *Alternaria* produce toxins that can damage the tissues of plants. Toxins are often categorized as either selective (host specific) or unspecific. Host-selective toxins (HSTs) are only toxic to host fungal plants that produce the toxin. Non-specific toxins, by contrast, can affect many plants regardless of whether they are a host or not of the pathogen producing. Most HSTs are considered pathogenicity factors which are required by the fungi that produce them to invade tissue and cause disease.

(Howlett, 2006) stated that all pathogenic isolates producing a HST are pathogenic to the specific host; all isolates which fail to produce HSTs lose pathogenicity to the host plants. Plants which are susceptible to the pathogen are toxin sensitive. These associations between HST production and pathogenicity in the pathogens and between toxin sensitivity and susceptibility to disease in plants provide compelling evidence that HSTs may be responsible for the development of host-selective infection and disease. On the other hand, the exact roles of non-specific toxins in pathogenesis are largely unknown but some are thought to contribute to virulence features, such as development of symptoms and propagation of plant-pathogen.

Johnson *et al*. (2000) documented that *Alternaria* HSTs involve a diverse group of low-molecular - weight substances, and most have been found as families of closely related compounds in culture filtrates. Genes encoding for various physiological parameters such as cell wall degrading enzymes, toxins, and transporter proteins involved in signal transduction cascades such as mitogenous activated protein (MAP) kinases are some of the various gene types responsible for pathogenicity. The toxins produced by the various *Alternaria* pathotypes are mainly secondary metabolites with low molecular weight. Some desipeptide-based molecules include other types of toxins.

Masunaka *et al*. (2005) reported that various *Alternaria* pathotypes reveal that fungi carrying additional chromosomes can be cured or lost by repeated subculture, indicating that they may not be needed for normal saprophytic development, implying that genes located on these elements may confer selective benefits in certain situations or ecological niches. It found a strong possibility of a genetic hybrid occurring.

Matic *et al*. (2008) reported that mycotoxins are secondary metabolites, produced by various species of fungi. In general, mycotoxins are chemically and thermally stable molecules. Koppen *et al*. (2010) stated that surviving storage, and most food processing environments, and thus survive to the final products. Because of their possible health threats to humans and/or animals, mycotoxins in cereal-based foods and feeds are a major global concern.

Battilani *et al*. (2009) stated, the genus *Alternaria* produces more than 70 mycotoxins and phytotoxins but few occur naturally in foodstuffs or are of major toxicological significance. *Alternata* is considered to be the most significant species producing toxins.

2.5.3. Biology of the Disease

Meena *et al*. (2002) reported that *Alternaria* Leaf Blight it is common to have fungal disease caused by *Alternaria* in carrot leaves and petioles. Although this disease does not directly harm but yield loss occurs when petioles become too brittle as a result of mechanical harvest leaving carrot foliage and root in the soil. *Alternaria* leaf spots spread rapidly in warm and moist weather, so that chemicals and frost may have injured the entire field. Crop loss severity would be additional if plants get contaminated early in the season. *Alternaria* leaf blight is mainly found on watermelon and muskmelon, but can occur on, squash cucumber, gourds, and pumpkin. This disease affects the foliage, and rarely the fruit. *Alternaria* leaf blight caused by *Alternaria dauci* fungus generally affects older plants, although it can also be associated with seedlings. *Alternaria* leaf blight is mainly found on watermelon and muskmelon, but can occur on, squash cucumber, gourds, and pumpkin. This disease affects the foliage, and rarely the fruit. *Alternaria* leaf blight caused by *Alternaria dauci* fungus generally affects older plants, although it can also be associated with seedlings.

2.5.4. Symptoms

Meena *et al*. (2002) reported that blight disease is one of the most dominant diseases caused by the *Alternaria* genus, causing an average yield loss of 32-57 percent. Symptoms of this disease include the appearance of irregular brown to dark brown leaf spots, frequently circular, with prominent lines in the spots. Symptoms of the disease first appear on older leaves as small necrotic spots which may be surrounded by a yellow halo. The circular spots often coalesce to form large patches which lead to the blight of the leaves. Small dark colored spots are also formed on pods and tender twigs in quite a few cases. For *Alternaria* blight management, early sowing of properly stored clean certified seeds after deep ploughing along with clean cultivation, appropriate weeding and optimal plant population, avoidance of irrigation at flowering stages and pod formation are just a few steps followed for well-organized disease management.

With the progression of the disease, the round spots may grow to 1⁄2 inch (1 cm) or more in diameter, and are usually gray, gray-tan, or near-black in colour, leaves curl and die, eventually leading to plant decay. Pathogen growth rate is not constant due to fluctuating environmental conditions, hence spots evolve in a target pattern of concentric rings. Where host leaves are wide enough to allow the production of unregulated symptoms, the target spots are diagnosed for *Alternaria* as there would be few pathogens causing diagnostic expression of this kind. Aside from the target pattern, the lesion was often covered by a fine, black, and fuzzy development. This development is the sporulating *Alternaria* fungus upon the dying host tissues. Many *Alternaria* species also produce toxins that diffuse over the fungus into host tissues. That is why it is not unusual to see Yellow Halo fade into the healthy host tissues that surround the mark spot. Shady, sunken lesions are generally the appearance on roots, tubers, stems and fruits of *Alternaria* infections. Fungus can sporulate in these cankers, causing fine, black, velvety fungal growth and spores to cover the affected area.

(Babadoost, 2001) reported that During moist weather the corresponding lower leaf surface is covered with a downy, pale gray to purple mildew. The colour of the mildew ranges from white to near black. The infection area spreads outward, causing defoliation and poor fruit development which reduces yield. In rainy humid weather entire vein is killed.

(Kuepper, 2003 and Rai and Yadav, 2005) stated that the symptoms of bottle gourd first appear as pale green areas on the upper leaf surface which change to yellow angular spots. A fine white to grayish downy growth soon appears on the lower leaf surface. Infected leaves generally die but may remain erect while the edges of the leaf blades curl inward. Usually, the leaves near the center of a hill or row are infected first. The infected area spreads outward, causing defoliation, stunted growth and poor fruit development. The entire plant may eventually be killed.

2.5.5. Cause and Disease Development

Deshwal *et al*. (2004) stated, *Alternaria cucumerina* causes leaf blight to winter in the soil as a saprophyte on rotting crop debris. When wind and splashing water carried conidia from diseased plants into susceptible tissues, it is spread from plant to plant. Germinating spores can directly penetrate the host, as well as through wounds and natural openings. Wet conditions and warm temperatures are ideal for the development of diseases. *Alternaria* has been found to influence all the aerial parts of the plants i.e. stem, leaves, fruits, pods, and heads. A comprehensive, comparative account of the morphological differentiation on *cucurbitaceous*, *brassicaceous*, and *solanaceous* crops of different *Alternaria* species.

Khalid *et al*. (2004) documented that it is found that the frequency of illness increases at all temperatures with increasing length of leaf wetness. The maximum observed mean severity of disease occurred at 180C after 24 hrs wetness duration. Ahamad *et al*. (2000) reported that the disease appeared in the first quarter of July and that the maximum intensity of the disease was noticed when the temperature ranged from 25 to 280C and the average relative humidity was over 80%. Rainfall was held highly responsible for the extent of infection and the emergence of diseases.

Matharu *et al*. (2006) stated that it found that the disease grew on radish leaves at a temperature range of 15-250C and a relative humidity of 100% for 10-12 hours.

2.5.6. Screening

Singh *et al*. (2009) reported that Cultivation of resistant or tolerant cultivars is one of the best options to minimize the losses due to disease occurrence. For the identification/development of a resistant variety, sources of resistance are the prerequisite. Resistant sources may be present in the indigenous cultivars, land races, folk cultivars, semi-wild relatives and allied species of the vegetable crops.

Mark *et al*. (2005) stated that it found Hannahs Choice F1 as resistance source against powdery mildew, Fusarium race 2 and potyviruses in muskmelon. Resistance source was reported in wild taxa such as *Abelmoschus crinitus, A. moschatus, A. angulosus* and *A. pungens* of okra against *Cercospora* blight. Borkar and Umaharan (2007) reported that found resistant cowpea genotypes, viz. IT97K-1069-8, IT97K-556-4 to *Alternaria* leaf spot. Singh and Gurha (2007) screened different genotypes/varieties of mung bean and they found that 5 genotypes (BM 4, CO 4, CO 5, ML 515 and TM 9850) were resistant against *Alternaria* leaf spot caused by *Alternaria* sp.

Kimber and Paul (2011) reported that all cultivars commercially available to the faba bean were susceptible to *Alternaria* leaf spot. Chauhan and Bhatia (2013) stated that screened 22 germplasm lines/cultivars of bottle gourd for resistance and found that lines such as GH 3 and GH 9 showed resistant to anthracnose disease.

Maheshwari *et al*. 2015 reported that cultivation of resistant or tolerant cultivars is one of the best options to minimize the losses due to diseases. Seventeen bottle gourd varieties/genotypes (Pusa Naveen, Pusa Samridhi, Udaipur Local, Pusa Santushti, Pusa Sandesh, PSPL, Chomu Local, Azad Harit, Panchmahal Local, Arka Bahar, Thar Samridhi, PN 22, DBG 5, DBG 6, Jodhpur Local, IC 567538 and Sriganganagar Local) were evaluated for resistance against *Cercospora* leaf spot during the rainy season of 2011 and 2012 under hot arid field conditions of Rajasthan. Among them, none was found immune or resistant, four varieties (Pusa Naveen, Pusa Santushti, Pusa Samridhi and Pusa Sandesh) were found to be moderately resistant and four (PSPL, Arka Bahar, PN22 and DBG6) were moderately susceptible and the remaining nine were susceptible.

Vivekanand *et al*. (2018) stated that Thirteen varieties of bottle gourd were evaluated for resistance against downy mildew disease during rabi 2017 at Horticultural Instructional-cum Research Farm, College of Agriculture, IGKV, Raipur, Chhattisgarh. Out of thirteen varieties tested three viz. Amrit, Ankit and Anmol were free from the disease and showed immune response against downy mildew. Two (Manya, Ns-433), four (Chutki, Haruna, Latto and Naveen) and three (Angad, Mahima and Sarita) varieties were found resistant, moderately resistant and moderately susceptible, respectively. One variety (Divya) was susceptible against the disease and none of the variety was found highly susceptible.

2.5.7. Disease management

Since a number of *Alternaria* species infect economically important crops, there is a strong need for this pathogen to be effectively controlled. There are numerous methods therefore required for its regulation. *Alternaria* is tough to monitor and prevention is the safest approach. Different species of *Alternaria* infect several family plant crops and reduce yield both qualitatively and quantitatively. So this polyphagous, destructive fungal community needs to be regulated effectively. Infected residues from an earlier crop retained on the surface should be avoided from planting susceptible varieties in field. By ground preparation Residues should be incorporated from previous crop. In addition, good crop nutrition should be given, in particular for potassium.

Singh *et al*. (2001) stated that one of the most effective measures to control the *Alternaria*-caused disease is the effective application of fungicides. Mancozeb 's effectiveness in controlling early tomato blight. Mancozeb also proved successful as seed dresser, followed by Thiram, Bavistin and Iprodione.

Maheshwari *et al*. (2017) reported the management of *Alternaria* leaf blight of bottle gourd in a field trial in the rainy seasons. For controlling the *Alternaria* leaf blight different types of fungicides and bio-agents are used to cure the bottle gourd disease. 17 different types of treatments used for this purpose, a carbendazim combined treatment 0.1%, mancozeb 0.25%, *pseudomonas fluorescens* 5.0% and neem leave extracts 5.0% used as a treatment. At the end of the trials the results showed that neem leaves 5.0% was the most effective to controlling the *Alternaria* leaf blight disease of bottle gourd with maximum disease control is 78.23%.

Javadian *et al*. (2014) reported that extracts of ethanol have an antimicrobial effect against *Streptococcus pyogenes, Streptococcus pneumonia*, *Streptococcus saprophyticus*. The effects can be determined by a method of micro dilution. The results of plant extracts have a maximum inhibition of the disease. The *Sesamum indicum* with ethanol extracts showed maximum effect to control the disease and *Satureja hortensis* with ethanol extracts have a minimum inhibition effects.

Mahesh and Satish *et al*. (2008) reported the anti-microbial activity of different plant extracts like *Acacia nilotica*, *Sida cordifolia*, *Tinospora cordifolia*, *Withania somnifera* and *Ziziphus mauritiana* with methanol against the *Bacillus subtilis, E. coli, P. fluorescens, S. aureus* and *X. axonopodis*. They also have an antifungal activity. *A. nilotica* leaf extract showing a maximum anti-bacterial activity against the *B. subtilis*. The leaf and root extract also showing a maximum activity to controlling the disease. *A. nilotica* leaf extract have a wide range of anti-fungal activity against the *A. flavus*.

Mekuria *et al*. (2005) documented the use of plant extracts as a crude, they are the new sources of fungicides and they lead to resistance against the pest pathogen. Organic pesticides are the point of point of interest todays. In crop protection different types of phytochemicals are used. A work was done by studying the 17 extracts of bryophytes because they have anti-fungal potential in vivo and invitro conditions. *Sphagnum quinquefarium*, *Bazzania trilobata*, *Dicranodontium*, *Diplophyllum albicans* and *Hylocomium splendens* have 50% inhibition level to control the *Botrytis cinerea* mycelial growth and also the *Alternaria solani*. *B. trilobata* also be reduced the *Phytophthora infestans* disease severity on the tomato plants. So, bryophytes extracts are the best natural products to controlling the plant pathogens.

Khair and Haggag (2007) examined the early blight (*Alternaria solani*) and late blight (*Phytophthora infestans*) are the main conditions that effect the vegetative growth of the potato.

Total 9 medicinal plant extract are used after drying in to the sun light like onion seeds (*Allium cepa*), garlic oil (*Allium sativum*), peppermint leaves (*Mentha piperita*), eucalyptus leaves (*Eucalyptus globules*), lemon foliage (*Cymbopogon citratus*), chilli fruits (*Capsicum jrutescens*) and tulsi leaves (*Ocinum bacilicum*). These medicinal plants extracts were assessed in-vitro against the *P. infestans* and *A. solani*.

Egel and Harmon, (2001) reported the fungicides application to controlling the *Alternaria* leaf blight of muskmelon. For controlling the *Alternaria* leaf blight disease a chlorothalonil was applied by a two different types spray nozzles, a flat-fan and other is a hollow nozzle was used for spraying. The results showed that unsprayed controls had a more disease severity as compared to other sprayed treatments. The nozzle type also had no effects on the disease severity and the production of the crop. So, there are no differences in the disease control by any application method of spray.

Prasad and Naik (2003) stated that have found effective in in vitro conditions among non-systemic fungicides Iprodione and Mancozeb, and among systemic fungicides thiophanate methyl under in vitro conditions. Singh and Singh (2006) reported that tested the effectiveness of seven fungicides at 2500, 2000, 1000, 500 and 250 ppm, chlorothalonil, copper oxychloride, azoxystrobin, propineb, copper hydroxide, mancozeb and hexaconazole at 1000, 500, 200, 100 and 50 ppm toward A. Alternate which causes tomato blight. Our findings showed that all of the fungicides reduced the radial growth of the fungus substantially. However, hexaconazole was very effective as it caused 100% growth inhibition.

Katiyar *et al*. (2001) reported the best control of *Alternaria* leaf spot disease of bottle gourd was obtained by spraying recommended @ 0.2% Indofil M45 followed by Chlorothalonil, Cuman L, Ridomil, Indofil Z-78, Copper oxychloride, Jkstein and Topsin-M.

Singh and Rai, (2003) examined that Indofil M-45 and Kavach have been found to be most effective in reducing the growth of A mycelium. *Alternata* brinjal in vitro infection preceded by Bavistin and Thiram.

Sidlauskiene *et al*. (2003) reported Amistar to be very effective in controlling *Alternaria* leaf spot in cucumbers, cabbages and tomatoes as it reduces disease incidence by 88-93 percent, whereas Euparen plus Bion was found to increase biological efficiency.

Singh and Singh (2002) stated that three sprays of 0.25% Dithane M-45 proved superior to other fungicides e. g., Kavach, Foltaf, Bayleton, Baycor and Contaf 5 EC, in terms of additional yield. They advocated three sprayings of Dithane M-45 (0.25%), Kavach (0.1%) or Foltof (0.25%) at 10 days' interval for adoption by the farmers for controlling *A. brassicicola* on cabbage.

Mei *et al*. (2007) reported the chitosan derivatives of sulfanilamide showed important inhibitory effect on *A. Solani* in concentrations 50 to 500 μg / ml. The potassium and sodium bicarbonate and nerol (a consumer component of fractions of the citrus essential oil) had a significant inhibitory effect on *A. Solani* which causes early potato blight.

Walker (1952) reported that this method is an effective measure in controlling *Alternaria* diseases as it helps to reduce primary inoculum. They recommended hot water treatment of seeds at 50°C for 30 min to control *Alternaria* diseases in cabbage while Ellis (1968) recommended the same temperature for 25 min to eliminate *Alternaria* infection from seeds of *Brassicaceae*. Seed treatment with 0.3 percent Thiram plus Captan (1:1) and four Zineb sprays (0.25 percent) was found to be quite effective in chilli control of this disease.

Katiyar *et al*. (2001) reported that founded three bottle gourd varieties to be resistant to A, namely Azad Harit, 7002 and 7003; Cummerina. Two highly resistant genotypes of chilli, CA 87-4 and CA 748, have been reported against fruit rot caused by *Alternaria.*

Matharu *et al*. (2006) reported that genotypes of tomatoes viz. Arka Alok, Arka Abha, Arka meghali, Arka Saurabh, IIHR-305, IIHR-308, IIHR-2266, IIHR-2285 and IIHR-2288 have been found to have early blight resistance. Likewise, researchers worldwide are focusing on the expression of various genes encoding proteins that are critical to inducing resistance in different crops.

Namanda *et al*. (2004) reported that *Alternaria leaf* blight involves avoiding long periods of leaf surface wetness, cultural scouting, drainage, and fungicide-resistant host plant growth.

Babu *et al*. (2000) reported the antagonistic properties of various bacteria and actinomycetes, the use of specific agents for bio-control is encouraged. The fact that they too are eco-friendly is another important reason for their increased application. Active inhibition of the growth of mycelial *A. Solani* causing leaf tomato blight were also identified as Bacillus subtilis and Trichoderma viridae.

Zhao *et al*. (2008) documented that both in vitro and in vivo conditions Bacillus and Pantoea had significant antifungal activity while Curtobacterium and Sphingomonas displayed antifungal activity only in vitro against *A. Solani* isolated tomato. The use of various herbal extracts and natural products is promoted by herbal extracts and natural products, since they do not cause any health threat or pollution. The extracts of *Canna indica*, *Convolvulus arvensis, Ipomoea palmata, Cenchrus catharticus, Mentha piperita, Prosopsis spicigera, Allium cepa, A. sativum*, *Lawsonia inermis*, *Argemone mexicana, Datura stramonium* and *Clerodendron inerme* completely inhibited the spore germination of *A. brassicae* isolated from leaves of cauliflower.

Datar (1996) stated the inhibitory effect of garlic bulb extract on the production of A in myceliums. Tenuis -causal organism of brinjal leaf spot. The effective inhibitory action of a speed weed extract of ethanol or methanol (Polygonum perfoliatum) against conidial germination. A. Ching (2007) reported *brassicicola* causing leaf spot of spoon cabbage. The leaf extract from the neem showed high efficacy inhibiting A's radial growth. *Solani* (43.3% and 26.7% respectively, 0.1% and 0.01%). Therefore, there are a number of herbal extracts and herbal products that are found to be effective in controlling diseases caused by *Alternaria* without any health or pollution hazards.

(Srinivas, 2006) reported the control of disease, fungicides are used in high dosages that cause environmental hazards and also leaving residues of fungicides which are toxic to the consumers. In the light of World Trade Organization, the major obstruction in the export of vegetables from India is the presence of excess amount of pesticide residues. So as to avoid the problem of pesticide residues and to reduce the cost of inputs and save the environment from pollution hazards, it is thought appropriate to look alternate methods like use of resistant varieties in the management of bottle gourd downy mildew.

Sultana *et al*. (2009) stated that Using ISTA techniques, the seed borne fungi of bottle gourd (*Lagenaria siceraria* was studied. A total of 22 genera and 45species of fungi were isolated, of which 35 have not hitherto been recorded from seeds of bottle gourd in Pakistan. Both blotter and deep-freezing methods yielded quantitatively as well as qualitatively more fungi than agar plate method. *Lasiodiplodia theobromae, Fusarium semitectum Macrophomina phaseolina* and *Fusarium oxysporum* were most frequently isolated from 33, 91, 50 and 66 % seed samples of bottle gourd respectively.

Begum and Momin (2000) reported that blotter method was found useful for detection of most infectious fungi of cucurbits. Deep-freezing method was found most suitable for detection of deep-seated as well as slow growing seed borne fungi like *Drechslera halodes, D. rostrata, Fusarium moniliforme, F. oxysporum, F. solani, Macrophomina phaseolina, Myrothecium spp., Penicillium purpurogenum* and *Sordaria* spp. These findings corroborate the reports that the deep-freezing method is more suitable for deeply seated seed borne fungi especially *Fusarium* spp.

Sanjay *et al*. (2006) documented that Induced resistance is an important component of disease-resistance response of plants, and is accompanied by increased capability for activating defense responses upon pathogen ingress or elicitor treatment. Aqueous leaf extract of neem (*Azadirachta indica*) provided the control of *Alternaria* leaf spot pathogen (*Alternaria sesami*) of sesame (*Sesamum indicum* L.). Treatment with this extract led to the changes in plant metabolism as leaves of the treated plants exhibited significantly high level of enzymes phenylalanine ammonialyase (PAL), peroxidase (PO) and content of phenolic compounds. Furthermore, germination of A. sesame spores was not significantly inhibited by neem extract. It is therefore, suggested that, protection of sesame plants against A. sesami by neem extract might be due to stimulation of plants natural defense response.

(Amadioha, 2003) stated that conventional fungicides and microbial biocontrol agents, plant products/extracts have been found effective against a wide range of pathogens. Furthermore, plant product based bio fungicides are systemic, specific in action, nonphytotoxic and have poor environmental retention. Studies on the mechanisms of disease control by plant extracts /products have revealed that the biologically active constituents present in them may have either direct antimicrobial activity or induce host plants defense response resulting in reduction of disease development.

Singh *et al*. (2015) documented that *Alternaria* and bacterial leaf blight are regulated by various effective materials described in the Commercial Vegetable Production Guidelines for Integrated Crop and Pest Management provided by the Cooperative Extension program of Cornell. Studies in Canada has shown that the 25 per cent disease exists as a guideline for timing the first application of fungicides to combat *Alternaria* and *Cercospora* disease.

Field data collected over a number of growing seasons in New York had confirmed and validated the use of the first application of fungicides as the cause for the incidence of 25 per cent disease under growing conditions in New York. Due to variations in susceptibility to variety, it is important to scout and make management decisions by variety, as different varieties can attain the disease threshold at different times. When the first application of the fungicide is made, subsequent sprays can be determined on the basis of:

1) Calendar foliar application, based on prescribed intervals for the particular fungicide used
2) Continue to investigate disease development and tracking temperature and forecast rainfall (a spray is mandatory if disease intensity has increased, rainfall is expected to reach 60oF for the following 5 days and/or night temperature) or
   using the Tom-Cast model (early blight model for tomato) available for some IPM weather stations via (NEWA). Tom-Cast model for measuring a disease severity value (DSV) takes into account temperature and leaf wetness. A fungicide application is recommended to be made after > 15 DSV's have accumulated after the earlier spray. The accumulated DSV's return to zero once a fungicide is made and the process begins again.

Research findings examed that the need for the next fungicide application may be postponed to control *Alternaria* application of a copper based bactericide.

Singh *et al*. (2015) stated that careful monitoring of the severity of disease incidence and weather conditions will decrease the number of sprays needed to manage blight diseases, allowing the grower to decrease the impact on the environment and decrease input costs too. It is recommended that alternation of fungicides be avoided to prevent resistance production and to reduce costs. Results indicated that alternating one of the registered fungicides with a copper material in the management of *Alternaria* and *Cercospora* was also successful and can be helpful in keeping bacterial leaf blight under control.

2.5.8. Natural products

The hypothetical setting of the use of beneficial microbes and beneficial plant leaf extracts should be based on. For alternative agricultural methods of production, therefore, lay emphasis on a desire. Analysis only paying attention to the two contrasting patterns (present and past) in this chapter. Significance and advancement of the use of beneficial microbes and various crop production practices was established through emphasis.

Pasche *et al*. (2004) reported that the resistant varieties could be affected by chemicals used for a long period of time. Those chemicals are causing health problems. Many clients and growers face environmental problems and face many ecological problems as well. Excessive use of chemicals on the field could increase the risk of an attack on resistant varieties by a pathogen. Some countries, such as the USA A. Such concerns were also faced by the use of excess chemicals on the ground. Growers are worried about such situations with excessive use of chemicals.

Choulwar and Datar (1992) stated they have shown that the Mancozed is the main minimizer against severity of Alternaria disease. It is also boosting production. Yield of crops also increased with the use of Mancozed. Also tested against disease were other nine fungicides such as Iprodione, captafol, Dithionon, Copper oxychloride, Carbendazim, zineb, man-cozed, and ziram, and thiophenate methyl. Such fungicides have provided good results for *Alternaria* diseases.

Sinha and Prasad (1991) reported they are conducteding the research in three (3) seasons at a concentration of 0.2 percent of Dithane among other seven (7) fungicides examined in research area. Product of this chemical (Mancozed) showed decreased regulation of disease incidence and disease infection.

Bhardwaj (1991) stated that at a concentration of 0.25 percent, all fungicides such as copper oxychloride, captofol and mancozeb yielded the greatest yield after transplantation using them. They also minimize the occurrence of *Alternaria spps* disease. Meena *et al*. (2004) documented that micro-climate terms vary in plant diseases at specific locations or specific conditions. The natural conditions are polluted by the use of fungicides and pesticides. Some non-toxic fungicides are not environmentally harmful, and are used against pathogens.

Proietti *et al*. (2004) reported that Environmentally friendly fungicides are used, and crop losses are reduced within 45 to 75 days of sowing. Plants Pathologists are looking for the non-toxic pesticides that have been suggested as chemical substances.

Maynard *et al*. (2003) reported that all fungal diseases can be handled by industrial use fungicides. Pesticides and fungicides may control the bacterial and fungal diseases. Such diseases occur on leafy plants, which are caused by pathogens. Those diseases are cured through commercial fungicide applications. Acacia leaf extracts which are used against *Alternaria* as a cured drug. Extracts of *allium satium* are used to treat *Alternaria* disease as consumer goods.

Dubuis *et al*. (2005) stated that the loss of sulphur reliantphyto anticipates that affect oilseed nutrients and mineral loss that adversely affect the resistance of diseases. Trichoderma is the best commercial and beneficial agent used in leafy crops for the cure of *Alternaria* diseases. Trichoderma is the best commercial product which used leafy vegetables against *Alternaria* blight.

Ching *et al*. (2007) reported that methanol and weed ethanol extracts that regulate cabbage conidia germination that the name of the disease is cabbage leaf spot. Extracts of methanol and ethanol which have an inhibitory effect on cabbage leaf spot. It was stated to have an inhibitory effect against cabbage leaf spot in China 0.02 per cent extract of ethanol. For this vegetable disease this concentration has the most effect. kumar *et al*. (2004) reported the neem products which also have the most effect on this disease. Bioneem and achook are best-effective compared to nimbicidin. Compared to bioneem, nimbicidin has less effect on cabbage leaf spot.

Sarker and Islam *et al*. (2017) stated that Trichocompost produced the highest percentage of germination in field condition of all therapies, and compacted the incidence and severity of *Cercospora* leaf spot disease to a greater degree. It also yielded acceptable results in improving the agronomic yields. Decomposed cow dung may also reduce the incidence of disease and achieve appropriate yield. Biskatali leaf extract was the best treating cause among the botanicals, since it could limit the incidence of a disease and severity of *Cercospora* leaf spot of Indian spinach. It also assisted in rising agronomic yields.

Gondal *et al*. (2012) reported that tomato (*Lycopersicon esculentum* L.) is a world vegetable of great importance. It is cultivated in Pakistan at an extremely low level of genetic resistance. Farmers cultivate certain high-yielding varieties that are less resistant. The five tomato varieties were planted in five tunnel replications, and varying doses of mancozeb were added in 7 days. After ten days, data on the disease was recorded from the flowering stage. After ten pickings an average yield for each variety was calculated. The disease was managed with the use of fungicides and mancozeb. As a comparison to Astra, Eurica and Ti-66 and Astra, yield of Litah545 and Litah514 have superior capitulate. Results suggested that mancozeb sprays at 12 g / L of water were an effective and biodegradable outlay intended for the management of tomato blight *Alternaria*.

Vadivel *et al*. (2006) stated that garlic (bulb) and Prosopis julifera extortion and gave moderate hang-up intensity. Palmarosa oil Neem 60 EC (3%), Neem oil (3%), and *Madhuca indica* oil (3%) also subdued mycelial increase. Bacillus subtilis, Trichoderma viride and Gliocladium virens were also among the antagonists who suppressed mycelial development of *A. Solani, A. alternata*.

Prasad and Naik (2003) reported that *Acacia nilotica, Anthocephalus, Aloe vera* and *Asparagus racemosus* root extracts are very effective against cabbage leaf spot disease. These root extracts have inhibitory action against family *brassicaceae* leaf spot. These root extracts show mild inhibitory action against cabbage leaf spot. Prosopis leaves and garlic bulb extracts inhibit *A. solani* growths on tomatoes or other vegetables.

Vadivel and Ebenezar (2006) reported that Trichoderma viride and Bacillus subtilis are halting mycelia growths. Maximum spore germination mycelial growth has been inhibited by the roots extract of various plants such as *prosopi sjulifera*, neem, *madhuca india*, and garlic which have an inhibitory effect on family *brassicaceae* leaf spot. Maximum spore germination and growth of mycelia inhibited by the extract from these plant roots.

Babu *et al*. (2000) reported they conducted work using crude plant extracts and plant oils as a therapy to control *Alternaria 's* early blight disease. Specific plant extract products were used against administering *Alternaria* disease in crops. These compounds do not pose any harmful or health problems to humans. Such compounds are environmentally friendly and are used in crops and vegetables to combat fungal diseases.

Leksomboon *et al*. (2001) reported the extracts from different plants used as a spray against strategies for disease management. Such crude plant extracts contain both airborne fungal pathogens and microbes. Usage of these compounds indicates different beneficial outcomes showing different beneficial outcomes as these are environment friendly and have no adverse health issues. These compounds directly destroy the fungal spores and completely kill them on plant surfaces (Govindappa *et al*., 2011).

Hassanein *et al.* (2010) reported that if the concentration of neem leaf extracts increased then the degree of suppression increased. Neem extracts minimize out of the field infection. When these crude extracts were added then pathogenic growth was inhibited. This is combined by irrigation application with neem spray that decreases disease incidence by up to 44 per cent. Using aqueous extract of neem products has increased crop yield and reduced the severity and incidence of *Alternaria* disease in vegetable crops and inhibited mycelial growth (Patil *et al*., 2001).

Nashwa and Abo-Elyousr (2012) stated that zimmu plant extracts reduce fungal pathogen growth and also expose hybrid *Allium sativum* and *Allium sepa* pathogens from the attack of zimmu plant. *Acoruscalamus* root extracts also reduce or stop the mycelia and spore formation production. Garlic (5 percent), *prosopis juliflora* 10 percent, bulb and leaf extract used to prevent the spore germination, respectively. Mycelia growth prevented by the use of garlic bulb extracts, zimmu plant extracts, and mesquite leaf extracts (Latha *et al*., 2009; Curtis *et al*., 2004; Krebs *et al*., 2006).

Abd-El-Khair and Haggage (2007) stated that leaf extracts from lemon grass are good inhibitors of mycelia, and are also used to regulate *Alternaria* spore's production. Haloxylon recurvum is used to control *Alternaria* spore's formation germination, and is used to suppress *Alternaria alternata* mycelia growth. To suppress mycelium growth, stem extracts of *Atriplex lentiformis* and *Haloxylon recurvum* are utilized. Eleven various halophyte plant extracts are used to inhibit the growth of mycelia. Various parts such as bark, root, leaf, and stem are used in extract preparation.

Latha *et al*. (2009) stated that leaf extracts of *Prosopis juliflora* and *Cocosnucifera* control the *Alternaria* diseases on plant leaf surfaces and various eighteen plant families used against tomato plant infection. The use of Chamgol and Sardasht oils regulates the production of *Alternaria solani* Hooshyari *et al*., (2013).

Mate *et al*. (2005) reported the recordeding at 10 percent under artificial and natural conditions of inoculation. Extracts of the eucalyptus leaf are used against *Alternaria solani* development. Extracts of the leaf prosopis and garlic bulbs are used to inhibit mycelia production. *Alternaria* spp. is managed using various plant extracts such as onion bulb, garlic, and neem.

Derbalah *et al*. (2011) stated that Bauhinia purpurea gave good results under green house and laboratory conditions and that leaf extracts of this plant were also most successful against *Alternaria* diseases. *Melaleuca alternifolia* essential oils have been used to minimize the frequency and severity of the *Alternaria* disease (Caolotanski *et al*., 2002).

Datar *et al*. (1996) stated the use of natural products are promoted because they do not cause any health threat or pollution. Such drugs are also cheaper than chemicals which have limited to practically no harmful side effects on hosts. The extracts of *Lawsonia inermis*, *Canna indica*, *A. sativum*, *Allium cepa*, *Argemone mexicana*, and *Clerodendron inerme* fully inhibited the germination of spore of *A. brassicae* isolated from leaves of cauliflower. Garlic bulb extract had inhibitory effect on *A*. *tenuis* mycelial growth i.e, causal organism of brinjal leaf.

Sharma *et al*. (2007) documented that there are therefore a number of effective herbal extracts and herbal products with no health hazards or pollution in controlling *Alternaria* diseases. It was surprising that the extracts from cold water were more successful than the extracts from the boiling water. Leaves extract of *Lawsonia alba*, *Datura stramonium* roots and *Mentha piperita* inflorescence had fungi-toxic activity against *A*. Isolated *brassicae* from the cauliflower stems. Two Aegle marmelos 10 percent leaf extract sprays combined with 0.01 M nickel sulphate at age 100 and 115 days after sowing substantially decreased the chili disease severity. An antifungal peptide, Ay-AMP was isolated from the seeds of Amaranthus hypochondriacus, and found to be effective in regulating *A*. *Alternata* at very low doses Ay-AMP degrades chitin and is extremely protease and heat resistant.

Sunita *et al*. (2013) documented that Aqueous extract of three botanicals as leaves of neem (*Azadirachta indica*), bulbs of garlic (*Allium sativum*) and rhizome of ginger (*Zingiber officinale*) at four different doses (5, 10, 15, 20%) were evaluated against *Alternaria* leaf blight of mustard under field condition. The disease was adequately managed by the application of these three botanicals irrespective of their doses in comparison to untreated control. Two years' data revealed that spraying of neem leaf extract @15% was more effective against this disease as well as increased the seed yield of mustard. Seed yield was significantly highest ($p < 0.05$) on application of neem leaf extract @15% over other treatments (1403.83 kg/ha) where as in check had only 820.33kg/ha. Garlic bulb extract@10% also gave better yield (1366.17kg/ha) which is similar to that of 10% of neem leaf extract (1383.00kg/ha). Cost benefit ratio was also highest (1:2.5) at 15% neem leaf extract which was similar to that of 10% of the same botanicals (1:2.0) and 10% garlic bulb extract (1:1.9). It indicating that the spraying of neem leaf extract on mustard was effective against *Alternaria* blight resulting superior performances in seed yield for consecutive two years.

(Chattapadhyay and Vhunia, 2003) stated that Different chemicals including systemic fungicides have been used for management of this disease. However, increase environmental pollution and present day public perception on pesticide contaminants of foods specially the edible oils, development of alternate economical and ecofriendly approaches for disease management is needed several plant products are known to have antifungal activities which are environmentally safe and non-phytotoxic.

(Vadivel and Ebelezer, 2006) stated that using different doses of aqueous extract (5, 10, 15, 20%) were prepared individually according to the treatments. the different doses of aquese extract of these three botanicals were selected as per doses used by different scientist on other crops in vitro condition on early blight of tomato and extract of dried roots of *Acorus calamus* against *alternaria solani* of tomato.

Paul (2003) reported the carried out in vitro tests against *Phytophthora cryptogea, Trichoderma virens, Aspergillus niger, Phoma sp., Fusarium oxysporium, Pythium ultimum, Cochliobolus heterostrophus, Rhizoctonia solani, Sclerotium rolfsii* and *Pyrenophora teresusing* extracts of *Maesalan ceolata var. goulun gensis* showed effective results against all the pathogens tested.

Harish *et al*. (2004) reported the working on rice brown spot (*Helminthosporium oryzae)* control with 15 seed extracts under laboratory condition found that 10% rhizome extract of turmeric (Curcuma longa), seed extracts of sundavathal (*Solanum indicum*) and vedpalai (*Wrightia tinctoria)* exerted maximum mycelial growth and spore germination inhibition.

Choi *et al*. (2004) reported the extract of *Rumex acetosella* roots reduced development of powdery mildew of barley.

Velluti *et al*. (2004) reported the evaluated 37 essential oils of which lemongrass, cinnamon, clove, palmarosa and oregano showed antifungal activity against *Fusarium* sp. showing no interspecific difference which suggests that EOs can be safe alternative. Rodriguez *et al*. (2005) reported the antifungal activity of Aloe Vera (*A. barbadensis*) on mycellium growth of *Rhizoctonia solani, Fusarium oxysporum*, and *C. coccodes* showed an inhibitory against fungi.

Boughalleb *et al*. (2005) stated that antifungal activity of volatile components extracted from flowers of *Lantana camara, Malvaviscus arboreus* and *Hibiscus rosa-sinensis* showed stronger antifungal activity against *Alternaria solani, Botrytis cinerea, Pythium ultimum, Rhizoctonia solani* and *Verticillium dahlia* than extracts from stems or leaves.

Doltsinis *et al*. (2006) reported the efficacy of *Milsanato* induce resistance to powdery mildew on cucumbers, against *Leveillula taurica* on greenhouse tomato which demonstrate that *Milsana* could play a supreme role in of powdery mildew management in organic and low resource tomato production.

Kumar *et al*. (2007) reported *Chenopodium ambrosioides* can inhibit two aflatoxigenic strains of *Aspergillus flavus* along with *A. fumigatus, Botryodiplodia theobromae, F. oxysporum, P. debaryanum* and *S. rolfsii*) and essential oil of Peumusbolduswas effective against *A. niger, A. flavus* and *Fusarium* spp.

Magro *et al*. (2006 reported that inhibition of *Aspergilluscandidus, A. niger, Penicillium* sp., and *F. culmorum* with Chamomile and malva aqueous extracts. Shirzadian *et al*. (2009) reported twenty-one moss species and two leafy liverwort species obtained by ethanol, water and petroleum ether solvents against *Alternaria alternata* showing broadest spectrum antifungal activity by the ethanolic extracts of six moss species.

Fawzi (2009) reported carried out in vitro studies of different plant extracts against *Fusarium oxysporum* showed radial growth inhibition of the fungi. Lakhdar (2010) stated antifungal activity of powdered extracts and essential oils of some local medicinal plants on *F. oxysporum* f. sp. lentil population in soil showed 10% and 5% powdered extracts of *I. viscosa* and *M. pepirita* and all the essential oil formulations of all the plant extract reduced the soil population densities of fungi and disease incidence in lentil.

Surender (2012) stated aqueous extract of 20 plants for antifungal activity against *F. solani* (dry rot of potato) showed differential activities of different plant extracts against the mycelial growth inhibition. Cheng *et al*. (2008) reported the antifungal activity of essential oil from Calocedrus macrolepis var. formosana and its constituents T-murolol and a-cadinol on the growth of plant pathogenic fungi which also inhibited the growth of *Rhizoctonia solaniand Fusariumoxysporum* and mycelial growths of *Colletotrichum gloeosporioides, Pestalotiopsis funerea, Ganoderma australe* and *F. solani*.

Razzaghi-Abyaneh *et al*. (2008) reported the inhibitory effect of carvacrol and thymol as aflatoxins producer obtained from essential oil of Satureja hortensis against *Aspergillusparasiticus* inhibit the growth of fungal.

Feng and Zheng (2007) studied the antifungal activity of essential oils of five plants (thyme, sage, nutmeg, eucalyptus and cassia) against *Alternaria alternata* at different concentrations and found that the cassia oil can completely inhibit *A. alternata* growth. Faria *et al*., (2006) reported that essential oil of aerial parts of Ocimum gratissimum obtained can inhibited growth of several fungi including *Botryosphaeria rhodina*, Rhizoctonia and *Alternaria* sp.

Abo El-Seoud *et al*. (2005) reported the essential oils of fennel, peppermint, caraway, eucalyptus, geranium and lemongrass for their antimicrobial activities against some plant pathogens *(F oxysporum, a alternata, P italicum and B cinerea*) and found that essential oils of fennel, peppermint and caraway can be used as active ingredients for formulating biocides.

Hassane *et al*. (2008) stated that ethanol, ethyl acetate and water extracts leaf of *Azadiracta indica* and Melia azedarach against two tomato fungal pathogens at different concentrations and found that both ethanol and ethyl acetate extracts of neem leaves assayed, completely suppressed the growth of *F. oxysporum* and *A. solani*. Hadizadeh *et al*. (2009) stated that working on antifungal effect of essential oils from some medicinal plants of Iran: neetle (Urticadioica), thyme (*Thymus vulgaris*), eucalyptus (*Eucalyptus* sp.), rute (*Ruta graveolens*) and common yarrow (*Achillea millefolium*) on *A*. *alternata* of potato as a model pathosystem.

Zabka *et al*. (2009) reported use of EOs obtained from Carum carvi, Cymbopogon nardus, Pelargonium roseum, Pimentadioica and Thymus vulgaris against growth *F. oxysporum, Fusarium verticillioides, Penicilliumexpansum, Penicilliumbrevicompactum, A. flavus* and *A. fumigatus*.

Vilela *et al*. (2009) stated the EO of Eucalyptus globulus showed inhibitory effect against fungal species, *A. flavusand Aspergillus parasiticus*. Deba *et al*. (2008) tested the fungitoxic activities of the flower essential oils of Bidens pilosa against *Fusarium spp., Fusarium solani* the most suppressed species, followed by *F. oxysporum.*

Matchima and Ampai (2009) stated that to investigate efficacy of crude dichloromethane extracts of pomelo albedo on radial growth and spore germination of Colletotrichum gloeosporioides at different concentrations which showed that crude extracts did not affect radial but reduced spore germination at 25% and 100% concentration.

Yasmin (2008) examined 55 angiospermic plants the in vitro vegetative growth of *Fusarium moniliforme* Sheldon where leaf extract of *Lawsonia inermis* showed maximum inhibition followed by roots extract of Asparagus racemosus. Yusuf (2011) evaluated Antifungal activities of Xanthium strumarium, Laurisnobilis, Salvia officinalis and Styrax officinalis which were the most active against mycelial growth of *P. infestans.*

Debjani *et al*. (2017) reported three plant extracts Ginger, Polyalthi and Clerodendrum shows good inhibitory effect on *Rhizoctonia solani* under in vivo condition and also observed dose response effect against growth of *Colletotrichum capsici* at three different concentrations.

Jantasorn (2016) reported the efficacy of Hydnocarpus, Caesalpinia and Carallia against five plant pathogenic fungi in in vitro conditions at various concentrations among which Hydnocarpus fruit extracts exhibited potential to growth inhibition, and recorded 100 % growth inhibition against *P. oryzae, P. palmivora* and *R. solani* followed by *S. rolfsii* (96.33 %). Bhardwaj (2012), reported the aqueous extract of twenty plants as sole and also in combination against *Fusarium solani* causal agent of dry root rot of potato. The mixtures of Lawsonia alba leaf extracts and Acacia catechu stem extracts showed an enhancement in activities over the individual extracts by 54.69 % and 62.07 % respectively. Nguefack *et al*. (2012) observed synergistic effect against *Penicillium expansum* by mixing fractions of essential oil from *Cymbopogon citrates*, *T. vulgaris* and *O. gratissimum*.

Akila *et al.* (2011) reported that they clearly found that combined application of botanical extracts and biocontrol agents effectively reduced *Fusarium* wilt of banana. Hsieh *et al*. (2004) documented that Botanicals are also being tried in the field of food borne microorganisms. The combined extracts of Corni fructus, cinnamon and Chinese chive were used to evaluate its antimicrobial activity on common foodborne microorganisms. The combined extract was not only found very stable under heat treatment and also showed an outstanding inhibitory effect against entire antimicrobial spectrum. They concluded that combined extract is suitable application where a naturally antimicrobial additive is desired.

Burtram *et al*. (2015) stated both synergistic and antagonistic interactions between the plant extracts and the kresoxim-methyl fungicide which showed both Synergistic and additive effects against one strain of B. cinerea. Some researchers also reported enhanced antifungal activity of cassia oil, essential oils when applied in combination with salt like KCl or NaCl, polysaccharide like chitosan. Tahany *et al*. (2010) stated that using combination have been done against human pathogens where synergistic effect of two plant extracts plants extracts and antibiotics were studied in different ways.

Parajapati *et al*. (2017) stated that phytoextracts have been shown to be safer botanical pesticides as they are environmentally friendly and cause substantial fungal growth reduction.

2.5.9. Some other methods

Among the various methods listed above, many other methods can also be used to help mitigate devastating effects caused by the species *Alternaria*.

Gomez-Rodriguez *et al*. (2003) found that the intercropping of marigold tomatoes (*Tagetes erecta* L.) induced a substantial reduction in A-caused early blight, *Solani*.

It was done by three distinct processes, such as:

(I) marigold allelopathic influence on A. Germination of conidial *solani*,

(ii) by modifying the microclimatic conditions around the canopy, in particular by reducing the amount of hours per day with relative humidity to 92%, thereby reducing conidal growth and

(iii) the creation of a physical barrier against conidia spread. Besides this, the introduction of residues after harvest as soon as possible is another step to reduce the adverse effects of *Alternaria.*

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental Area

Experiment was carried out during May to September 2019 in the research area of the Department of Plant Pathology, University of Agriculture Faisalabad. For this experiment the overall study period was six months. The site 's location is around half a kilometer away from the Department of Plant Pathology. Soil in all agricultural areas is ideal for farming purposes. The experimental site climate is subtropical in nature during August to September, with slight rainfall.

3.2 Collection of materials

For this experiment which was purchased from Vegetable Research Institute, Faisalabad (AARI), three varieties (Anmol F1, Bemisal and Green Gold) were used. This experimental study was conducted with three Replications (R1, R2 and R3) within a Randomized Complete Block Design (RCBD). The experimental plot was partitioned according to the experimental design into rows and columns in. Experimental design was designed as:

Date of sowing : 14/05/2019

Experimental design : RCBD

Plot length : 10 feet

Plot width : 30 feet

Replications : 3

Varieties : 3(V1 Anmol F1, V2 Bemisal, V3 Green gold)

Plant to plant distance : 30cm / 1feet

Row to row distance : 90cm / 3feet

Path to path distance : 90cm / 3feet Treatments : 5

Fig 3.1: **Prepared experimental area**

3.3 Intercultural Operation

In the experimental plot weeding was performed for three times. First weeding was performed one month after sowing followed by two more at interval of 20 days.

Figure 3.2: **Germination start**

3.3: **Cultivation of Bottle gourd**

3.4 Isolation and identification of pathogen

3.4.1 Preparation of media

For the preparation of potato Dextrose Agar (PDA) media such as: Potato starch = 20 g, Glucose = 20 g, Water = 1 L, Agar agar = 20 g. Only1L of distilled water was taken into a flask and put on the magnetic stirrer for 10 minutes. In this flask, ingredients were carefully mixed, and media was sterilized along petri plates at 12 ° C for 20 minutes. Both petri plates had been covered in newspaper prior to autoclave. Sterilized cool media was decanting in sterilized petri sheets at a rate of 20ml per plate. These petri plates were positioned at last in the incubator. All work was done in a chamber with laminar flow (Sarwar *et al.,* 2005).

3.4.2 Pathogen isolation and identification

After the appearance of disease on the leaves, sampling was taken from field area. The leaves were converted into pieces. These leaves were then washed with sterilized water and 70 percent ethyl alcohol was used for immersion. Those leaves were dried on filter paper surface after sterilization, and then immunized on PDA media. Those plates were incubated at 25 ° C for 7 days. Fungi have been isolated and analyzed based on spores such as size and colour. (Figure.3.4) A. Spores were multicellular, creating in chains and branches. Polymorphic conidia pattern also singles, long, small chains or provided with longitudinal, fractious and tilted septa. (Figure. 3.5).

Figure3.3: **Diseased leaves of Bottle Gourd**

Figure 3.4: **Culture of *Alternaria Cucumarina***

Figure3.5: ***Microscopic spores' identification***

3.5 Recoeding of Disease Incidence

Following the initial appearance of *Alternaria* leaf blight disease on bottle gourd field, the experimental plot was monitored at 15days intervals. The occurrence of the disease was reported four times (DAS 20, 35, 50 and 65). Identified infected plants, and calculated *Alternaria* leaf blight incidence using the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{No. of total plants}} \times 100$$

3.6 Assessment of Disease Severity

For scoring four infected plants were randomly selected from each board. Treatments were administered at a 15-day interval. The first treatment was done on the initial indication of disease appearance. Disease data was collected in advance of each treatment. Scored at 20, 35, 50 and 65 DAS with infected plants using (0-5) rating scale as follows: Prasada *et al*., (1973).

Rating	Description	Status
0	No infection	Highly Resistant (HR)
1	10% leaf area infection	Resistant (R)
2	11-30% leaf area infection	Moderately Resistant (MR)
3	31-50% leaf area infection	Moderately Susceptible (MS)
4	51-70% leaf area infection	Susceptible (S)
5	71 and above leaf area infection	Highly Susceptible (HS)

(Prasada *et al*., 1973)

Percent disease index (PDI) was calculated using the recorded data according to:

$$\text{Infection Index} = \frac{\text{Category No x Number of leaves per category}}{\text{Total No. of leaves counted x Maximum possible disease category x 100}}$$

3.7 Management of *Alternaria* leaf blight of Bottle gourd by plant extracts

3.7.1 Collection of plant extracts

Fresh leaves of Neem T1 (*Azadirachta indica* L.), Ginger T2 (*Zingiber officinale* L.), Onion T3 (*Allium cepa* L.), were collected from University of Agriculture Botanical Garden, Faisalabad for management purposes. After collecting the leaf, using an electronic balance, weighted the parts of the plant and then washed with distil water. After washing the leaves, all leaves were dried thoroughly.

Fig3.5: **Leaves of Neem,**

3.7.2 Preparation of plant extracts

All crushed tissues of plants were grasped through 4 folds of fine fiber rags. After these leaves had been dried, all the leaves were crushed with grinder machine and then dipped separately into distilled water for 24/7 hours. Slanting sections of plants were mixed together to get extracts, and distilled water was added. For field spraying purposes, ratios 1:4 (w / v) were made by adding 400 ml of distilled water to 100 g of sections of the plant. These plant extracts were after all applied in the field as management purposes against bottle gourd leaf blight disease at intervals of 15 days after disease appearance.

Fig 3.6: **Dry pastes of plant extracts**

3.7.3 Plant extracts preparation and applications

Plant extracts (neem, onion, and ginger) were prepared for spraying on infected bottle gourd field. These plant extracts were prepared with a ratio of 1:4(w / v), adding 400ml of distilled water in 100 g of plant parts for spraying purposes. Using Randomized Complete Block Design (RCBD), effective concentration of plant extracts was applied to bottle gourd research area. First spray was applied on the bottle gourd field after the appearance of disease and three other treatments were applied at an interval of 7 days. First treatment was applied at 7 days, and data on incidence of disease were also recorded before and after the spray.

Fig. 3.7: **Spray**

3.8 Statistical Analysis

Statistically analyzed data obtained from different treatments (parameters) to find out the significant difference between the treatment. Using ANOVA tables, data was analyzed and correlated at 5 per cent probability level. The difference between the treatment means was estimated at 5 per cent probability level by LSD (Least Significance Difference).

CHAPTER 4

RESULTS

4.1. Screening of Bottle gourd against *Alternaria* leaf blight under field conditions

Ten varieties of bottle gourd (Hazarvi, Bemisal, Green gold, King, Anmol F1, Surbhi, Punjab long, Punjab large, Royal and Advanta-205) were screened against *alternaria* leaf blight. No variety was resistant. Green gold, Anmol F1 and Bemisal showed moderately resistant response having rating (2) with disease severity 20.05, 21.50 and 21.88 respectively. While Hazarvi, Surbhi showed moderately susceptible having rating (3) with disease severity of 44.05 and 48.05. Further two variates Punjab large or Punjab long showed susceptible having (4) rating with disease severity 55.05 and 66.50, as compared to three varieties King, Advanta-205 and Royal showed highly susceptible having (5) rating with disease severity of 76.72, 76.72 and 78.88. (Table 1) (fig 1.)

Table 1: Response of Bottle gourd against *Alternaria* leaf blight under field conditions

Sr.#	Varieties	Disease incidence (%)	Rating	Response
1	Green Gold	20.05 ab	2	MR
2	Anmol F1	21.50 d	2	MR
3	Bemisal	21.88 bcd	2	MR
4	hazarvi	44.05 ab	3	MS
5	Surbhi	48.05 d	3	MS
6	Punjab Large	55.05 abc	4	S
7	Punjab Long	66.50 a	4	S
8	king	76.72 cd	5	HS
9	Advanta-205	76.72 d	5	HS
10	Royal	78.88 abc	5	HS

Means sharing similar letters are statistically non significant (P>0.05).

R = Resistant
MS = Moderately susceptible
S = Susceptible
HS = Highly susceptible

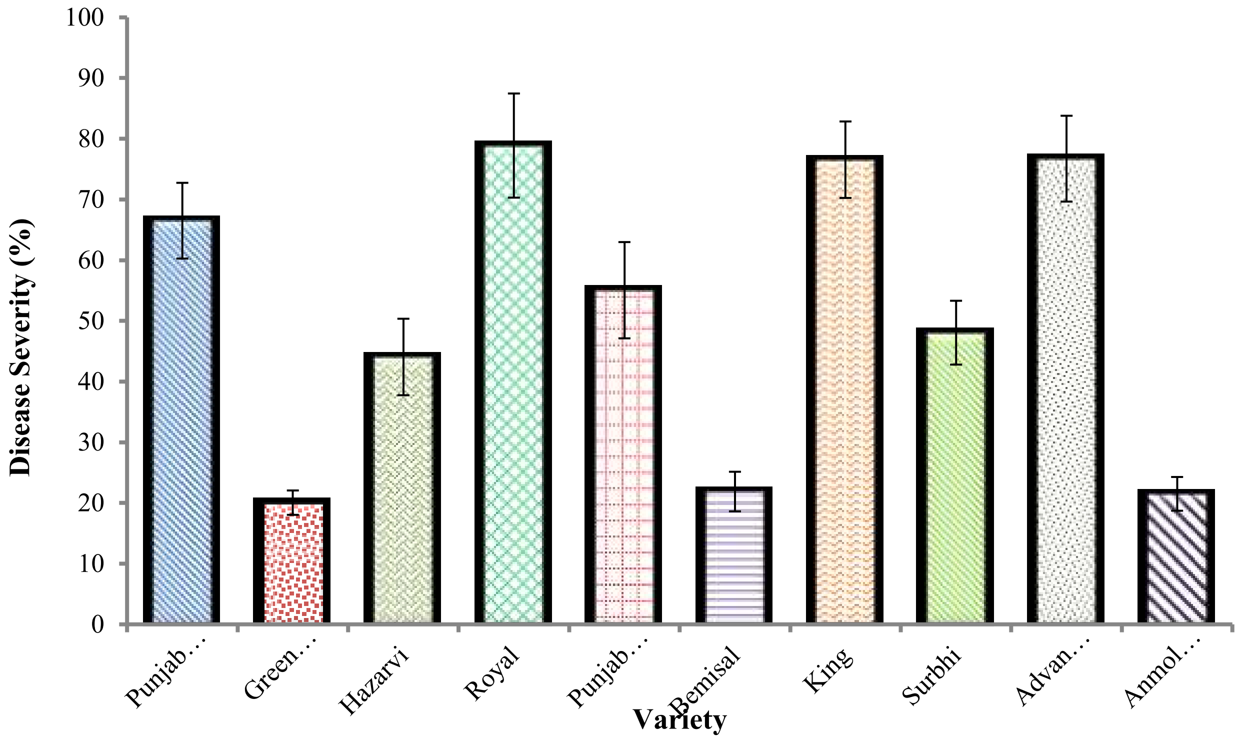

Fig.1. **Response of Bottle gourd against *Alternaria* leaf blight under field conditions**

4.2. Evaluation of plant extracts against *Alternaria* leaf blight under field Conditions

ANOVA table showed that all days, treatment, concentration, and their interaction T×C**,** expressed significant result while interaction between D×T, D×C and D× T× C indicated non-significant results (Table.2).

Table 2**: ANOVA for evaluation of plant extracts against *Alternaria* leaf blight under field Conditions**

SOV	**DF**	**SS**	**MS**	**F**	**P**
Days (D)	2	381.9	190.96	4.43	0.0136*
Treatment (T)	7	25254.1	3607.73	83.64	0.0000**
Conc. (C)	2	7196.5	3598.23	83.42	0.0000**
D×T	14	417.9	29.85	0.69	0.7795NS
D×C	4	3.5	0.86	0.02	0.9992NS
T×C	14	1143.9	81.71	1.89	0.0314*
D×T×C	28	60.7	2.17	0.05	1.0000NS
Error	144	6211.3	43.13		
Total	215	40669.7			

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01)

4.3. Evaluation of interaction b/w treatments and days against *Alternaria* leaf bligh under field conditions

The table expressed that combination of treatments *Allium cepa* + *Azadirachta indica* + *Zingiber officinale* showed minimum disease incidence (16.21mm) (10.70mm) (13.44mm), followed by *Zingiber officinale* + *Azadirachta indica* (27.17mm) (22.30mm) (4.62mm), *Zingiber officinale* + *Allium cepa* (31.38mm) (27.33mm) (29.42mm), *Zingiber officinale* (31.74mm) (27.76mm) (29.42mm), *Allium cepa* + *Azadirachta indica* (37.52mm) (33.10mm) (35.30mm), *Azadirachta indica* (39.37mm) (35.02mm) (37.23mm), *Allium cepa* (42.09mm) (37.562mm) (39.934mm) showed maximum disease incidence as compared to control. (Table 3) (Fig 3).

Table.3 **Evaluation of interaction b/w treatments and days against *Alternaria* leaf blight under field conditions**

Treatment	Disease incidence (%)			Mean
	D_1 (7 days)	D_2 (14 days)	D_3 (21 days)	
A.c+A.i+Z.o	16.21	10.70	13.44	29.68C
Z.o+A.i	27.17	22.30	4.62	37.23B
Z.o+A.c	31.38	27.33	29.42	39.93B
Z.officinale	31.74	27.76	29.68	24.62C
A.c+A.i	37.52	33.10	35.30	29.42C
A.indica	39.37	35.02	37.23	35.30B
A.cepa	42.09	37.562	39.934	13.44D
Control	49.88	55.53	52.70	52.70A
Mean	34.42 A	32.78AB	31.16B	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Z.o abbreviates ***Zingiber officinale*** (Ginger)

A.c abbreviates ***Allium cepa*** (onion)

A.i abbreviates ***Azadirachta indica*** (neem)

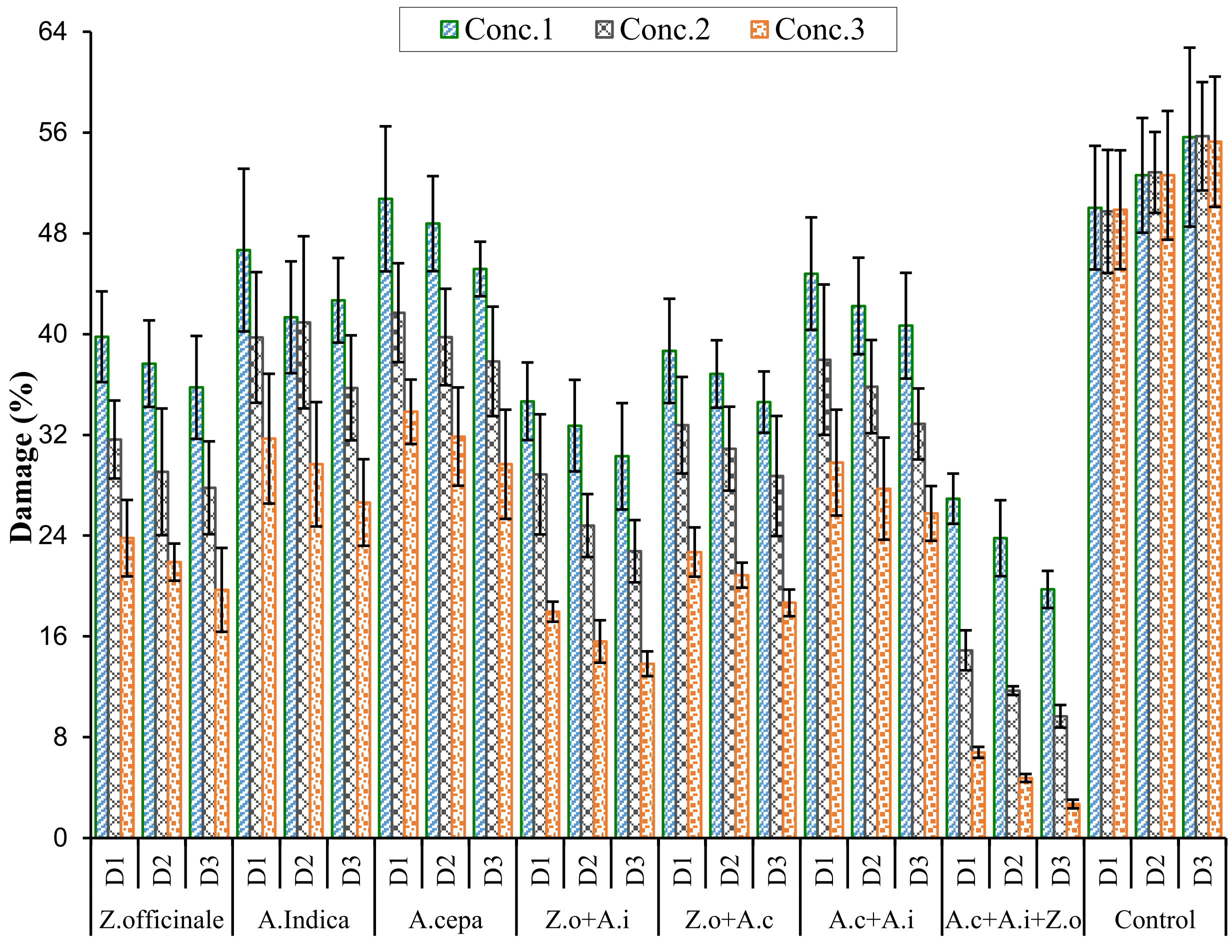

Fig.3 **Investigation of interaction between treatments and days**

4.4. Evaluation of Interaction between concentrations and days against *Alternaria* leaf blight.

This showed that interaction between concentration and days. The C1 showed maximum disease incidence (41.54) (39.91) (38.07) and C2 showed disease incidence (34.66) (32.81) (31.39) while C3 showed minimum disease incidence (27.06) (25.63) (4.03) as compared to control concentration. (Table 4) (Fig 4).

Table.4 **Evaluation of Interaction between concentrations and days against *Alternaria* leaf blight**

Concentration	Disease incidence (%)			Mean
	D_1 (7 days)	**D_2 (14 days)**	**D_3 (21 days)**	
C1	41.54	39.91	38.07	39.84A
C2	34.66	32.81	31.39	32.95B
C3	27.06	25.63	4.03	25.57C
Control	49.88	55.53	52.70	52.70A
Mean	34.42 A	32.78AB	31.16B	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

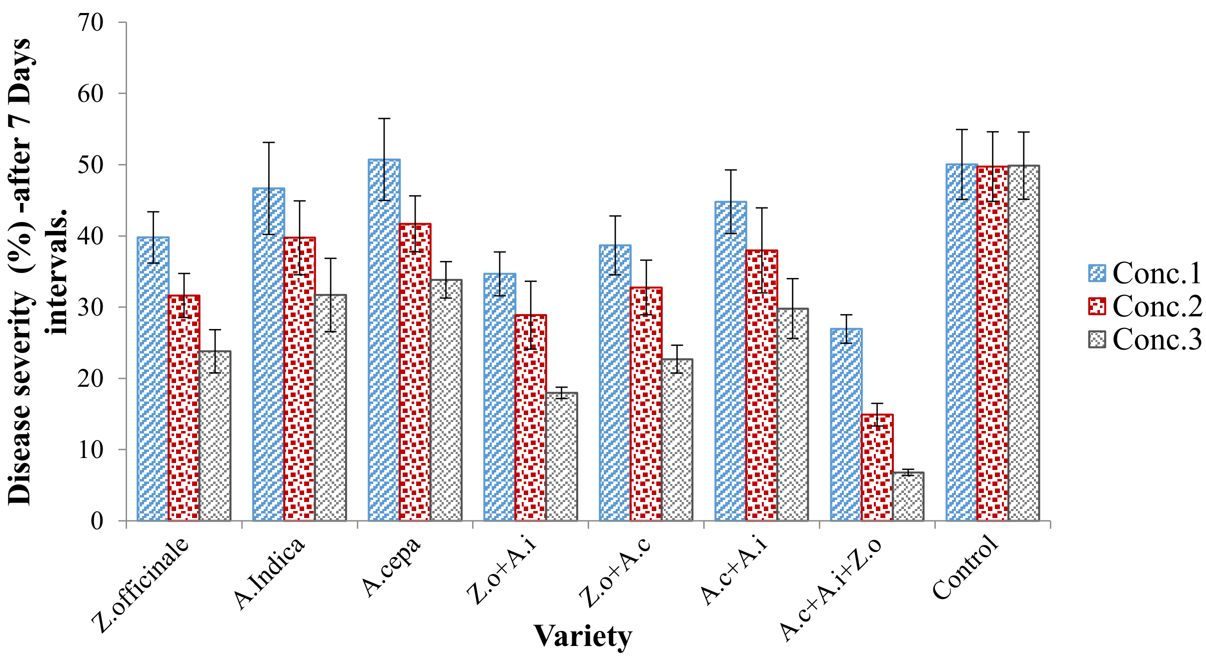

Fig. 4 **Assessment of combined effect of different plant extract concentrations after 7 days' interval**

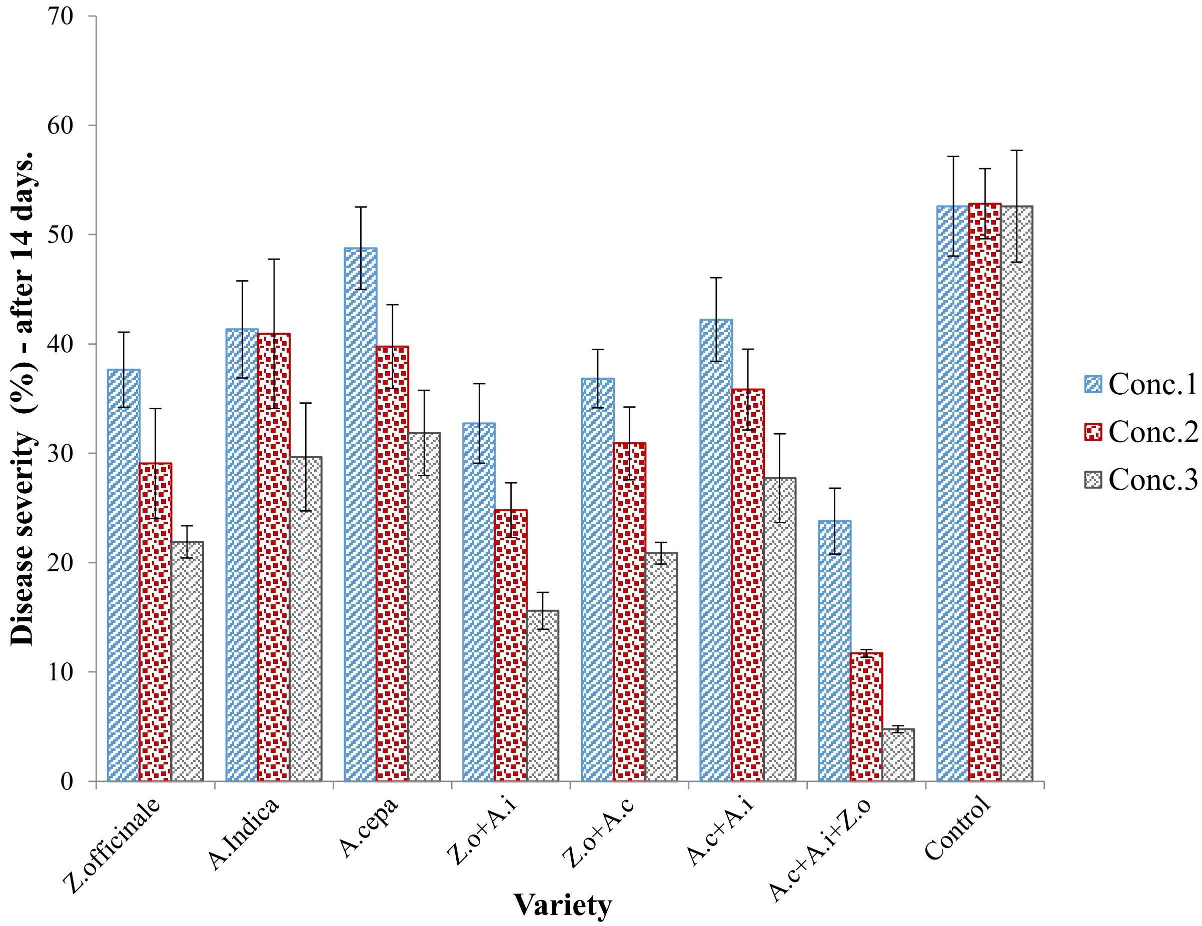

Fig.5 **Assessment of combined effect of different plant extract concentrations after 14 days' interval**

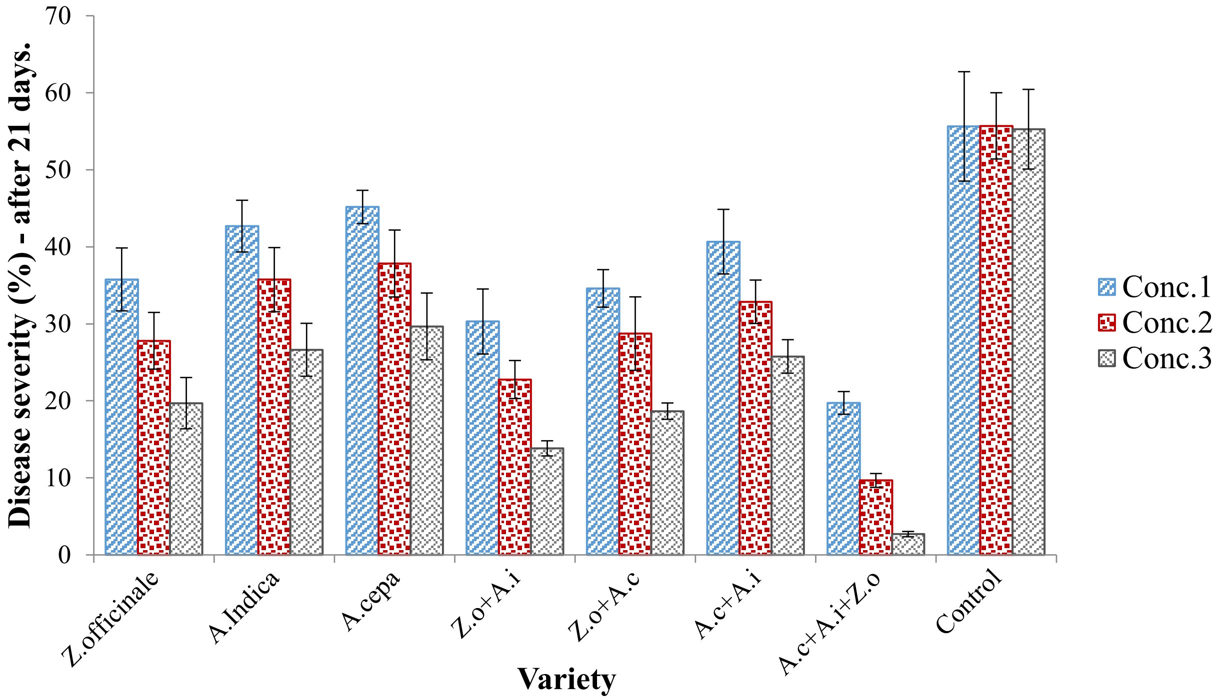

Fig.6 **Assessment of combined effect of different plant extract concentrations after 21 days' interval**

(C) Days × variety × concentration (%) interaction mean±SE

Conc. (%)	Variety	Days D1	D2	D3	Mean Conc. × Variety
C1	***Z.officinale***	39.79	37.65	35.77	37.74B-F
	A.Indica	46.67	44.67	42.68	44.67ABC
	A.cepa	50.73	48.77	45.17	48.22AB
	Z.o+A.i	34.67	32.73	30.30	32.57C-H
	Z.o+A.c	38.67	36.83	34.60	36.70C-G
	A.c+A.i	44.80	42.23	40.67	42.57A-D
	A.c+A.i+Z.o	26.93	23.80	19.73	23.49HIJ
	Control	50.03	52.60	55.63	52.76A
C2	***Z.officinale***	31.69	29.07	27.80	29.50E-I
	A.Indica	39.73	37.60	35.73	37.69B-F
	A.cepa	41.70	39.77	37.83	39.77B-E
	Z.o+A.i	28.87	24.80	22.77	25.48G-J
	Z.o+A.c	32.77	30.91	28.73	30.80E-I
	A.c+A.i	37.97	35.83	32.87	35.56C-G
	A.c+A.i+Z.o	14.90	11.70	9.67	12.09KL
	Control	49.73	52.83	55.70	52.76A
C3	***Z.officinale***	23.80	21.90	19.70	21.80H-K
	A.Indica	31.70	29.67	26.63	29.33E-I
	A.cepa	33.83	31.87	29.67	31.79D-I
	Z.o+A.i	17.97	15.60	13.83	15.80JKL
	Z.o+A.c	22.70	20.87	18.67	20.74IJK
	A.c+A.i	29.80	27.73	25.77	27.77F-I
	A.c+A.i+Z.o	6.80	4.77	2.70	4.76L
	Control	49.87	52.60	55.27	52.58A

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Discussion

Bottle gourd, *Lagenaria siceraria* is a cosmopolitan cucurbitaceous vegetable for its young, tender and non-bitter fruits, which is successfully grown in tropical and subtropical regions. Bottle gourd is high in nutritional value, it contributes substantially to the dietary intake of vitamins (Vitamin A, B, C) minerals (0.5%) such as calcium, magnesium, potassium, phosphorus and carbohydrates (2.9%), proteins (0.25%) and fats (0.5%) by virtue of the amount ingested (Morimoto *et al*., 2004).

Various viral and fungal pathogens attack bottle gourd crop. Among fungal diseases, the most common are *Alternaria* leaf blight, downy mildew, powdery mildew, *Cercospora* leaf spot and anthracnose, from which *Alternaria* leaf blight caused by *Alternaria cucumerina* is one of the most serious diseases and causes significant losses to bottle gourd crops. *Alternaria* blight to the leaf caused by *A. Cucumerina,* Cooke was observed on cucurbits (Sarbhoy 2006). Mukhtar *et al*., (2013) reported that bottle gourd disease symptoms mainly occur on leaves. Small, circular to irregular circular spots appear on older leaves, with tan to light brown lesions with dark margins. *Alternaria cucumerina* is a fungus that occurs worldwide and can infect most cultivated cucurbits. Initially the disease emerged in the field only after the seedling arose. Small, scattered, water-soaked, yellowish, circular to oval shaped spots appear on the leaves. Subsequently, these spots are transformed into brownish in color surrounded by chlorotic area with normal clustered circles, which coalesced to form large irregular patches on leaves showing a blighted appearance that can cause leaves to become wilted and shed. Economic yield losses caused by disease are 20 to 60 per cent (Bruce 2013).

Chemical control is costly, and not environment friendly. The residual effect of the fungicides causes human health hazards. It is particularly relevant as public perception of environmental contamination and residual effects on goods is rising due to the indiscriminate use of toxic substances and the advent of new races. Plant resistance is an efficient and environmentally safe means of reducing the fungal disease losses. The disease 's cheapest, most practical, and most economic control can be achieved by identifying the disease's resistant genetic stock (Jadhav and Sharma, 1983). Cultivating resistant or tolerant cultivars is one of the best ways to minimize the losses caused by disease. Sources of resistance are prerequisite for identifying a resistant variety. The indigenous cultivars, land breeds, folk cultivars, semi-wild relatives and allied vegetable crop species may have resistant origins (Singh *et al*., 2009). So far, information on the varieties / genotypes of screening bottle gourd against *Alternaria* leaf blight in field conditions is done. Considering the above facts in background, the present study was undertaken to evaluate and identify resistant bottle gourd varieties / genotypes against *Alternaria* leaf blight under field conditions.

10 bottle gourd genotypes were collected from various sources such as Faisalabad AARI Institutes for conducting screening research. The Bottle Gourd content collected, viz. Green gold, Anmol F1, Royal, Bemisal, Advanta -205, Broad Punjab, Long Punjab, Surbhi, King, and Hazarvi were screened against *Alternaria* leaf blight resistant. The seed of each variety / genotype was sown under field conditions. Of the 10 genotypes, none were found to be highly resistant to this condition. Green gold, Anmol F1 and Bemisal exhibited moderately resistant responce. While Hazarvi showed a moderate susceptibility to Surbhi. Additionally, two varieties Punjab large or Punjab long showed highly susceptible compared to three varieties king, advanta-205 and royal.

Carmody *et al*., (1985) who screened muskmelon cultivars and recorded resistant cultivars against *Alternaria* leaf blight (TAM-Mayan Sweet, TAM-Uvalde, and Greenflesh Honeydew). Our findings are similar to Chauhan and Bhatia (2013) who screened 22 bottle gourd germplasm lines / cultivars for resistance and found that lines like GH 3 and GH 9 display *Alternaria* resistance. Cultivation of resistant or tolerant cultivars is one of the best choices for reducing the losses caused by disease. Green gold, Bemisal, and Anmol-F1 are best against *A. cucumerina*, and for better growth as well as bottle gourd production. This disease may reach an alarming status and may wreak havoc in growing areas of bottle gourd unless care is taken well in time. Therefore, identifying resistant varieties for an effective management strategy against this dreaded crop disease needs the hour.

Plant extracts are preferred because chemical compounds have direct or indirect effects on human health by destroying the crop and polluting the atmosphere. Although plant extracts are environmentally friendly, cost-effective and less harmful compared to high-concentration fungicides that cause phytotoxicity. Therefore, plant extracts are environmentally friendly. Therefore, the present experiment was conducted to find out the efficacy of natural phytoextracts.

Results indicated that treatment combinations of *Allium cepa, Azadirachta indica and Zingiber officinale* were effective by combination of *Zingiber officinale* and *Azadirachta indica.*

Individuals extracts of *Azadirachta indica*, *Allium cepa* and *zingiber officcinale* also showed promising results.

Our results were similar to those of Obongoya *et al*., (2010), who also treated seventeen extracts to assess their impact on many diseases, and it was found that extract obtained from neem was significantly effective against alternative diseases due to the presence of an antifungal compound that reduced micro-conidial germination. *A. indica* (neem) extracts (Santos *et al.,* 2005) which prevent spore germination of *A. cucumerina*.

Azadirachta indica, and *Zingiber officinale* respectively showed the best inhibitory effects since these extracts contain several anti-fungal activities against bottle gourd *Alternaria* leaf blight. *Azadirachta indica* shows therapeutic role due to the rich source of antioxidant and other valuable active compounds such as azadirachtin, nimbolinin, nimbin, and quercetin. Neem plants parts shows antimicrobial role through inhibitory effect on microbial growth/potentiality of cell wall breakdown. Azadirachtin, a complex tetranortriterpenoid limonoid present in seeds, is the key constituent responsible for both antifeedant and toxic effects in insects. (Nisbet et al., 2002). Ginger is abundant in active constituents, such as phenolic and terpene compounds. Ginger rhizome contains several constituents which have antibacterial and anti fungal effects. The gingerol and shagelol are identified as more active agents to killing the fungus growth. (Nile et al., 2015).

These naturally occurring ingredients are less phytotoxic, safe, ecofriendly, easily biodegradable, and translocated in plants with adequate concentrations. Comprehensive biochemical and molecular biology studies of plants for the detection of metabolites, enzymes and other active ingredients are required to investigate the hidden features of medicinal plants against *Alternaria* bottle gourd leaf blight.

CHAPTER 5

SUMMARY

Bottle gourd (*Lagenaria siceraria* L.) is a gourd of white colored flowers, also known as Cucurbitaceous family climbing vine. It is the largest group of vegetables from summer season, of which more than 20 are commercially grown in India. It is an important vegetable from home garden and occupies a unique place among cucurbits in Pakistan. Bottle gourd is one of the excellent vegetables that nature gives to humans having composition of all the essential components required for good health and quality of human life. It is an important plant that can be cultivated in tropical and subtropical countries.

It is one of the first plant species to be domesticated for human use, providing food, medicine and a wide variety of tools and utensils made from the large, hard-shelled fruit. The fruits have medicinal values and are used as cardiotonic, aphrodisiac, hepatoprotective, analgesic, anti-inflammatory, expectorant, diuretic and antioxidant agents. *Lagenaria* seeds have a high linoleic acid content and sterolic compounds.

Bottle gourd is attacked by a number of diseases such as *Alternaria* leaf blight caused by *Alternaria cucumerina. Alternaria* leaf blight is found primarily on watermelon and muskmelon, but mostly occur on squash cucumber, gourds, and pumpkin can occur. This disease has an effect on foliage and rarely on fruit. It affects mostly older plants, while seedlings may also be infected.

Symptoms of *Alternaria cucumerina* disease are irregular, circular dark brown leaf spot colors on the leaves, and contain concentrated rings in the leaf spot as well. *Alternaria cucumerina* culture microscopic observation shows that conidiophores cover widths from obclarate to pyriform. *Alternaria cucumerina* is a worldwide occurrence fungus that can infect most cultivated cucurbits. The disease first appeared in the field just after the emergence of seedlings. On the leaves there are scant, scattered, water-soaked, yellowish and circular to oval shaped spots. Subsequently, these spots are turned into brownish in color surrounded by chlorotic area with typical concentrated rings, which coalesced to form large irregular patches on leaves showing blighted look that can result in leaves being withered and shedded. Disease-induced economic yield losses are 20 to 60 percent.

This experiment was carried out during May to September 2019 in the field conditions of the Department of Plant Pathology, Faisalabad University of Agriculture. Use of resistant varieties is successful management strategy. Ten varieties of bottle gourd against *Alternaria* leaf blight were assessed for this purpose. Three varieties showed moderately resistant response Green Gold, Bemisal, and Anmol F1. So they used in this experiment. The experimental study was conducted with three Replications (R1, R2 and R3) within a Randomized Complete Block Design (RCBD). Disease severity data were recorded after sowing at an interval of 7 days, and analyzed the data.

Plant extracts (*Azadirachta indica, Zingiber officinales,* and *Allium cepa* and their different concentrations were evaluated under field conditions for management of *Alternaria* leaf blight of bottle gourd. Among these treatments, *Allium cepa, Azadirachta indica* and *Zingiber officinale* expressed maximum reduction in *A. cucumerina* colony growth. All treatments expressed significant results but minimal disease severity were expressed using combination of three concentrations as compared to other treatments. Plant extracts were used in place of fungicides, because fungicides are harmful to health and harmful to the environment. *Alternaria cucumerina* is a disease pathogen present within the environment and caused *Alternaria* disease in vegetables. All analysis and graphs from the ANOVA table showed that disease severity decreased as a result of spraying of plant extract concentrations and crop yield also increased with the use of these treatments. In Bottle gourd these plant extracts were better at curing diseases.

REFERENCES

Abd-El-Khair, H., and W.M. Haggag. 2007. Application of some Egyptian medicinal plant extracts against potato late and early blights. Res J Agric Biol Sci. 3: 166-175.

Abo-El- Seoud, M.A., M.M. Sarhan, A.E. Omar and M.M. Helal. 2005. Bioside formulation of essential oils having antimicrobial activity. Archiv. Phytopathol. Pl. Protec. 38: 175-184.

Agarwal, A., R. Prajapati, S.K Raza and L.K Thakur. 2017. GC-MS Analysis and Antibacterial Activity of Aerial Parts of *Quisqualis indica* Plant Extracts. Int. J Pharm Sci Rev Res. 51: 329-36.

Ahamad S., and U. Narain. 2000. *Alternaria* diseases of vegetable crops and its management control to reduce the low production. Ann. Pl. Protec. Sci. 8: 114-115.

Akila R, L. Rajendran, S. Harish, k. Saveetha, T. Raguchander and R. Samiyappan. 2011. Combined application of botanical formulations and biocontrol agents for the management of *Fusariumoxysporum* f. sp. cubense causing *Fusarium* wilt in banana. Biol. Control. 57: 175-183.

Amadioha AC. 2003. Evaluation of some plant leaf extracts against *Colletotrichum lindemuthianumin* cowpea. Acta - Phytopathologica-et-Entomologca – Hungarica.38: 259-265.

A Ching., W.T. Yu, S.H. Jen and K.W. Hsiung. 2007. Effect of oriental medicinal plant extracts on spore germination of *Alternaria brassicicola* and nature of inhibitory substances from speed weed. Plant Dis. 91: 1621-1624.

Babu S., K. Seetharaman, R. Nandakumar and I. Johnson. 2000. Effect of selected plant extracts/oils against tomato leaf blight. Int J. Trop Agric. 18: 153-157.

Badadoost M. Downy mildew of cucurbits. 2001. University of Illinois Ag. Services. 13: 345-365.

Battilani, P., L.G, Costa, A, Dossena, A., M.L Gullino, R, Marchelli, R., G, Galaverna, A. Pietri, C, Dall'Asta, P, Giorni, D Spadaro and A, Gualla. A. 2009. Scientific information on mycotoxins and natural plant toxicants, Scientific/Technical report submitted to EFSA. 3: 810-814.

Begum, H.A. and A. Momin. 2000. Comparison between two detection techniques of seed-borne pathogens in cucurbits in Bangladesh. Pak J. Sci. and Inds. Res. 43: 244-248.

Bhardwaj S.K. 1991. Evaluation of Plant Extracts as Antifungal Agents against *Fusariumsolani* (Mart.) Sacc. World J. Agric sci. 8: 385-388.

Bhatt JC, A. Gahlain and SK Pant. 2000. Record of *Alternaria alternata* on tomato, capsicum and spinach in Kumaon hills. Indian Phytopathol. 53: 495-496.

Borkar H M., and P. Umaharan. 2007. Identification of resistance to *Cercospora* leaf spot of cowpea. Eur J. Plant Pathol. 118: 401-410.

Boughalleb, N., J. Armengol and M. El Mahjoub. 2005. Detection of races 1 and 2 of *Fusarium solani f.*sp. *Cucurbitae* and their distribution in watermelon fields in Tunisia. J. Phytopat. 153: 162-168.

Bourgaud, F., A. Gravot, S. Milesi and E. Gontier. 2001. Production of plant secondary metabolites: a historical perspective. Pl. Sci. 161: 839-851.

Bruce A. Fact Sheets *Alternaria* Leaf Blight of Cucurbits. 2013. Extension Plant Pathologist, (extension.umaine.edu.) accessed. Eur J. Plant Pathol. 56: 101-105.

Burtram C. F, L. K, Cindy, A. V Filicity and A. K Jeremy. 2015. Testing of Eight Medicinal Plant Extracts in Combination with Kresoxim Methyl for Integrated Control of *Botrytis cinerea* in Apples. Pl. Sci. 5: 400-411.

Cavigelli, M.A., J.R. Teasdale and A.E. Conklin. 2008. Long-term agronomic performance of organic and conventional field crops in the mid-Atlantic region. Agron. J. 100: 785–794.

Chattopadhyay, A. K. and C. K. Bhunia. 2003. Management of *Alternaria* leaf blight of rapeseed-mustard by chemicals. J. Mycopathol Res. 41: 181-183.

Chauhan R S and J.N Bhatia. 2013. Screening of bottle gourd genotypes against anthracnose disease under natural as well as artificial epiphytotic conditions. Plant Dis Res.28: 92-3.

Ching, H.W., W.T. Yu, S.H. Jen and K.W. Hsiung. 2007. Effect of oriental medicinal plant extracts on spore germination of *Alternaria brassicicola* and nature of inhibitory substances from speed weed. Plant Dis. 91: 1621-1624.

Choi, J.H, E., Brummer and D.A. Stevens. 2004. Combined action of micafungin, a new echinocandin, and human phagocytes for antifungal activity against *Aspergillus fumigatus*. Microbes Infect. 6: 383-389.

Carmody B E, Miller M E and Grisham M P. 1985. A technique to screen muskmelons for resistance to *Alternaria* leaf blight. Plant Dis. 69: 426-428.

Choudhary, B.R., R.S. Dhaka and M.S. Fageria. 2002. A textbook on production technology of vegetables, Kalyani Publishers, New Delhi.

Choulwar, A.B. and U.V. Datar. 1992. Cost linked spray scheduling for the management of tomato early blight. Indian Phytopathol. 41: 603-606.

Clark, A.C., M.K., Burtenshaw, P.A., McLenachan, D.A Erickson and D, Penny. 2006. Reconstructing the origins and dispersal of the polynesian bottle gourd (*Lagenaria siceraria*). Molecular Biology and Evolution. 23: 893-900.

Conn K.L. and Tewari J.P. 1990. *Alternaria* disease of vegetable crops and its management control to reduce the low productivity. Plant Dis. Survey. 70: 66-67.

Crous PW, GJM Verkley, JZ Groenewald, RA Samson (eds). 2009. Fungal Biodiversity. CBS laboratory Manual Series 1. CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands. 6: 9-14.

Crous PW, W, Gam, JA Stalpers, V Robert, G Stegehuis. 2004. MycoBank: an online initiative to launch mycology into the 21st century. Stud. Mycol. 50: 19-22.

Dass Anchal, N., K. Lenka, U.S. Patnaik and S. Sudhishi. 2008. Integrated nutrient management for production, economics, and soil improvement in winter vegetables. Int. J. Veg. Sci. 14: 104-120.

Datar, V. V. 1996. Efficacy of growth regulators and fungitoxicants on fruit rot of chilli. Indian J. Mycol. Plant Pathol. 26: 239-242.

Deba, F., T.D., Xuan, M., Yasuda, S., Tawata. 2008. Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from Bidenspilosa Linn. var. Radiata. Food Control. 19: 346-352.

Debjani C, R.A. Yumlembam, K. Susamoy, N. Ranjan, K.K. Ramen and S. Jayanta. 2017. Effect of plant extracts against sheath blight of rice caused by *Rhizoctonia solani*. J. Pharmacogn. Phytochem. 6: 399-404.

Decker-Walters, D.S., J.E, Staub, A. Lo´pez-Se´se and E. Nakata. 2001. Diversity in landraces and cultivars of bottle gourd (*Lagenaria siceraria*; *Cucurbitaceae*) as assessed by random amplified polymorphic DNA. Genetic Resource and Crop Evolution. 48: 369-380.

Decker-Walters, D.S., M.W. Ellert, S.M. Chung, J.E. and Staub. 2004. Discovery and genetic assessment of wild bottle Gourd [*Lagenaria siceraria* (Mol.) Stand; *Cucurbitaceae*] from Zimbabwe. Econ Bot. 58: 501-508.

Derbalah, A.S., M.S. El-Mahrouk and A.B. El-Sayed. 2011. Efficacy and safety of some plant extracts against tomato early blight disease caused by *Alternaria solani.* Plant Pathol. J. 10: 115-121.

Deshpande, J.R., A.A. Choudary, M.R. Mishra, V.S. Meghre, S.G. Wadodkar and A.K. Dorle. 2008. Beneficial effects of *Lagenaria siceraria* (Mol.) Stand. Fruit epicarp in animal models. Indian J. Exp. Biol. 46: 234-42.

Deshwal K. 2004 Taxonomy and parasitism of *Alternaria* species associated with *Solanaceous* hosts. M.Sc. (Ag.) Thesis, C.S.A. Univ. Agric. And Technol.11: 31-36.

Doltsinis, S.K., E., Markellou, A.M., Kasselaki, M.N.F, anouraki and C.M. Koumaki. 2006. Efficacy of milsana, a formulated plant extract from *Reynoutriasachalinensis*, against powdery mildew of tomato (*Leveillulataurica*). Biolcontrol. 51: 375-392.

Dubuis, P.H., C. Marazzi, E. Stadler and F. Mauch. 2005. Sulphur deficiency causes a reduction in antimicrobial potential and leads to increased disease susceptibility of oilseed rape. J. Phytopathol. 153: 27-36.

Egel, D. S., and P. Harmon. 2001. Effects of nozzle type and spray pressure on control of *alternaria* leaf blight of muskmelon with chlorothalonil. Plant Dis. 85: 1081-1084.

Ellis. M. B. 1971. *Demataceous hyphomycetes*. CAB International, Wallingford, Oxon, England. 608-612.

Emina, M., J. Berenji, V. Ognjanov, M. Ljubojeviæ and J. Èukanoviæ. 2012. Genetic variability of bottle gourd *Lagenaria siceraria* (Mol.) Standley and its morphological characterization by multivariate analysis. Arch. Biol. Sci. 64: 573-583.

Enonch, G., A. Dako, J. Fuchs, A. Ahanchede and F.R. Blattner. 2008. Flow cytometric analysis in *Lagenaria siceraria* (*Cucurbitaceae*) indicates correlation of genome size with usage types and growing elevation. Plant Syst. Evol. 276: 9-19.

Erickson, D.L., B.D. Smith, A.C. Clarke, D.H. Sandweiss and N. Tuross. 2005. An Asian origin for a 10,000-year-old domesticated plant in the Americas. Proceeding of National Academic Science. 102: 315-318.

FAO, F. 2018. Agriculture Organization. 2018. Livestock Primary. Food and Agriculture Organization of the United Nations.

Fawzi E.M, A.A. Khalil and A.F Afifi. 2009. Antifungal effect of some plant extracts on *Alternaria* alternate and *Fusariumoxysporum*. Afr. J. Biotechnol. 2: 2590-2597.

Feng W and Zheng X. 2006. Control of *Alternaria alternata* by cassia oil in combination with potassium chloride or sodium chloride. J. Appl. Microbiol. 101: 1317-1322.

Garcia-Mas, J., A. Benjak and Sanseverino. 2012. The genome of melon (*Cucumis melo* L.) USA. Proc. Natl Acad. Sci. 109: 11872-11877.

Ghule, B.V., M.H. Ghanti, A.N. Saoji and P.G. Yeole. 2007. Anti-hyperlipidemia effects of metabolite extract from *Lagenaria siceraria* stand. Fruit in hyperlipidemic rats. J. Ethnopharmacol. 124: 333-337.

Ghule, B.V., M.H. Ghanti, P.G. Yeole and A.N Saoji. 2009. Diuretic activity of *Lagenaria siceraria* fruit extracts in rats. Indian J. Pharm. Sci. 69: 817-819.

Gomez-Rodriguez O., E. Zavaleta-Mejia , M. Livera-Munoz and E. Cardenas-Soriano. 2003. Field Crops Res. 83: 27-34.

Gondal, A., M. Ijaz, K. Riaz and A. Khan. 2012. Effect of different doses of fungicide (Mancozeb) against *Alternaria* leaf blight of tomato in tunnel. J. Plant Pathol. Microbiol. 3: 2-7.

Green, R.C. 2000. A range of disciplines support a dual origin for the bottle gourd in the Pacific. J. Polyn Soc. 109: 191-197.

Green, R.C. 2005. Sweet potato transfers in Polynesian prehistory. In: The sweet potato in Oceania: a reappraisal (Eds. Ballard, C., Brown, P., Bourke, R.M. and Harwood, T.), Oceania Publications, University of Sydney, Sydney, Australia. 18: 43-62.

Hadizadeh, I., B. Pivastegan and H. Hamzehzarghani. 2009. Antifungal activity of essential oils from some medicinal plants of Iran against *Alternaria alternata*. Am. J. Applied Sci. 6: 857-861.

Han, J.S., C.K. Kim, S.H. Park, K.D. Hirschi and I.G. Mok. 2005. Agrobacterium-mediated transformation of bottle gourd (*Lagenaria siceraria* Standl.) Plant Cell Rep. 23: 692-698.

Hanif, R., Z. Iqbal, M. Iqbal, S. Hanif and M. Rasheed. 2006. Use of vegetables as nutritional food: role in human health. J. Agric Biol Sci. 1: 18-22.

Haque, M.M., M. Hasanuzzaman and M.L. Rahman. 2009. Effect of light intensity on the morpho-physiology and yield of bottle gourd. Acad. J. Plant Sci. 2: 158-161.

Harika, M., V.D. Gasti, T. Shantappa, R. Mulge, A.M. Shirol, A.B. Mastiholi and M.S. Kulkarni. 2012. Evaluation of bottle gourd genotypes [*Lagenaria siceraria* (Mol.) Standl.] for various horticultural characters. Karnataka J. Agr. Sci. 2: 18-23.

Hassane, N.M., M.A. AbouZeid, I.F. Youssef and D.A. Mahmoud. 2008. Efficacy of leaf extracts of neem (*Azadirachtaindica*) and chinaberry (*Meliaazedarach*) against early blight and wilt diseases of tomato. Austr. J. Basic Applied Sci. 2: 763-772.

Hassanein, N.M., M.A. Abou Zeid, K.A. Youssef and D.A. Mahmood. 2010. Efficacy of leaf extracts of Neem (*Azadirachta indica*) and Chinaberry (*Melia azedarach*) against early blight and wilt diseases of tomato. Aust. J. Basic Appl. Sci. 3: 763-77.

Hegazy, E. M., and O.S. El Kinawy. 2011. Characteristics, of pumpkin and bottle gourd in Egypt and potentially their contamination by Mycotoxins and Myco-toxigenic fungi. Int. J. Agric. Biol. Sci. 1: 18-22.

Howlett BJ. 2006. Secondary metabolite toxins and nutrition of plant pathogenic fungi. Curr. Opin. Plant Biol. 9: 371-375.

Jadhav V M R and B L, Sharma. 1983. Field reaction of mungbean (*Vigna radiata* L.) Wilczek varities to *Cercospora* leaf spot in north Madhya Pradesh. Legume Res. 6: 99-100.

Jantasorn. A, M. Boontida and D. Tida. 2016.In vitro antifungal activity evaluation of five plant extracts against five plant pathogenic fungi causing rice and economic crop diseases. J. Biopestic. 9: 01-07.

Johnson RD, L. Johnson, Y. Itoh, M. Kodama, H. Otani and K. Kahmoto. 2000. Cloning and characterization of a cyclic peptide synthetase gene from *Alternaria alternata* apple pathotype whose product is involved in AM-toxin synthesis and pathogenicity. Mol. Plant-Microbe Interac. 13: 742-753.

Jones, T.L. and K.A. Klar. 2005. Diffusionism reconsidered: Linguistic and archaeological evidence for prehistoric Polynesian contact with southern California. American Antiquity. 70: 457-484.

Joshi, D.P. and S.K.S, Gour. 1971. Floral biology studies of *Lagenaria siceraria* Standl. (Bottle gourd). J. Res. PAU. 8: 420-426.

Javadian, F., Z., Sepehri, S., Saeidi, M. and Hassanshahian. 2014. Antifungal effects of the extract of the *Withania somnifera* on Candida albicans. Adv.Herb.Med. 2: 31-37.

Katiyar, A., S., Kant, S.S. Chauhan and S. Alka. 2001. Sources of resistance in bottle gourd to *Alternaria* leaf spot. Ann. Plant Protec. Sci. 9: 155-157.

Kausik, B., I. Chattopadhyey, R.K. Benerjee and U. Bandyopdyey. 2002. Biological activities and medicinal properties of neem. Curr. Sci. 82: 1336-1344.

Khalid A., Akram Mohd, U. Narain and M. Srivastava. 2004. Characterization of species and parasitism in the genus *Alternaria*. Farm Sci. J. 13: 195-196.

Kimber R.B. E and J.G Paul. 2011. Identification and genetics of resistance to *Cercospora* leaf spot (*Cercospora zonata*) in germplasm of faba beans (*Vicia faba*). Euphytica. 117: 419-29.

Kirk PM, PF. Cannon, DW Minter and JA. Stalpers. 2008. Dictionary of the Fungi. 10th edition Wallingford, CABI. 6: 19-22.

Kirk WW, DS Flecher, JM Douchs, KM Coombs and R, Hammerschmidt. 2001. Effect of host plant resistance and reduced rates and frequency of fungicides application to control potato late blight. Plant Dis. 85: 1113-1118.

Koike, S.T., P. Gladder and A.O. Paulus. 2007. Vegetable diseases-a colour handbook. London, UK. Manson Publishing Ltd. Pp. 5: 220-228.

Köppen, R., M., Koch, D., Siegel, S., Merkel, R. Maul and I. Nehls. 2010. Determination of mycotoxins in foods: current state of analytical methods and limitations. Appl. Microbiol. Biotechnol. 86: 1595-1612.

Kuepper G. Downy mildew control in cucurbits. 2003. National Center for Appropriate Technology through a grant from the Rural Business-Cooperative Service, U.S. Department of Agriculture. 4: 1-6.

Kumar A, Srivastava and HL Thakur. 2004. Evaluation of some plant extracts against *A. brassicae* (Berk.). J. Oi l seeds. Res. 15: 198-199.

Kumar, R., A.K., Mishra, N.K., Dubey and Y.B., Tripathi. 2007. Evaluation of Chenopodiuambrosioides oil as a potential source of antifungal, antiaflatoxigenic and antioxidant activity. Int. J. Food Microbiol. 115: 159-164.

Lakhdar Belabid, LeilaSimoussa and BassamBayaa. 2010. Effect of some plant extracts on the population of *Fusarium oxysporum* f.sp. lentis, the causal organism of lentil wilt. Adva. Environ. Biol. 4: 95-100.

Latha, P., T., N., Anand, V. Ragupathi, R. Prakasam and Samiyappan. 2009. Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against *Alternaria solani*. Biol Control. 50: 85-93.

Levi, A., J., Thies, K., Ling, A.K., Simmons, C. Kousik and R. Hassell. 2009. Genetic diversity among *Lagenaria siceraria* accessions containing resistance to root-knot nematodes, whiteflies, ZYMV or powdery mildew. Plant Genet. Resour. 7: 216-226.

Levi, A., K. Ling and A.R. Davis. 2008. Est-SSRs of watermelon (*Citrullus* sp.) useful in assessing genetic diversity among *Lagenaria siceraria* accessions. Plant and Animal Genome XVI Conference Proceedings. Biol Control. 6: 64-63.

Leksomboon, C., N. Thaveechai and W. Kositratana. 2001. Effect of Thai medicinal plant extracts on growth of phytopathogenic bacteria. In: Proc. 36th Kasetsart Univ., Ann. Conf., Plant Sec., February, 3-5, 1998, Kasetsart Univ., Bangkok. 7: 379-398.

Magro, A., M. Carolino, M. Bastos and A. Mexia. 2006. Efficacy of plant extracts against stored products fungi. RevistaI beroamericana de Micología. Plant Genet. Resour. 23: 176-178.

Mahapatra, S., and S. Das. 2013. Bioefficacy of botanicals against *Alternaria* leaf blight of mustard under field condition. *The Bioscan*, 8: 675-679.

Mahesh, B., and Satish. 2008. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World J. Agric. Res. 4: 839-843.

Maheshwari, S. K., B.R., Choudhary, B.D., Sharma and P.L. Saroj. 2017. Management of *Alternaria* Leaf Blight of Bottle Gourd in Western Rajasthan, India. Int J. Curr. Microbiol. Appl. Sci. 6: 1272-1277.

Maheshwari, S. K., B.R., Choudhary, D., Singh, B.D., Sharma and S.K. Sharma. 2015. Evaluation of resistance in different varieties/genotypes of bottle gourd (*Lagenaria siceraria*) against *Cercospora* leaf spot under field conditions.17: 56-63.

Maigandi, S.A. and F.I. Ngang. 2002. Performance of rabbits fed varying levels of calabash seed cake. J. Agric. Environ. 3: 201-208.

Malkhan, S.G., A. Shahid, A. Masood and S. Kangabam. 2012. Efficacy of plant extracts in plant disease management. Agric. Sci. 7: 425-433.

Mark J H, M M, Henry and M J. Moly. 2005. Hannahs Choice F1: a new muskmelon hybrid with resistant to powdery mildew, *Fusarium* race 2 and potyviruses. Hort Sci. 40: 492-310.

Masunaka A, K Ohtani, TL Peever, LW Timmer, T Tsuge and M Yamamoto. 2005. An isolate of *Alternaria alternate* that is pathogenic to both tangerines and rough lemon and produces two host selective toxins, ACT and ACR toxins. Phytopathol. 95: 241-247.

Matchima. R, and Ampai. 2009. Preliminary study on antimicrobial activity of crude extracts of pomelo albedo against Colletotrichum gloeosporioides. J. Food Ag-Ind. 11: 138-142.

Mate GD, VV. Deshmukh, DJ. Jiotode, NS. Chore and M. Dikkar. 2005. Efficacy of plant products and fungicides on tomato early blight caused by *Alternaria solani*. Res Crops 6: 349-351.

Matharu B.K., J.R. Sharma and M.R. Manrao. 2006. Synthesis and antifungal potential of 2-chlorobenzal derivatives. Pesticide Res. J. 18: 113-115.

Matic, J., A. Mandić, J. Mastilović, A. Mišan, B. Beljkaš and I. Milovanović. 2008. Contaminations of raw materials and food products with mycotoxins in Serbia. Food Processing, Quality and Safety. 35: 65-7.

Meena PD, C. Chattopadhyay, F. Singh, B. Singh and A. Gupta. 2002. Yield loss in Indian mustard due to white rust and effect of some cultural practices on *Alternaria* blight and white rust severity. Brassica. 4: 18-24.

Meena PD, RP. Awasthi, C. Chattopadhyay, SJ. Kolte and A. Kumar. 2010. *Alternaria* blight: a chronic disease in rapeseed mustard. J. of Oilseed Brassica. 1: 1-11.

Mei, Z.Z., C. Rong, C. Ronge, X. Lin, L. Song, G.Z. Yong, J. Xia, W. Lin and L.P. Cheng. 2007. Synthesis and antifungal properties of sulfonilamide derivatives of chitosan. Carbohydrate Res. 342: 2390-2395.

Mekuria, T., U., Steiner, H., Hindorf, J.P, Frahm and H-W. Dehne. 2005**.** J Appl Bot Food Qual. 79: 89-92.

Milind, P., and k. Satbir. 2011. Is bottle gourd a natural guard. Int. Res. J. Pharm. 2: 13-17.

Mohan, R., R., Birari, A., Karmase, S. Jag tap and K.K. Bhutani. 2012. Antioxidant activity of a new phenolic glycoside from *Lagenaria siceraria* Stand. Food Chemistry, 132: 244–251.

Mohana DC, KA. Raveesha, 2007. Anti-fungal evaluation of some plant extracts against some plant pathogenic field and storage fungi. Journal of Agricultural Technology. 4: 119-137.

Morimoto, Y., P., Maundu, M., Kawase, H. Fujimaki and H. Morishima. 2004. RAPD polymorphism of the white-flowered gourd (*Lagenaria siceraria* (Molina) Stand L). Landraces and its Wild Relatives in Kenya. Genet Resour Crop Ev. 53: 963-974.

Mukhtar, I., S., Mushtaq, I., Khokhar and A. Hannan. 2013. First record of *cercospora citrullina* leaf spot on lagenaria siceraria in Pakistan. Phytopathol. 12: 73-76.

Marraiki, N. N., I., Siddiqui, H., Rizwana and A. Javaid. 2012. First report of *Alternaria alternata* leaf spots on spinach in Saudi Arabia. J. Anim. Plant Sci. 22: 247-248.

Namanda S, OM. Olanya, E. Adipala, JJ. Hakiza and RE. Bedewy .2004. Fungicide application and host resistance for potato late blight management: benefits assessment from onfarm studies in S.W. Uganda. Crop Protect. 23: 1075-1083.

Nashwa SM, KA. Abo-Elyousr. 2012. Evaluation of various plant extracts against the early blight disease of tomato plants under greenhouse and field conditions. Plant Prot Sci. 48: 74-79.

Neeraj and S. Verma. 2010. *Alternaria* diseases of vegetable crops and new approaches for its control. Asian J. Exp. Biol. Sci. 1: 61-692.

Nile, S. H., S.W and Park. 2015. Chromatographic analysis, antioxidant, anti-inflammatory, and xanthine oxidase inhibitory activities of ginger extracts and its reference compounds. Indust. Crops. Prod. 70: 238-244.

Nisbet, A. J. 2002. Azadirachtin from the neem tree Azadirachta indica: its action against insects. Anais da Sociedade Entomológica do Brasil. 29: 615-632.

Nguefack J, O. Tamgue, JBL. Dongmo, CD. Dakole, V. Leth, HF. Vismer, PH. AmvamZollo and AE. Nkengfack. 2012. Synergistic action between fractions of essential oils from Cymbopogoncitratus, Ocimumgratissimum and Thymus vulgaris against Penicilliumexpansum. Food Control. 23: 377-383.

Ogunbusola, M.E., T.N. Fagbemi and O.F. Osundahunsi. 2010. Amino acid composition of *Lagenaria siceraria* seed flour and protein fractions. J. Food Sci.Technol. 47: 656-661.

Obongoya, B. O., S. O., Wagai and G. Odhiambo. 2010. Phytotoxic effect of selected crude plant extracts on soil-borne fungi of common bean. Afr. Crop Sci. J. 18: 1-4.

Ostry, V. 2008. *Alternaria* mycotoxins: an overview of chemical characterization, producers, toxicity, analysis and occurrence in foodstuffs, World Mycotoxin J. 1: 175-188.

Pasche, J. S., C.M. Wharam and N.C. Gudmestad. 2004: Shift in sensitivity of *Alternaria solani* in response to QoI fungicides. Plant Dis. 88: 181-187.

Pati PK, M. Sharma, RK. Salar, A. Sharma, AP. Gupta and B. Singh. 2008. Studies on leaf spot disease of *Withania somnifera* and its impact on secondary metabolites. Indian J. Microbiol. 48: 432-437.

Patil, M.J, S.P. Ukey and B.T. Raut. 2001. Evaluation of fungicides and botanicals for the management of early blight (*Alternaria solani*) of tomato. PKV-Res. J. 25: 49-51.

Paul O. Okemob, P.B. Harsh, M. Jorge and Vivancoa. 2003.In vitro activities of Maesalanceolata extracts against fungal plant pathogens. Fitoterapia. 74: 312-316.

Pawar, R. R., R. T., Sapkal and K. B. Pawar. 2014. Epidemiology, Symptomatology and Management of the Fruit Rot of Bottle Gourd (*Lagenaria siceraria* Standl.) Caused by *Alternaria alternata* (Fr.) Keissler. The Bioscan. 9: 363-370.

Ping, W.L., W.U. Xiao-hua, B.G. Wang, X.U. Pei and L.I. Guo-jing. 2011. SCAR marker linked to resistance gene of powdery mildew in bottle gourd [*Lagenaria siceraria* (Molina) Standl.] breeding line J083. J. Zhejiang Univ.37: 119–124.

Prajapati, V. P., N.K. Gajre, D.H. Tandel, A.J. Deshmukh and R.C. Patel. 2017. Evaluation of Phyto-extracts against *Curvularia Eragrostidis* Causing Leaf Tip Blight of Spider Lilly in Vitro Condition. Int. J. Econ. Plants. 4: 119-120.

Prasad Y and MK. Naik. 2003. Evaluation of genotypes, fungicides and plant extracts against early blight of tomato caused by *Alternaria solani*. Indian J. Plant Protec. 31: 49-53.

Prasad Y. and M.K. Naik. 2003. Evaluation of genotypes, fungicides and plant extracts against early blight of tomato caused by *Alternaria solani*. Indian J. Plant Protec. 31: 49-53.

Prasada, R., G.L. Khandelwal and J.P. Jain, 1973. Epidemiology, forecasting and control of *Alternaria* blight of cucurbits. P. Natl. Sci. Acad. 37: 301-308.

Puri, R., R. Sud, A. Khaliq, M. Kumar and S. Jain. 2011. Gastrointestinal toxicity due to bitter bottle gourd (*Lagenaria siceraria*) a report of 15 cases. Indian J. Gastroenterol. 30: 233-236.

Rai N, DS. Yadav. 2005. Advances in vegetable production. Researchco Publishing, New Delhi. 4: 325-337.

Razzaghi-Abyaneh, Shams-Ghahfarokhi, T. Yoshinari, Rezaee, K. Jaimand, H. Nagasawa and S. Sakuda. 2008. Inhibitory effects of *Saturejahortensis* L. essential oil on growth and aflatoxin production by Aspergillusparasiticus. Int J Food Microbiol. 123: 228-330.

Rodríguez, D. Jasso de, D. Hernández-Castillo, R. Rodríguez-García, and J L. Angulo-Sanchez. 2005. Antifungal activity in vitro of Aloe verapulp and liquid fraction against plant pathogenic fungi. Ind. Crops Prod. 21: 81-87.

Sahraei, S., Z. Mohkami, F. Golshani, F. Javadian, S. Saeidi and G.S. Baigi. 2014. Antibacterial activity of five medicinal plant extracts against some human bacteria. Eur. J. Exp. Biol. 4: 194-196.

Santos GR, MD. Castro Neto, LN. Ramos, AC. Café-Filho, A. Reis, VG. 2005. melon genotypes to the gummy stem blight and the downy mildew. Hort. Bras. 27: 160-165.

Saharan, PR, Verma, GS. 1994. Monograph on *Alternaria* Diseases of crucifers. Agriculture and Agri-Food Canada. 4: 1-146.

Sarbhoy A K. 2006. Text Book of Mycology. IBH Publishing Ltd, New Delhi. 7: 242-249.

Sarkar, M. 2004. Characterization of the bottle gourd (*Lagenaria siceraria*) genotypes collected from Mediterranean region. Master Thesis, University of Mustafa Kemal, Institute of Natural and Applied Science, Hatay, Turkey. 81: 321-416.

Sarker, S., M. Haque, M. Islam, S. Dey and A. Biswas. 2017. Evaluation of different botanicals and biological agents to control *Cercospora* leaf spot of Indian spinach in field condition. JALSI. 13: 1-8.

Seshadri, V.S. and T.A. More. 2004. History and antiquity of cucurbits in India. In: Progress in Cucurbit Genetics and Breeding Research. Proceedings of the 8th EUCARPIA Meeting of Cucurbit Genetics and Breeding. 41: 81-90.

Sharma A, A. Dass and MS. Pau. 2007. Antifungal effect of neem extract on some common phytopathogenic fungi. Adv. Plant Sci. 20: 357-358.

Sheikh, R.A. and J.P. Agnihotri. 1997. Antifungal properties of some plant extracts. Ind. J. Mycol. Plant Pathol. 2: 143-146.

Sunita., M, and S, Das. 2013. Bioefficacy of botanicals against *Alternaria* leaf blight of mustard under field condition. The Bioscan. 8: 675-679.

Shirzadian, S., H.A. Azad and J. Khalghani. 2009. Introductional study of antifungal activities of bryophyte extracts. Iran. J. Pl. Pest Dis. 77: 1-22.

Shuping, D.S.S. and J.N. Eloff. 2017. The use of plants to protect plants and food against fungal pathogens: a review. Afr J. Tradit Complem. 14: 120-127.

Sidlauskiene, A., A. Rasinskiene and E. Surviliene. 2003. Effect of various protection means on *Alternaria* diseases of tomato, cucumber and cabbage seed plants. 22: 388-394.

Sinha, P.P. and R.K. Prasad. 1991. Evaluation of fungicides for control of early blight of tomato. Madras Agric. J. 78: 141-143.

Singh B, S K. Sanwal, Rai, Mathura and A B. Rai. 2009. Sources of biotic stress resistance in vegetable crops: a review. Veg. Sci. 36: 133-46.

Singh N.K., R.P., Saxena. S.P., Pathak and S.K.S., Chauhan. 2001. Management of *Alternaria* leaf spot disease of tomato. Ind. Phytopathol. 54: 501-508.

Singh P.C and D. Singh. 2006. In vitro evaluation of fungicides against *Alternaria alternata*. Ann. Plant Protec. Sci. 14: 500-502.

Singh R A and S N. Gurha. 2007. Stable sources of resistance to *Cercospora* leaf spot in mung bean. Ann. Plan. Protect. Sci. 15: 501-512.

Singh, A. and D. Singh. 2002. Efficacy of fungicides against *Alternaria* leaf spot of cabbage. Crop Res. 23: 192-193.

Singh, D.K., S. Padiyar, H. Choudhary, A. Tiwari and H.H. Ram. 2010. Utilization of novel of bottle gourd for identification of hybrid seed with RAPD marker. Acta Hort. 871: 491-498.

Singh, D.P., U.C. Mishra, H.G. Prakash and O. Mishra. 2012. Role of organic farming on yield and economics of bottle gourd after vegetable pea. Int. J. Agric Sci. 8: 165-167.

Singh, K. and M. Rai. 2003. Evaluation of chemicals against *Alternaria* leaf spot of brinjal. Ann. Plant Protec. Sci. 11: 394-395.

Singh, S.P., Maurya, I.B. and Singh, N.K. 1996. Occurrence of andro monoecious form in Bottle gourd (*Lagenaria siceraria*) exhibiting monogenic recessive inheritance. Curr. Sci. 70: 458-459.

Singh, V. 2015. *Alternaria* diseases of vegetable crops and its management control to reduce the low production. Int. J. Agri. Sci. 2: 2975-3710.

Sivaraj, N., and S.R. Pandravada. 2005. Morphological diversity for fruit characters in bottle gourd germplasm from tribal pockets of Telangana region of Andhra Pradesh, India. Asian Agrihist. 9: 305-310.

Smith, B.D. 2005. Reassessing coxcatlan cave and the early history of domesticated plants in Mesoamerica. Proceedings of the National Academy of Sciences. 102: 9438-9445.

Souza, E.L.d, E.d.O. Lima, K.R.d.L. Freire and C.P.d. Sousa. 2005. Inhibitory action of some essential oils and phytochemicals on the growth of various moulds isolated from foods. Brazi Arch. Biol.Technol. 48: 245-250.

Srinivas S.2006. Integrted management of powdery mildew of bottle gourd using compost teas, bioagents and systemic acquired resistance. Plant Dis. 11: 61-67.

Steel, R. 1997. Analysis of variance I: The one-way classification. Pri. Proced. Stat. Biometry Appr. 139-203.

Subbarao, K. V., Z. Kabir, F.N. Martin and S.T. Koike. 2007. Management of soil borne diseases in strawberry using vegetable rotations. Plant Dis. 91: 964-972.

Sultana, N. A. S. R. E. E. N. and Ghaffar. 2009. Seed-borne fungi associated with bottle gourd [*Lagenaria siceraria* (Mol.) Standl]. Pak. J. Bot. 41: 435-442.

Surender K.B. 2012. Evaluation of plant extracts as Antifungal Agents against *Fusarium solani* (Mart.) Sacc. World J. Agric. Res. 8: 385-388.

Sarwar, A., Z., Latif, S., Zhang, J., Hao and A. Bechthold. 2005. A Potential Biocontrol Agent Streptomycesviolaceusniger AC12AB for Managing Potato Common Scab. Front. microbiol. 4: 202-213.

Tahany MA, AK. Hegazy, AM. Sayed, HF. Kabiel, T. El-Alfy and SM. El-Komy. 2010: Study on combined antimicrobial activity of some biologically active constituents from wild Moringaperegrina Forssk. J. Yeast Fungal Res. 1: 15-24.

Tan, Y. C., J.S. Lai, J. K. R., Adhikari, S. M., Shakya, A. K., Shukla and K.R. Sharma. 2009. Efficacy of mulching, irrigation and nitrogen applications on bottle gourd and okra for yield improvement and crop diversification. Irrig. Drain. systems. 23: 25-41.

Thamburaj, S. and N. Singh. 2000. Textbook of Vegetables, Tuber crops and Spices. Published by DIPA, ICAR, and New Delhi. 12: 469-473.

Thomma, B. 2003. *Alternaria* spp. from general saprophyte to specific parasite. Mol. Plant Pathol. 4: 226-236.

Vadivel, S and E.G. Ebenezar. 2006. Eco-friendly management of leaf blight of tomato caused by *Alternaria solani*. J. Mycol. Plant Pathol. 36: 79-83.

Valkonen JPT and H. Koponen. 1990. The seed-borne fungi of Chinese cabbage (*Brassica pekinensis*), their pathogenicity and control. Plant Pathol. 39: 510-516.

Velluti, A., S. Marín, P. Gonzalez, A.J. Ramos and V. Sanchis. 2004. Initial screening for inhibitory activity of essential oils on growth of *Fusariumverticillioides*, *F. proliferatum* and *F. graminearum* on maize-based agar media. Food Microbiol. 64: 621-656.

Verma N. and S. Verma. 2010. *Alternaria* pathogenicity and its strategic controls. Asian J. Exp. Biol. Sci. 1: 681-692.

Verma, P. R., and G.S. Saharan. 1994. Saskatoon Research Station Technical Bulletin 1994-6E. Monograph on *Alternaria* diseases of crucifers. 31: 49-52.

Vilela, G.R., G.S., de Almeida, M.A.B.R., D'Arce, M.H.D., Morae's, J.O., Brito, M.D.d.G.F., da Silva, S.C., Silva, S.M., de Stefano Piedade, M.A., Calori-Domingues, E.M., da Gloria. 2009. Activity of essential oil and its major compound, 1,8-cineole, from Eucalyptus globulusLabill., against the storage fungi *Aspergillusflavus* Link and *Aspergillusparasiticus* Speare. J. Stored Prod. Res. 45: 108-111.

Vivekanand., U. 2018. Studies on disease of Bottle gourd, *[Lagenaria siceraria (Molina) standl]* and its management strategies (Doctoral dissertation, Indira Gandhi Krishi Vishwavidhyalaya, Raipur). Asian J. Exp. Biol. Sci. 4: 9-13.

Wagh, P., S. Sinha, H.K. Singh and U.K. Khar. 2013. Pathogenic behaviour of *Alternaria alternata* and phyto-toxicity of its culture filtrates on *Lepidium sativum*: a medicinal herb of immense pharmacological potential. The Bioscan. 8: 643-647.

Walker, J.C. 1952. Diseases of crucifers. In: Diseases of vegetable crops, McGraw Hill Book Co., New York, USA. 7: 150- 152.

Whistler, W.A. 1990. The other Polynesian gourd. Pacific Science. 44: 115-122.

Wightwick, A., R. Walters, G. Allinson, S. Reichman and N. Menzies. 2010. Environmental risks of fungicides used in horticultural production systems. Fungicides. 11: 273-304.

Wolpert TJ, LD Dunkle and LM. Ciuffetti. 2002. Host-selective toxins and avirulence determinants. Annual Review of Phytopathol. 40: 251-285

Xu, P., X. Wu, J. Luo, B. Wang, Y. Liu, J.D. Ehlers, S. Wang, Z. Lu and G. Li. 2011. Partial sequencing of the bottle gourd genome reveals markers useful for phylogenetic analysis and breeding. BMC Genom. 12: 467-477.

Yasmin. M., K.S. Hossain and M.A. Bashar. 2008. Effects of some angiospermic plant extracts on in vitro vegetative growth of *fusariumm oniliforme*. Bangladesh J. Bot. 37: 85-88.

Yetisir, H.M., M. Sakar and S. Serce. 2008. Collection and morphological characterization of *Lagenaria siceraria* germplasm from the Mediterranean region of Turkey. Genet. Resour. Crop Evol. 55: 1257-1266.

Yusuf.Y, K. Izzet. G. Ayhan. D. brahim, G. Nezhun, C. Halit and W. Mark. 2011. In vitro antifungal activities of 26 plant extracts on mycelial growth of Phytophthora infestans (Mont.) deBary. Afr. J. Biotechnol. 10: 2625-2629.

Zabka, M., R. Pavela and L. Slezakova. 2009. Antifungal effect of *Pimentadioica* essential oil against dangerous pathogenic and toxinogenic fungi. Ind Crops Prod. 30: 250-253.

Zhao, Y., K. Tu, X.F. Shao, W. Jing, W. Yang and Z.P. Su. 2008. Biological control of the post-harvest pathogens *Alternaria solani*, *Rhizopus stolonifer* and *Botrytis cinerea* on tomato fruit by Pichia guilliermondii. J. Hort. Sci. Biotech. 83: 132- 136.

Zitter, D.L., D.L. Hopkins and C.E. Thomas. 1996. Compendium of Cucurbit Diseases. APS Press, St. Paul, MN. pp. 4: 729-732.