Studies on the incidence and management of guava decline involving root-knot nematode and fungi

By
Madhu, M. R.
2015A25D

Thesis submitted to the Chaudhary Charan Singh Haryana Agricultural University in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

IN

NEMATOLOGY

DEPARTMENT OF NEMATOLOGY
CCS HARYANA AGRICULTURAL UNIVERSITY
HISAR-125 004, HARYANA, INDIA

2019

CERTIFICATE – I

This is to certify that thesis entitled, **"Studies on the incidence and management of guava decline involving root-knot nematode and fungi"** submitted for the degree of **Doctor of Philosophy** in the subject of Nematology to the Chaudhary Charan Singh Haryana Agricultural University, Hisar is a bonafide research work carried out by **Mr. Madhu, M. R.,** Admission No. **2015A25D** under my supervision and no part of this thesis has been submitted for any other degree.

All the assistance and help received during the course of investigation have been fully acknowledged.

Dr. K. K. VERMA
(Major Advisor)
Department of Nematology
CCS Haryana Agricultural University
Hisar, Haryana

CERTIFICATE-II

This is to certify that thesis entitled **"Studies on the incidence and management of guava decline involving root-knot nematode and fungi"** submitted by **Mr. Madhu, M. R.,** Admission No. **2015A25D** to the Chaudhary Charan Singh Haryana Agricultural University, Hisar in partial fulfilment of the requirement for the degree of **Doctor of Philosophy** in the subject of Nematology has been approved by the Student's Advisory Committee after an oral examination on the same.

MAJOR ADVISOR **EXTERNAL EXAMINER**

HEAD OF THE DEPARTMENT

DEAN, POST-GRADUATE STUDIES

ACKNOWLEDGEMENTS

"At times, our own light goes out and is rekindled by a spark from another person. Each of us has cause to think with deep gratitude of those who have lighted the flame within us." I put on record my compliments to all whose grace, glory and blessings allowed me to complete this endeavor. Without blessing of the "Almighty God" this effort would have remained a far-fetched dream and a sheath of notes.

Firstly, I would like to express my sincere gratitude to my advisor Dr. K.K. Verma, Principal Scientist, Deptt. of Nematology, CCS Haryana Agricultural University, Hisar, for the continuous support of my Ph.D study and research. The research work undertaken would have remained unaccomplished without his valuable guidance, keen interest, continuous persuasion and remarkable patience during the entire course of study. Hunceasing encouragement, untiring efforts, inspiration, ever-willing help, precise and constructive criticism and meticulous suggestions throughout the course of this investigation enabled me in executing my research work and preparation of this manuscript. His affection and sincerity towards work has been much beyond her formal obligation as a major advisor for which I shall ever be indebted to his.

I would like to show my gratitude to members of my advisory committee, Dr. Anil Kumar, Assistant Scientist (member from major subject), Dr. Yogesh Kumar, Principal Scientist (member from minor subject), Deptt. of Entomology, Dr. Kushal Raj, Assistant scientist (member from supporting subject), Deptt. of Plant pathology and Dr. S. K. Sethi, Principal Scientist (Dean PGS nominee), Deptt. of Genetics and Plant Breeding for their valuable suggestions, everlasting help and constant encouragement during my research work.

I extend my sincere thanks to Dr. R. S. Kanwar, Professor and Head, Department of Nematology, for his untiring support for me in many ways. I extend my sincere thanks to Dr. Sewak Ram, Dr. Arvind Malik Assistant professor, Deptt of Horticulutre, Dr. Prakash Banakar, Assistant Professor, Dr.Jaideep Patil, Assistant Nematologist, Dr. Vinod Kumar, Assistant Nematologist, Miss Priyanka, Junior nematologist and non-teaching staff members, Deptt. of Nematology for providing me the necessary help and suggestion during the course of this study. I wish to thank all from the deepest core of my heart, for providing homely atmosphere.

I thank my fellow labmates, friends, seniors and juniors especially Harjot Singh, Mahanthesh, Harsha sir, lingana, Basanna, Ganapanna, Shivanna, Bharat, Pavan, Shankar, Ramesh sir, Swami, Dilip, Vijay, Somu, Baskar, Praveen, Gurpreet, Babita, Anshul, Sujatha, Deepak, Shweta, Lochan, Vipul, and Promila for the suggestions, for working together before deadlines, timely help and a great company. I am thankful to all my friends for their cooperation, affection, support and encouragement during my Ph.D programme.

The credit of my rise in academic career goes entirely to my loving parents, ***Shri. Rudrappa, M.*** *and* ***Smt. Manjula,*** *my most lovable and affectionate uncle Basavarajappa, M., aunty Sunitha, brothers Manu, Naveen, sister Nayana and all other family members for their constant enthusiastic encouragement which spurred me towards higher studies.*

I express my sincere thanks to ICAR for providing financial support for my doctoral programme.

Last but not the least I thank all those who helped me directly or indirectly during period of my stay in this CCS HAU Hisar,Haryana

PLACE: Hisar ***Madhu, M. R.***

Dated: November, 2019

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER- I

INTRODUCTION

Guava (*Psidium guajava* L.) is an important fruit crop in India which belongs to the family Myrtaceae. It was originated from Mexico or Central America and later spread to Asia, Africa and Europe. There are 150 species in genus *Psidium* of which twelve are commercially important. Common Guava (*P. guajava*) is cultivated for its edible fruits and some other species of genus *Psidium* are ornamental. Guava cultivars are diploid in nature (2n=22) and some are triploid (2n=3x=33) which are used to produce seedless fruits. It is mainly grown in tropical and subtropical countries *viz*., India, China, Thailand, Pakistan, Mexico, Indonesia, Brazil and Bangladesh.

Guava is known as ‘Poor man's apple' for being nutritionally rich in vitamins and minerals. It is a good source of vitamin C, carotene, calcium, phosphorus, iron, and carbohydrates. Guava is used in the production of jam and jelly due to the presence of pectin and also used in cardiovascular treatment as it reduces the cholesterol in the human body. It is used in the production of fruit juice, ice creams, canned products, and fruit beverages. The leaves are being used to heal wounds, ulcers, toothache, cough treatment, skin diseases, gastroenteritis and diarrhoea. It contains antioxidant factors and can control systolic blood pressure. It is a good source of roughage and helps in the removal of constipation. Because of its tannin content in the leaves and bark, it's being used as a coloring agent in silk and cotton industries.

Guava is tolerant to a wide range of climates and is well adapted to tropical and subtropical climate, hence it is called as ‘Apple of tropics'. It is drought-resistant, salt tolerant and susceptible to frost. It requires an optimum rainfall of 1000 to 2000 mm, temperature between 23 to 28^0 C, well-drained sandy loams to clay loams with 6.5 to 8.5 pH for good growth and yield. Because of the hardy nature of the crop, the cost of cultivation of guava is low and gives a good yield even under the poor management practices. The improved varieties, Allahabad Safeda and Sardar (L-49) are adopted throughout the Indian guava cultivation and were suited for both table and processing purposes. Hisar Surkha and Hisar Safeda two varieties have been developed by CCS HAU, Hisar and popularly grown throughout Haryana state.

India is the largest and leading producer of guava in the world, shares 44.4 per cent of world production and China is in second place. In India, guava is grown in an area of 2.65 lakh hectares with the production of 40.54 lakh MT and an average productivity of 15.3 MT/ha. Uttar Pradesh, Madhya Pradesh, Bihar, West Bengal, Punjab, Haryana, Maharashtra, Gujarat, and Karnataka are the major guava producing states in the country. Uttar Pradesh

ranks first in both area (0.49 lakh ha) and production (9.28 lakh MT), however, productivity is highest in Andhra Pradesh of 24.12 MT/ha. Haryana is one of the largest producer of guava with the production of 1.37 lakh MT, an area of 0.12 lakh hectares and productivity of 11.33 MT/ha. In Haryana, Hisar, Jind, Fatehabad, Sirsa, Sonipat, Yamuna Nagar, and Karnal are major guava producing districts (Anonymous, 2018).

Guava cultivation is affected by many biotic and abiotic factors. Among biotic factors, insect pests *viz*., Fruit fly (*Bactrocera correcta)*, Fruit borer (*Conogethes punctiferalis*), Tea mosquito bug (*Helopeltis antonii)*, Mealybug (*Ferrisia virgata*, *Maconellicoccus hirsutus*) and Guava aphid (*Aphis punicae)*; Fungal diseases *viz*., Guava wilt (*Fusarium oxysporum* f. sp. *psidii, F. solani*), Anthracnose (*Colletotrichum gloeosporioides*), Rust (*Puccinia psidii*), Algal leaf spot (*Cephaleuros virescens)*, Stem canker and dry fruit rot (*Pestalotiopsis psidii*), Phytophthora fruit rot (*Phytophthora citricola*), Damping-off (*Rhizoctonia solani*) and plant-parasitic nematodes *viz.,* Root-knot nematodes (*Meloidogyne* spp.), Spiral nematode (*Helicotylenchus dihystera*), Lance nematode (*Hoplolaimus indicus*) and Lesion nematode (*Pratylenchus coffeae*) are major constraints for profitable guava production. Among these, the complex infestation of *Meloidogyne* spp. and *F. oxysporum* f. sp. *psidii* causes the sudden death of plants and severe loss in terms of quality and quantity of fruits and its gaining importance at the national level for want of management.

Guava decline, a complex disease syndrome, is a serious threat to guava production in India. The combined infection by the pathogens causes the sudden and severe decline of plants, which has become a national problem for guava cultivation. Guava decline is caused due to the infestation of root-knot nematode, *Meloidogyne* spp. and wilt causing pathogen *Fusarium oxysporum* f. sp. *psidii* of which first symptoms are guava wilt. The guava wilt was first reported in 1935 from Allahabad. Dasgupta and Rai (1947) reported association of *Fusarium* spp. with guava wilt for the first time from India. Regarding estimation of losses due to guava wilt, Singh and Lal (1953) reported 5-15 per cent death of the trees and loss of one million rupees due to wilt every year in Uttar Pradesh. Chattopadhyay and Bhattacharya (1968) reported yield losses of 80 per cent from West Bengal due to wilt in commercial guava orchards. There was report on the uprooting of 150 and 300 acres of wilted guava orchards in Haryana and Punjab respectively (Jhooty *et al*., 1984). Misra and Shukla (2002) also reported 5 to 60 per cent losses due to guava wilt around Lucknow. Initially it was believed that *F. oxysporum* f. sp. *psidii* is causal agent of guava wilt (Prasad e*t al.,*1952; Pandey and Dwivedi 1985) but later on, *F. solani*, *Macrophomina phaeseoli* and *Rhizoctonia solani* and many other pathogens were reported from the guava wilted samples (Dwivedi and Dwivedi, 1999).

Among plant-parasitic nematodes, root-knot nematode is wide spread and has a host range of more than 2000 plant species. Root-knot nematode is most problematic in horticultural crops, particularly in fruit crops such as pomegranate, guava, papaya, grapes *etc*.,

and cause the average annual losses of 10-69 percent in India (Walia and Poornima, 2017). Eighty per cent of guava trees were infected by root-knot nematode, *Meloidogyne* spp., and was the major limiting factor of guava production in Thailand (Sontirat, 1989). The root-knot nematode, *Meloidogyne incognita* has been reported from Brazil, Cuba, Malaysia and Venezuela (Fernandez and Silveira, 1975; Razak and Lim, 1987) and *M. incognita* races 1 and 2 were also reported on guava (De Moura and de Moura, 1989; Crozzoli and Casassa, 1998). Yield loss of around 50 per cent in terms of quality and quantity and complete distruction of 7-years old infested orchards was reported due to infestation by *M. incognita* in Malaysia (Razak and Lim, 1987). Khan *et al*. (2007) recorded *M. incognita, M. javanica* and *M. graminicola* on guava orchards from west Bengal. Ansari and Khan (2012) observed that guava orchards infected by *M. incognita* had symptoms of yellowing, stunting, dieback and numerous small to big size galls from Aligarh district, Uttar Pradesh.

Another emerging root-knot nematode species, *Meloidogyne enterolobii* causes huge losses to guava in many countries. Earlier, *M. enterolobii* was identified as *M. mayaguensis* and believed that infestation restricted only to guava plants, but recently has been reported on tomato, chilli, potato tubers from South Africa (Onkendi and Moleleki, 2013; Marais, 2014). Karssen *et al.* (2012) re-studied the holo and paratypes of both species and confirmed *M. mayaguensis* as a synonym for *M. enterolobii.* In India, the report of sudden decline and severely infested sampling revealed the incidence of *M. enterolobii* from Tamil Nadu (Poornima *et al.,* 2016). Kumar and Rawat (2018) also reported the decline in the growth of guava due to incidence of *M. enterolobii* from Uttarakhand.

Plant-parasitic nematodes (PPN) are becoming important co-factor in the initiation of guava wilt, as these facilitate the entry of fungi by causing physical injuries to roots which leads to the severe cause of ‘guava decline' (Suarez *et al.,* 1999; Khan *et al*., 2001). The combined infection of root-knot nematode *M. enterolobii* and wilt fungus *Fusarium solani* is the predominant cause of guava decline in Brazil (Gomes *et al.,* 2014). Poornima *et al.* (2016) reported that slow decline due to the infestation of *M. enterolobii* alone and sudden death of guava plants must be due to presence of wilt/rot causing pathogens. The incidence of root-knot nematode *M. enterolobii* and *F. solani* caused loss of US$ 66 million and destructed area of approximately 5000 ha spread over 16 States of Brazil (Pereira *et al.,* 2009). Infected plants show the symptoms of yellowing scorching of margin, wilting and dropping of leaves which resembles symptoms of nitrogen, phosphorus and potassium deficiency and small to compound galls along with root rotting symptoms associated with the disease (De Siqueira *et al.,* 2009; Gomes *et al.,* 2011).

The main pathogen involved in the guava decline is still difficult to be understood, but root-knot nematode *Meloidogyne* spp. *Fusarium oxysporum* f. sp. *psidii*, *F. solani*, *Macrophomina phaeseoli*, *Rhizoctonia bataticola*, and *Rhizoctonia solani* have been reported

and they are predominant pathogens in India. Due to the complex nature of guava decline, there was a need to work on this very important disease of guava particularly management aspects. Since chemical control is expensive and health-hazardous resulting in environmental pollution, therefore present investigation was conducted to detail the study of guava decline distribution and incidence in few districts of Haryana, identification of pathogens involved, interaction studies between pathogens and management through biological control methods using organic amendments and bio-control agents.

The present investigation was udertaken into consideration of all these above points and the set the following objectives for the best management and other studies of guava decline in Haryana.

1. Survey for the incidence of guava decline caused by root-knot nematode and fungi in guava orchards of western districts of Haryana
2. Culturing and pathogenicity studies of *Meloidogyne* spp. and fungi associated with guava decline
3. Studies on interaction between root-knot nematode and fungi on guava seedlings
4. Management of guava decline by various management practices under screen house conditions

CHAPTER- II

REVIEW OF LITERATURE

Guava is fourth most important fruit crop of India after mango, banana, and citrus. The area under cultivation of guava has been increased from 2.55 to 2.65 lakh ha but the production varied only 40.48 to 40.54 lakh MT in the past three years (Anonymous, 2018). Even though increasing in the area under guava cultivation, the production remains unchanged due to many factors. Among these, guava decline is an important factor involved in decreasing the guava production in the country.

Many investigations have been conducted on guava wilt caused by *F. oxysporum* f.sp. *psidii*, but there is limited availability of literature on guava root-knot nematode interaction with wilt pathogen and its management strategies. The literature available on guava decline caused by root-knot nematode and *F. oxysporum* and relevant literature is reviewed here in this chapter:

2.1 Survey for the incidence of guava decline caused by root-knot nematode and fungi

2.1.1 Survey for the occurrence of root-knot nematode, *Meloidogyne* spp.

Razak and Lim (1987) reported the decline in the growth of guava due to infestation of root-knot nematode, *Meloidogyne incognita* for the first time from Malaysia. The infected guava plants showed symptoms of stunted growth, yellowing, absence of fine roots, a poorly developed root system, small to large galls and decline in yield quality and quantity. The extraction of nematode from infested soil samples revealed the presence of plant-parasitic nematodes *viz*., *Meloidogyne* spp., and *Pratylenchus* spp. with an average population of 9300/200cc soil and 33/200cc soil respectively. The extremely high soil population of *M. incognita* on guava could be attributed to host susceptibility, soil conditions and the indigenous population of the nematode in the area. About 50 per cent of yield loss in terms of quality and quantity was caused by *M. incognita* and also showed the complete distruction of 7-years old orchard.

Moura *et al.* (1989) reported the severe incidence of *M. incognita* race 2 in guava orchards of Pernambuco State, Brazil. The pathogenicity test showed that *M. incognita* race 2 was the only pathogen to cause disease, as other pathogen *Agrobacterium tumefaciens* failed to infect the guava plants. Willers and Welgemoed (1993) observed the symptoms of *Meloidogyne* spp. infection to guava plants for the first time from South Africa and those species were different from ten species known to occur in South Africa.

The extensive survey of guava orchards conducted in Assam, Orissa, Nagaland, Jharkhand, and West Bengal showed the prevalence of *Meloidogyne* spp, *Helicotylenchus dihystera*, *Rotylenchulus reniformis*, *Hoplolaimus* spp., *Pratylenchus* spp., other tylenchids

and dorylaimids in healthy and wilted plants. Among plant-parasitic nematodes, the population frequency and density of *Helicotylenchus dihystera* was predominant in the wilted guava plants. Population dynamic studies revealed that relatively higher population density of *H. dihystera* was observed during June to September/October. The high nematode population was observed in unweeded guava orchards. Weeds *viz*., *Ageratum conyzoides, Commelina benghalensis* and other members of Compositae family were found to act as collateral host of *H. dihystera* (Khan *et al.*, 2001).

A survey conducted by Khan *et al.* (2007) in West Bengal revealed the occurrence of *M. incognita, M. graminicola, M. javanica, Pratylenchus coffeae, P. brachyurus, R. reniformis, Hoplolaimus indicus, Helicotylenchus goodi, H. indicus, H. abunamai, Tylenchorhynchus mashhoodi, T. nudus* and *Aphelenchus avenae* from guava orchards. Among plant-parasitic nematodes, the maximum population density of *R. reniformis* was 1052/200cm^3 soil and *Helicotylenchus* 233/200cm^3 soil was recorded. The decrease in yield was due to the infestation of guava plants by *Meloidogyne* spp., *R. reniformis, Pratylenchus, Xiphinema,* and *Longidorus* individually or in association with soil-borne pathogens.

Ansari and Khan (2012) conducted the survey and observed symptoms of stunting, yellowing, dieback, patchy growth, and several small to big size galls due to the infestation of root-knot nematode, *Meloidogyne incognita* in guava orchards of Aligarh district, Uttar Pradesh.

In Thailand, constant observations and survey revealed the occurrence of root-knot nematode spp., in guava orchards. The infected guava plants showed symptoms of decline in the growth, yellowing, stunted growth and reduction in the fruit yield. The decline in guava plants in the Cha-um district of Phetchaburi Province was due to infestation of root-knot nematodes, *M. incognita*. In 2003 and 2004, *M. incognita* severely damaged guava trees in a wide area of Nakhon Pathom Province (Sukhakul, 2006). But in 2012, an emerging and destructive root-knot nematode species, *M. enterolobii* was identified in guava roots collected from orchards of Nakhon Patho Province (Jindapunnapat, 2012).

Jindapunnapat *et al.* (2013) surveyed heavily infested guava orchards with root-knot nematode in Nakhon Pathom and Samut Sakhon provinces of Thailand. The symptoms of immature fruits, yellowing, wilted leaves, brittle branches, stunted growth, galled and moderately rotted roots was due to root knot-nematode infestation. Nurseries were also seriously infested with root-knot nematode and found several nematode-infected guava seedlings for sale.

Poornima *et al.* (2016) observed the three to four-year-old trees showed the symptoms of sudden wilting and yellowing, shedding of leaves, decrease of fruit size, heavily galled roots, root rotting and complete death of trees from Ayakudi village, Dindigul district, Tamil Nadu. The assessment of infested galled roots under laboratory conditions revealed the occurrence of root-knot nematode, *Meloidogyne enterolobii* and was identified by using

morphometry and Polymerase Chain Reaction (PCR) common 18S primer and further confirmed by sequencing the 18s rRNA gene.

Guava orchards of Uttarakhand showed a sudden decline and the infested plant observed symptoms of reduction in plant growth, absence of fine roots, poor root system, decrease in the yield in terms of quantity and quality. Highly infested trees shows symptoms of small leaves, browning of leaves, drying and falling of leaves, small to large and multiple galls and finally sudden death of a tree. The diagnostic results showed the presence of *M. enterolobii* from infected samples (Kumar and Rawat, 2018).

2.1.2 Survey for the occurrence of other plant parasitic nematodes

Willers and Grech (1986) conducted a survey of guava orchards in South Africa and results revealed that Cape Province plantings and Transvaal plantings were infected by the spiral nematode, *Helicotylenchus dihystera* with an average nematode population density of 2200/250 cc of soil. The pathogenicity test revealed that the pathogenic level of *H. dihystera* (1200/pot) significantly reduced the plant height (53.30 %) and also inhibited leaf size when compared to control.

The survey results of guava orchards in Allahabad, revealed that the 50 to 60 per cent wilting of guava plants due to infestation by plant-parasitic nematodes *viz., Tylenchulus semipenetrans, Longidorus* sp., *Xiphinema* sp., *Tylenchorhynchus brassicae, Ditylenchus dipsaci, Hoplolaimus indicus, Helicotylenchus indicus, and Hemicriconemoides* sp., and wilt causing fungi *viz., F. oxysporum, F. solani, F. equiseti, F.moniliforme, F. accuminatum*. The use of nitrogen fertilizers are found congenial for the multiplication of *Fusarium* spp. and plant-parasitic nemtodes (Ruchi *et al.,* 2002).

Ansari and Khan (2012) conducted an extensive survey and collected 164 samples from guava orchards of Aligarh district and results revealed that the presence of twelve genera *viz., Meloidogyne, Pratylenchus, Helicotylenchus, Hoplolaimus, Tylenchorhynchus, Tylenchus, Longidorus, Trichodorus, Hemicriconemoides, Aphelenchoides, Xiphinema,* and *Rotylenchulus*. Among plant parasitic nematodes, the highest absolute density, relative density was recorded in *Meloidogyne* spp. (density range 95-3234) followed by *Hoplolaimus* spp. The survey results also revealed that *Meloidogyne* spp., *Hoplolaimus* spp., *Rotylenchulus* spp., *Helicotylenchus* spp. and *Hemicriconemoides* spp., are the most frequently occurring and highly pathogenic to guava plants.

2.1.3 Survey for the occurrence of pathogenic fungi

Chattopadhyay and Bhattacharya (1968) collected samples on a large scale from infected guava plants of Gangetic alluvium and red lateritic soils of India. They isolated *Fusarium solani* f. sp. *psidii* and *Macrophomina phaseoli* from the infected samples and it was found that *F. solani* f. sp. *psidii* and *M. phaseoli* are capable of causing wilt either individually or in combination. In Haryana, the guava decline revealed that the isolation of

two fungi, namely *Fusarium* sp. and *Rhizoctonia* were obtained but the pathogenicity tests proved to be negative (Suhag, 1976).

Mehta (1987) conducted the survey for incidence of guava wilt in eight districts of Haryana and reported the disease incidence ranged from 1.97 per cent in Sonepat to 40.0 per cent in Jind, with an average incidence of 26.11 per cent. None of the commercially grown cultivars showed resistance or tolerance to the wilt/dieback. The disease did not occur in plants less than five years old and increased in plants up to 20 years old, subsequently decreasing as plants mature. Heavy soils with high moisture content were conducive to the disease.

The rhizosphere and rhizoplane region of wilted guava roots were collected from more than 26 orchards and seedling nurseries of different localities in Varanasi. Results found that, *F. oxysporum* f.sp. *psidii* and *F. solani* f.sp. *psidii* were primary casual pathogens followed by *Macrophomina phaseoli* and *Rhizoctonia solani* (Dwivedi *et al.,* 1990).

Gupta *et al.* (2003) conducted a survey during 2001-2003 in major guava growing areas of Uttar Pradesh and isolated *Fusarium oxysporum* f.sp. *psidii* and *Verticillium* spp. from the wilted plants. The diagnostic studies confirmed and identified the *Verticillium* spp. as *V. albo-atrum.*

The survey of guava orchards during 2009-2011 for wilt incidence in severely affected areas of India *viz.,* Allahabad, Agra, Farukhabad, Lucknow, Punjab, Ranchi and recorded the incidence of guava wilt varies from 75 to 90 per cent, while severity ranged between 30 and 55 per cent on infected plants. The isolation of *Fusarium* spp. from wilted samples found that *F. oxysporum* f. sp. *psidii* and *F. solani* were the predominant pathogens causing wilt of guava (Gupta *et al.,* 2010; Sharma *et al.,* 2011; Srivastava *et al.,* 2011).

Mishra *et al.* (2013) had conducted survey and collected seventeen soil samples from the different agro ecological regions of India *viz.*, Allahabad, Muzaffar nagar, Lucknow and Puskar. The survey results showed that all the guava orchards were severely infected with *F. oxysporum* f. sp. *psidi.* The disease incidence was recorded up to 100 per cent but the minimum (50 per cent) in the region of Lucknow.

2.1.4 Survey for the occurrence root-knot nematode, *Meloidogyne* spp. and fungi

In Brazil, maximum guava cultivated area was infested by root-knot nematode *Meloidogyne mayaguensis* and has become a threat to guava production. The parasitized guava roots were rotting which leads to disease progress due to soil borne pathogens. Therefore, to find out the soil borne pathogen involved in disease progress, 2000 root fragments were collected from nematode infected and nematode free orchards and were tested. The results showed that isolation of *Fusarium* sp. from nematode infested orchards and it confirmed *Fusarium* sp. a predominant soil borne pathogen involved in guava decline (Gomes *et al*., 2011).

Gomes *et al.* (2012) received root samples of root-knot nematode infected orchards from Northeastern, Southern and Midwestern Brazil, to access the pathogen involved in guava decline. The isolation results showed that half of nematode infected samples had an association of *Fusarium* sp., and only 5 per cent were other fungi. The virulent isolates were identified as *F. solani* by morphological characterization and also from molecular identification using ITS4 and ITS5 gene sequencing.

2.2 Pathogenicity studies of *M. incognita* and *F. oxysporum* associated with guava decline

2.2.1 Pathogenicity studies of *M. incognita* on horticultural crops including guava

Babatola and Oyedunmade (1992) conducted the experiments to establish the host parasite relationship between guava cultivars and *M. incognita*. The nematode population of 5, 10, 20 and 40 (thousands) inoculated to 28 days old seedlings of guava cultivars of Webber supreme, Supreme, Branca, and Allahabad. All the cultivars inoculated with 5000 eggs of *M. incognita* showed a significant reduction in dry matter and dry matter accumulation also decreased with increasing inoculum levels irrespective of the cultivars. All the cultivars were successfully infected by *M. incognita*, however cultivar Allahabad, took significantly more days from the infection to reproduction than other cultivars.

Arredondo (1992) conducted the pathogenicity test of *Meloidogyne incognita* on three-month-old grapevine seedlings at different inoculum levels *viz*., 0, 500, 1000, 2000, 4000 and 8000 j_2 under greenhouse conditions. The galls produced in the all five inoculation levels, but significant reduction of total dry weight was recorded in plants inoculated with 2000 and 8000 juveniles, 189 days after inoculation.

Singh and Nath (1996) observed the pathogenic level of *M. incognita* by inoculating at 0, 10, 100, 1000 and 10000 j_2 per 500 g of soil on papaya (Cv. Ranchi) under glass house conditions and results revealed that an inoculum level of *M. incognita* at 1000 j_2 per 500 g in sandy loam soil to be pathogenic level for papaya plant; and increasing inoculum level of the nematode, there was a gradual reduction in plant variables except at 10 j_2. Better plant growth at 10 j_2 nematode population can be attributed to production of more roots to counteract the light infection of plant which was much below the damaging threshold level. Similarly, Khan *et al.* (2001) conducted the pathogenicity test of *Helicotylenchus dihystera* on two-month-old seedling of guava (cv. Allahabad Safeda) at different inoculum levels *viz*., 0, 100, 500, and 2000 juveniles under greenhouse conditions. The significant reduction in plant height and number of leaves was at higher inoculum level *i.e.* 2000 juveniles per pot and also indicated that there was significant increase in population at lower initial inoculum levels.

Milan (2005) evaluated 19 accessions of guava against root-knot nematode *i.e. Meloidogyne incognita.* Sixty days old seedlings inoculated with 5000 eggs of *M. incognita* and resistance evaluated 60 days after inoculation and results showed that, only three out of 19 accessions were shown resistant to *M. incognita*. In the same way, guava seedlings of

different accessions were evaluated against *M. mayaguensis* for resistance by inoculating 10,000 eggs per plant and the observations were taken eight months after inoculation. The rootstocks of *P. guajava* were highly susceptible (RF=59.2), *P. friedrichsthalianium* was moderately resistant (RF=1.9) and three accessions of *P. cattleyanum* were resistant (RF=0) to *M. mayaguensis* (Carneiro *et al.*, 2007).

Burla *et al.* (2010) reported that the inoculum level of 500 or 2,000 eggs per plant was suitable for screening of guava genotypes instead of the high inoculum level of 15,000 eggs per plant. They also suggested that screenings should be evaluated 135-180 days after inoculation. Miranda *et al.* (2010) improved protocols for the screening of guava genotype for resistance to *M. enterolobii*. They suggested the inoculum level of 500 eggs per plant and genotypes should be evaluated 135 days after inoculation based on the final nematode population.

Martin *et al.* (2013) cultured of *M. enterolobii* was maintained and multiplied on tomato seedlings for the screening of guava genotypes and evaluated the eleven genotypes of guava collected from Federal University of Lavras, Brazil and were inoculated with 10,000 eggs and j2 of *M. enterolobii.* After 120 days of inoculation, Surinam cherry genotypes *(Psidium* spp.) such as AUFLA1, AUFLA4, AUFLA5 and APASTO and the genotypes of guava *(P. guajava)* such as G-PURPLE and G-AMA showed a susceptible reaction. The Surinam cherry genotypes ALU1, ALU2, ALU3, AROXO-C, and AROXO-U were resistant to *M. enterolobii.*

2.2.2 Pathogenicity studies of *F. oxysporum*

Pandy and Dwivedi (1985) conducted the pot experiment on the pathogenicity test to confirm the causal organism of guava wilt *(F. oxysporum* f.sp. *psidii)*. Guava seedlings showed the symptoms of chlorosis, wilting of entire plant and presence of mycelia in root xylem vessels by histopathological studies. The isolation of *F. oxysporum* f. sp. *psidii* from the inoculated seedlings and symptoms were confirmed with original symptoms and conidial character. Edward and Srivastava (1957) and Dwivedi *et al.* (1988) had tried pathogenicity of *F.oxysporum* f.sp. *psidii* in potted guava seedling.

The inoculation procedure was standardized for the pathogenicity test on guava by Misra and Pandey in 1992. They placed the inoculum of *Gliocladium roseum* by making a hand-drilled hole of 7.5 cm and placed the inoculum in holes and covered by moist cotton and again covered by polythene sheet. The symptoms were observed and confirmed with original symptoms after two to three months of inoculation. Suarez *et al.* (1999) proved the pathogenicity of *Macrophomina phaseolina* ($1.1x10^6$ conidia/ml), *F. oxysporum* f. sp. *psidii* ($3.9x10^6$ conidia /ml) on guava plants by soil inoculation method.

Mahapatra (1995) conducted the pathogenicity test of *F. oxysporum* on blackgram at five inoculum levels *i.e.,* 0.5, 1.0, 2.0, 4.0 and 8.0 g of mycelial mat per kg soil in 15 cm diameter earthen pots along with an non-inoculated check. The damaging threshold level of *F. oxysporum* was recorded at the initial inoculum level of 4.0 g mycelial mat/kg soil.

Significant reductions in plant growth and nodulation were observed at this level. However, plants inoculated with 8.0 g mycelium/kg soil exhibited wilting symptoms 40 days after seedling emergence.

Bokhari (2009) conducted a pathogenicity test on one-year-old guava plants by soil inoculation of *B. theobromae*, *F. oxysporum* f.sp. *psidii* and *F. solani* f.sp. *psidii* under pot conditions. The inoculums were placed in the hole at different depth of 2.5, 5.0 and 7.5 cm and were covered by moist cotton. The pathogens were re-isolated and confirmed with the original morphological characters of the pathogen and also with symptoms. Gomes *et al.* (2012) conducted trail on the stem cuttings with two leaves of guava cultivar 'Paluma' immersed in a conidial suspension of *F. solani* (10^7/ml) for five seconds to test the virulence of different isolates.

Gupta and Misra (2010) conducted a pathogenicity test of 51 isolates of *Fusarium* spp. on seven-year-old Allahabad Safeda using stem hole inoculation technique for the reproduction of symptoms of guava under field conditions. In the stem hole inoculation technique, a 5 mm disk of pure cultures of each isolate was inserted inside a 5 mm hole made in the stem portion, at about 10-12 cm the above soil level, of healthy guava plants. It was noticed that different isolates caused wilting at a variable period of time indicating the difference in their virulence. However, the stem hole inoculation technique was found reliable and most suitable and is recommended.

Gupta and Misra (2012) conducted a field trial for the pathogenicity test of *F. chlamydosporum* on guava seedlings of Allahabad Safeda under greenhouse conditions. Stem hole inoculation technique was employed in order to produce typical symptoms of wilt. Koch's postulates were proved by isolating of *F. chlamydosporum* from inoculated plants.

2.3 Studies on the interaction between root-knot nematode, other plant parasitic nematodes and fungi on guava

Hamiduzzaman *et al.* (1997) conducted interaction studies on four guava cultivars, Kazipeyara, Sharupkatti, Mukundhupuri and Deshipeyara in Bangladesh by artificial inoculation of guava seedlings with *F. oxysporum* f.sp. *psidii* and nematodes *Helicotylenchus dihystera* and *Hoplolaimus indicus.* The wilts symptoms were induced successfully in all four guava cultivars due to the interaction of *F. oxysporum* f.sp. *psidii, Helicotylenchus dihystera* and *Hoplolaimus indicus.*

Suarez *et al.* (1998) reported that the interaction of *Meloidogyne* spp. (*M. arenaria, M. incognita, M. hapla, M. javanica*) and fungi *Macrophomina phaseolina, F. oxysporum* and *Phytophthora* sp. have been implicated for the guava decline in Venezuela. Suarez *et al.* (1999) conducted the experiment in screen house conditions to evaluate the synergistic effect of *F. oxysporum* and *M. phaseolina* individually or in combination with *Meloidogyne* spp (*M. incognita and M. arenaria*) on guava seedlings. Statistical analysis showed no significant

difference in plant growth parameters when plants were inoculated with nematode and fungi individually, however, the simultaneous presence of fungi and nematodes caused a greater detrimental effect than each pathogen alone.

Banana plants inoculated with *M. incognita* and *F. oxysporum f. sp. cubense* or *M. incognita* and *Pseudomonas solanacearum* or *M. incognita* along with both fungus and bacterium recorded significant increase in wilt. In the presence of nematodes 10 days prior to fungus and bacterium accelerated the wilt disease development to the maximum. Presence of all the three pathogens together had more deleterious effects on banana plants than one or two pathogens were inoculated, indicating a positive interaction between all the three pathogens (Pathak *et al.,* 1999).

Khan *et al.* (2001) observed the synergistic interaction of *H. dihystera and Fusarium oxysporum* on guava cv. Allahabad Safeda. The simultaneous inoculation of *H. dihystera* and *Fusarium oxysporum* revealed a maximum reduction in plant height, plant girth, number of branches, highest leaf fall and significant increase of nematode and fungal population when compared with individual inoculation. Mejia *et al.* (2002) reported that association of *M. incognita, M. javanica* and *M. arenaria,* with the four fungi (*F. solani* f. sp. *psidii, Pythium aphanidermatum, Verticillium dahliae, Trichothecium roseum)* and some viral particles were observed with guava decline incidence. The plants inoculated with a combination of *Meloidogyne* spp., *F. solani, V. dahliae, P. aphanidermatum,* and *T. roseum* showed the disease syndrome. Root-knot nematode initiates the damage and severity of the disease is due to the presence of fungi which cause root rot.

Gomes *et al*. (2011) conducted two micro plot experiments to observe the effect of interaction between *M. mayaguensis* and *F. solani* on guava seedlings. In the first experiment, guava seedlings were inoculated with *M. mayaguensis* and 11 isolates of *F. solani* individually or in combination. Out of 11 isolates, four isolates of *F solani* were associated with guava root rot. In the second experiment, four isolates of *F. solani* were inoculated separately or in combination with *M. mayaguensis* and physical injuries to guava roots. There was no root rot and no effect on plant growth was observed in the seedling inoculated with four isolates alone or fungus with physical injuries. But, the maximum root rot and effect on plant growth parameters were observed in the seedlings inoculated with *M. mayaguensis* 21 days prior to isolates of *F. solani.* The experimental results revealed that the complex nature of guava decline and synergistic interaction between organisms was the main cause of guava decline.

The root exudates of guava seedlings inoculated with *M. enterolobii* and non-inoculated were collected and were tested on *F. solani* growth and sporulation. Nematode inoculated root exudates influence the mycelial growth and sporulation of *F. solani* than root exudates collected from non-inoculated plants. The results revealed that *M. enterolobii*

parasitization changes chemical composition in the root exudates of guava trees and induce pathogenicity of *F. solani* to cause infection (Gomes *et al*., 2013).

Safdar *et al.* (2013) conducted experiment on interaction of *Fusarium semitectum* and a nematode *Tylenchulus semipenetrans* (individually and concomitantly) on citrus and results revealed that maximum reduction in the plant growth parameters was observed in the plants having combined inoculation of *Fusarium semitectum* and *Tylenchulus semipenetrans*. Nematode reproduction parameters were maximum in the individual inoculation of *T. semipenetrans* and were reduced in combined inoculation of *F. semitectum* and *T. semipenetrans*.

Gomes *et al.* (2014) in another experiment, aiming to find out the mechanisms between *M. enterolobii* and *F. solani*, used bipartite root technique for the study. In this experiment half of the roots were inoculated with *M. enterolobii* and *F. solani* separately or in combination and another half of roots were non-inoculated. The nematode infection did not trigger a systemic effect on the plant to become susceptible to fungus. Therefore, it was concluded that, parasitism by *M. enterolobii* affect the pathogenicity of *F. solani* in guava roots, making it necessary for the two pathogens to occupy the same space at the same time for occurrence of guava decline.

The interaction studies of root-knot nematode and soil borne fungus on pomegranate revealed that the combined or sequential inoculation of *M. incognita, Ceratocystis fimbriata,* and *F. oxysporum* increased the wilt incidence in pomegranate. The maximum reduction in shoot and root length, shoot weight and fresh root weight was noticed in simultaneous inoculation of *M. incognita, C. fimbriata* and *F. oxysporum* over untreated control (Sonyal, 2015).

Ashok (2017) studied the interaction of *Meloidogyne incognita* and *F. oxysporum* on cucumber in polyhouse conditions. The results revealed that plant growth parameters *i.e.* shoot and root length, fresh shoot and root weight were significantly less in treatment inoculated with nematode 7 days prior to fungus. The number of galls, number of egg masses and the final nematode population were significantly less in plants inoculated with nematode 7 days after fungus.

2.4 Management of guava decline by various management practices under screen house conditions

2.4.1 Effect of deoiled cakes on the growth of guava under root-knot nematode and fungus infected conditions

Reddy *et al.* (1997) assessed the effects of neem cake and two bio-control agents (*Paeciliomyces lilacinus* and *Pasteuria penetrans*) in combination and results found that significant decrease in parasitization of *M. incognita* and least root galling and nematode multiplication was observed when bio-agents were integrated with neem cake. The application of neem cake (1.5 t/ha) or press mud (15 t/ha) significantly reduced the soil population of *M.*

incognita and *Helicotylenchus multicinctus* in banana plants and enhanced fruit yield (Jonathan *et al*., 2000)

Raguchander *et al.* (2001) reported that, application of *Pseudomonas fluorescens* and *Trichoderma viride* significantly lower the Panama wilt incidence (*F. oxysporum* f. sp. *cubense*) than the control in banana cv. Rasthali under field conditions. However, application of *P. fluorescens* at 3 and 5 months after planting recorded the lowest mean wilt incidence of 3.5 per cent and the highest wilt reduction over the control (80.6%). Logani *et al.* (2002) assessed the different botanicals against guava wilt pathogen (*F. oxysporum* f.sp. *psidii*) and reported that *Azadirachta indica*, along with the other botanicals, not only prevented the plant from infection by wilt causing fungi *F. oxysporum* f. sp. *psidii* but also increased better growth of guava plants by providing rich nutrients.

Srivastava (2002) tested efficacy of different organic amendments against root-knot nematode (*M. incognita*) infecting papaya. Mahua, castor, neem, karanja cake and biogas sludge were applied @ 2.5 t/ha and carbofuran @ 4 kg a.i./ha also evaluated as the control. All treatments significantly reduced infestation of *M. incognita* and increased fruit yield and yield components. Carbofuran was recorded lowest nematode reproduction and maximum plant growth parameters. Among the organic amendments, neem cake resulted in the lowest root-knot index (2.7) and the greatest stem girth (29.5 cm), leaf number (44.2) and fruit number (58.6) but highest fruit yields were obtained with the application of neem cake and mahua cake (51.8 and 51.7 kg/tree). Mishra *et al.* (2004) reported that the management of guava root-knot nematode and fungus complex was achieved by drenching of bavistin @ 0.1 per cent and neem cake @ 400 g/tree.

Rahman and Somers (2005) recorded the incorporation of mustard as green manure and seed meal in the inter-row or vine row suppressed nematode population densities of *M. javanica* in vineyards. Decreased nematode population due to presence of biologically active compounds *i.e.* glucosinolates which hydrolyses the toxic compounds such as isothiocyanates, organic cyanides and ionic thiocyanates during decomposition in soil.

Shreenivasa *et al.* (2005) managed effectively to brought down the population of the phytonematdes *viz., M. incognita, R. similis* and *H. multicinctus* of banana by paring and hot water (55 °C for 20 minutes) along with the application of neem seed cake (1 kg/plant) and carbofuran (16.6 g/plant) during planting and enhanced the growth, development and yield of banana.

Gomes *et al.* (2010) conducted experiments on infested orchards with *M. mayaguensis* with five treatments that combined with chemical fertilizers and organic soil amendments such as cow manure (30 kg/plant), poultry manure (40 kg/plant) and sugarcane filter cake (40 kg/plant) in two years experiment period. The results from the experiments revealed that the application of poultry compost and cow manure applied homogenously under tree canopy provided the highest nematode suppression and productivity. The economic

analysis indicated being unworthy to manage the heavily infested guava orchards and on the other hand, it indicated being feasible and profitable to manage moderately infested orchards with proper chemical fertilization coupled with applications of organic amendments.

Almeida *et al.* (2012) evaluated organic amendments viz., meat and bone meal (3%), chitosan (0.05%), shrimp shell (2%) and neem cake (0.1%) against *Meloidogyne enterolobii* and *Fusarium solani* on guava in Brazil. The observations recorded after ninety days of treatment applications showed that meat and bone meal @ 3 per cent significantly reduced the nematode reproduction. Neem cake @ 0.1 per cent was effective on plant growth parameters, but it presented no nematicidal effect, since final nematode population and reproduction factors actually increased in comparison with the control check. The application of meat and bone meal @ 25 kg/plant for six months (bimonthly), reduced the soil and root population of *M. enterolobii, Helicotylenchus* spp., other plant-parasitic nematodes and the fungus.

Ashok (2017) evaluated the different deoiled cakes viz., neem, mustard, and castor cake to manage *M. incognita* and *F. oxysporum* f. sp. *cucumerinum* (alone or in combination) infesting cucumber under polyhouse conditions. The results showed that maximum plant growth parameters and reduction in nematode reproduction factors (number of galls, number egg mass and final nematode population) were observed in treatment applied with neem cake @ 30g followed by mustard cake 30 g per kg of soil.

2.4.2 Effect of different bio-agents against root-knot nematode and fungus complex on guava

Misra *et al.* (2004) evaluated three bio-agents (*Aspergillus niger* AN17, *Penicillium citrinum* and *T. harzianum*) *in-vitro* and field experiments against guava wilt pathogens *viz., Gliocladium roseum, F oxysporum* f.sp. *psidii* and *F solani*. The results found that all three bio-agents were effective *in-vitro* evaluation and these bio-agents were multiplied on FYM and applied in basin @ 10 kg per plant. The field experiment results found that *P. citrinum* controlled the disease upto 52.67 per cent only, while *A. niger* ANI7 and *T. harzianum* controlled it upto 78.34 and 75.34 per cent respectively.

Senthilkumar and Rajendran (2004) conducted field trials for the management of disease complex caused by *Meloidogyne incognita* and *Fusarium moniliforme*, on grapevine. The plants were treated with bio-control agents *viz., Trichoderma viride* (100g/plant) and *Pseudomonas fluorescens* (100g/plant) alone and in combination with farmyard manure (20 kg/plant) and carbofuran 3G (60g/plant). All the treatments significantly reduced the final soil nematode population and wilt disease incidence. The highest reduction in final soil nematode population (76.9%), least root gall index (1.8) and least wilt disease incidence (24.8%) and increase in bunch weight of grapevine by 155.4 per cent were observed in FYM (20 kg) + *P. fluorescens* (100 @vine) treated vines compared to untreated control.

Bokhari (2008) conducted the experiment to study the effect of *Trichoderma harzianum* and different fungicides for the control of guava decline *in-vivo.* Statistical analysis revealed that disease intensity was zero and 0.74 per cent when treated with *T. harzianum* and Topsin-M as drenches in sterilized and unsterilized soil and showed maximum control of disease.

Gupta and Misra (2009) evaluated the bio-agents, *Aspergillus niger* isolates, *T. virens* and *T. viride* against guava wilt pathogens (*Fusarium oxysporum* f. sp. *psidii* and *Fusarium solani)* under *in-vitro* and field conditions. The results showed that, all bio-agents inhibited growth of the pathogen significantly but maximum inhibition was observed in *T. virens* and *T. viride.* The inhibition in the growth of *F. oxysporum* f. sp. *psidii* and *F. solani* due to toxic metabolites released by them or action of volatile compounds. Based on *in-vitro* results, isolates of *Trichoderma* (Tvd-P) and *Aspergillus* (AN9) were evaluated in field conditions. During the three years tested, two bioagents completely suppressed wilt guava wilt disease incidence (5%) and enhanced the plant growth.

Srivastava *et al.* (2009) conducted the pot experiment to evaluate different bio-control agents for management of guava *fusarium* wilt. Four strains of *Trichoderma viz., T. harzianum, T. hamatum, T. viride* and *T. virens* and also *Aspergillus niger* and *Gliocladium virens* were tested against *Fusarium* spp., and results found that, *T. harzianum* reduced the disease incidence than the other bio-agents.

The application of farm yard manure enriched with *Paecilomyces lilacinus* and *Pseudomonas fluorescens* bio-pesticides @ 1kg/seedling during planting and subsequently four more applications for 6 months reduced the root population of *M. incognita* and *R. reniformis* by 78 and 73 per cent respectively. These bio agents also increased the yield of the papaya by 26 per cent. The increase in the yield could be because of the management of nematodes and other pathogens in the rhizosphere of papaya and also due to growth promoting effects of these bio-pesticides (Rao, 2010)

Almeida *et al.* (2011) isolated 120 rhizobacterial isolates from guava orchards infected by *M. enterolobii*, of these, 44 isolates were tested on guava plants by inoculating plants with 2000 nematode eggs. All 44 rhizobacterial isolates reduced the number of galls, number of egg masses and the final nematode population.

Mervat *et al.* (2011) conducted experiment to control the root-knot nematode; *M. incognita* infecting grapevines by inoculating bio-agents (*T. harzianum* and *Arbuscular mycorrhiza*), plant oil extracts (orange and jojoba oil) and plant aqueous extracts (*Origanum majorana* and *Tagetes erecta*) oxamyl (24% EC) under field conditions. Results showed that all the treatments had a significant effect in reducing the total population *M. incognita* and increased the plant growth parameters.

The combined application of *Trichoderma viride* (15g/plant) + *Pseudomonas fluorescens (*15g/plant) + *Paecilomyces lilacinus* (15g/plant), effectively managed the banana wilt complex (*Radopholus similis* and *Fusarium oxysporum* f.sp. *cubense*) with a lowest wilt incidence and severity, nematodes population, lesions and maximum growth and yield (Dinesh, 2014).

Jindapunnapat *et al.* (2013) evaluated a commercially available fungal bio-agent, *T. harzianum* against *M. incognita* and *M. enterolobii* which were seriously damaging guava orchards in Thailand. The results found that inoculation of guava plants with *T. harzianum* reduced the soil and root population of nematodes when compared to untreated plants. Moreover, *T. harzianum* inoculation enhanced the plant growth through induced resistance, stimulates the adventitious root growth.

Singh and Singh (2015) assessed the effects of non-host crops intercropping, bio-agents and oil cakes, on population dynamics of *F. oxysporum* f. sp. *psidii* and wilt of guava. The lowest population of fungus was observed in intercropping with garlic (84.9%) followed by marigold (83.9%). Among oil cakes tested, neem cake significantly reduced the population of fungus followed by mahua cake. The combination of neem cake + *T. harzianum* + garlic intercropping significantly reduced fungus population followed by neem cake + *T. harzianum* + marigold.

Zaitoun *et al.* (2015) evaluated bio-agents (*Bacillus subtilis*, *Pseudomonas fluorescens* and *Trichoderma harzianum)* against *Fusarium oxysporum*, *Botryodiplodia theobromae* and *Rhizoctonia solani* causing guava decline. All bio-agents inhibited the mycelial growth of pathogens, but four isolates of *T. harzianum* showed an average of 58 per cent inhibition of all pathogens *in-vitro.* Based on *in-vitro* studies, *T. harzianum* isolate-T4 was tested at different densities (50, 100 and 150 ml, 10^7 spores/ml) and the results showed that the disease severity was significantly decreased with increased plant height, dry weight of shoot and root and total pigments in guava trees in comparison with infected trees. The application of bio-control agents decreased guava decline disease and improved the growth of guava trees.

Removal and destroying of root-knot nematode, *M. enterolobii* affected trees, applying 100 kg of farmyard manure, 250 g of neem cake and 25 g of *P. lilacinus* and carbofuran @ 60g per tree at an early stage of nematode infestation were effective in the nematode management and to enhance fruit yield (Anonymous, 2105).

Sonyal (2015) evaluated combination of bio-control agents along with chemicals for managing pomegranate wilt complex caused by *M. incognita*, *C. fimbriata* and *F. oxysporum.* The combination of *P. fluorescens* + *T. harzianum* + *P.lilacinus* + difenconazole was significantly superior over all the treatments in reducing the wilting of branches, plants and managing the *M. incognita* population in soil and root followed by *B. subtilis* + *P. fluorescens*

and *B. subtilis* + *T. harzianum*. For managing the nematode population, *P. lilacinus* and carbofuran were effective.

Chormule *et al.* (2017) observed the reduction of *M. incognita* population when grapevine plants were treated with bio-agents *viz.*, *P. fluorescens, P. lilacinus, Trichoderma* plus, *T. viride* and *Pochonia chlamydosporia* @ 20 kg/ha and organic amendment, neem cake @ 2 t/ha and were effective in reducing number of root galls and egg masses and increasing the yield. Among bio-agents, *P. fluorescens* was found to be most effective in reducing nematode population (38.6%), number of root galls (26.1%) and number of egg masses (28.8%).

Ashok (2017) evaluated three bio-agents such as *T. viride, P. fluorescens, P. lilacinus* and combined formulation of all three bio-agents against *M. incognita* and F. *oxysporum* f.sp. *cucumerinum* (alone or in combination) infesting cucumber. The results depicted that the maximum plant growth parameters and minimum nematode reproduction factors were observed in combined formulation of bio-agents (*T. viride, P. fluorescens and P. lilacinus* @ 15 ml/pot) followed by *P. lilacinus* @ 0.5 g/pot.

Dawabah *et al*. (2019) carried out the field experiments to study the efficacy of different bio-control agents against *M. javanica, Tylenchorhynchus mediterraneus, Hoplolaimus seinhorsti, Longidorus latocephalus* and *Xiphinema elongatum* on guava and fig trees in Saudi Arabia. The highest reduction of nematode densities was recorded in treatment with carbofuran 10 G followed by a combination of *P. lilacinum* + *P. penetrans* + urea and combination of *T. harzianum* + *P. penetrans* + poultry manure.

CHAPTER- III

MATERIALS AND METHODS

Guava decline, a complex disease caused by root-knot nematode and soil-borne fungi *Fusarium oxysporum* f. sp. *psidii* is gaining national importance due to its impact on production and productivity of guava. The present investigation included a survey of four districts of western Haryana to understand the distribution of guava decline. The series of experiments viz., pathogenicity studies of *M. incognita* and *F. oxysporum* f. sp. *psidii,* interaction of root-knot nematode and fungus and their management studies using deoiled cakes and bio-agents were carried out in the screen house, Department of Nematology, CCS HAU Hisar, Haryana. Hisar is situated in western Haryana at 29^0 09' N latitude, 75^0 43' E longitude and it has an average elevation of 215 m (705 ft) above mean sea level. The materials and methods and experiment protocols used in experiments are discussed in this chapter.

3.1 General laboratory procedures

3.1.1 Extraction of nematodes from soil samples collected during the survey

Cobb's decanting and sieving (Cobb, 1918) combined with Modified Baermann's funnel technique (Schindler, 1961) was used for the extraction of nematodes from soil samples collected during the survey for quantitative and qualitative analysis of nematode population. Soil sample of 200 cc mix thoroughly in 1000 ml of water in the beaker or pan and wait for 10-15 seconds to settle down heavier particles. The soil suspensions were passed through a set of 20, 100 and 300 mesh sieves respectively. The soil suspension was passed through 20 mesh sieve to remove the roots, stubbles and other inert materials from the suspension. Collected the filtrate from 20 mesh sieve in other pan and continue the procedure for 60, 100 and 300 mesh sieves. Discarded the filtrate from 300 mesh sieve and collect the residues in the beaker by backwashing of 300 mesh sieve. Then placed contents collected from the 300 mesh sieve on the Petri dish supporting a moulded wire gauge having two layers of tissue paper and kept undisturbed for 48 hours. After 48 hours, collected the nematode suspension and made the volume of nematode suspension to 100 ml and nematode suspension was used for qualitative and quantitative analysis of the nematode population by examining under the stereo binocular microscope.

3.1.2. Preparation of permanent slides for identification of nematodes

The nematode suspension collected from samples was further processed for the preparation of permanent slides by killing, fixing and clearing method (Seinhorst, 1966). Killing and fixing of the nematodes was done by adding an equal amount of boiling fixative (8% formalin) in the vials. Closed the lid tightly and kept it for 24 hours, nematodes were killed and fixed for further processing for the preparation of permanent slides. These

nematodes were transferred to the cavity block containing 0.5 ml Seinhorst's solution -I and partially covered cavity blocks were placed in a desiccator containing 96 per cent alcohol. The desiccators were placed in an oven at 40^0 C for 12 h. Then refilled the cavity block with Seinhorst's solution–II, covered it partially and placed again in an oven at 40^0 C for 4 h. The permanent slides were prepared by transferring the nematode from the cavity block to a clean glass slide on which a drop of glycerol was placed. The small pieces of glass wool were cut into small bits of the same size and were placed in the glycerol drop and then round coverslip was placed carefully over the drop of glycerol. Excess of glycerol was removed and the coverslip was sealed with nail polish.

3.1.3 Preparation of perineal pattern

The root samples infected with root-knot nematodes collected during the survey were washed in running tap water to remove the soil particles. Infected roots were cut into small bits of 2 cm and boiled in 0.1% acid fuchsin lactophenol stain for 2 to 3 minutes. Root bits were removed and then washed in running water to remove the excess stain and kept overnight in plain lactophenol for destaining. Then matured females were dissected out from galls of the roots under a stereo binocular microscope and the posterior portion of the female was cut and the body contents were cleaned. The posterior portion of the female was further trimmed and the perineal pattern was mounted on a glass slide in a drop of lactophenol and coverslip was placed on it, sealed with nail polish. The species confirmation was done based on the basis of perineal pattern as described by Chitwood (1949). Estimation of root population of root-knot nematode was made by counting the stained nematodes under the microscope.

3.1.4 Culturing and maintenance of *M incognita*

A single egg mass technique was used for raising the pure culture of the identified female *M. incognita* on tomato plants. The egg masses collected during the survey from infected guava plants were hand-picked and placed on Modified Baermann's funnel for hatching. After 24-48 hours second-stage juveniles collected from Petri plates were inoculated on tomato seedlings grown in pots containing sterilized soil. Tomato plants were removed 45 days after inoculation from the pots gently, washed and egg masses were collected for further multiplication and sub-culturing of *M .incognita* periodically. After mass multiplication of *M. incognita*, eggs were separated by stirring roots in 5 per cent sodium hypochloride solution for five minutes. Eggs were collected using 500 mesh sieve and washed two to three times on running water to remove the excess chlorine. The eggs were placed on Modified Baermann's funnel for hatching and collected nematode suspension for experiment purposes.

3.1.5 Isolation and identification of fungi from infected root samples

3.1.5.1 Glassware cleaning

The glasswares cleaning were done by keeping in the container having 60.0 g of potassium dichromate, 60.0 ml of concentrated sulphuric acid in 1000 ml of water for 24

hours. Glasswares were washed thoroughly with a detergent solution followed by rinsing with tap water and finally with distilled water.

3.1.5.2 Sterilization

The glasswares were sterilized in an autoclave at 121.6^0 C for 20 minutes and then kept these in a hot air oven at 60^0 C for further sterilization. The media used for the laboratory studies were sterilized by autoclaving at 121.6^0 C for 20 minutes. All cultural studies were conducted in the aseptic condition under laminar airflow. The sterilization of tips of inoculation needle, cork borers and forceps was done by using flame.

3.1.5.3 Preparation of the medium

Potato Dextrose Agar (PDA)

For isolation and culturing of the fungal pathogen, the Potato Dextrose Agar medium was used. The media was prepared by using Potato (200 g), dextrose (20g), agar-agar (20g) and distilled water (1000 ml). The peeled potatoes (200g) were cut into small pieces and boiled in 500 ml of water for 20 minutes. After boiling for 20 minutes, extract was collected by filtering through a double-layered muslin cloth. Then dextrose (20 g) and agar-agar (20 g) was dissolved in potato extracts and volume was made up to 1000 ml by adding distilled water. The freshly prepared media was poured into flasks and test tubes, plugged with cotton and then sterilized at 121.6^0 C for 20 minutes. After sterilization, test tubes were taken out and placed on the slanting board to prepare slants.

3.1.5.4 Isolation and identification of fungi

The roots were washed properly in water to remove the soil particles and cut in small pieces of 5 mm of each sample collected during the survey. The small pieces of roots were cleaned and surface sterilized by using about 0.1 per cent sodium hypochlorite for two to three minutes and rinsed two to three times with sterile distilled water (Dhingra and Sinclair, 1995). The small root samples were transferred aseptically to petri plates containing sterilized potato dextrose agar (PDA) medium with 500 ppm of streptomycin sulfate and incubated at 27 ± 2^0 C for seven days. After the incubation period of seven days, colonies were checked under the compound microscope for identification. Fungi were identified based on the sporulation, mycelia character according to the descriptions by Booth (1971), Ellis (1971). After identification of fungi, pure culture of *F. oxysporum* was obtained by the hyphal tip method and pure culture was maintained on PDA slants.

3.1.5.5 Mass multiplication of fungal inoculum

For mass multiplication of *F. oxysporum* f. sp. *psidii*, sand maize medium and potato dextrose broth were used. The flask containing sand maize medium was sterilized at 121.6^0 C for 20 minutes and were inoculated with freshly growing 2 to 5 mycelial discs of *F. oxysporum* f.sp. *psidii.* These flasks were incubated for 15 days at 27 ± 2^0 C and flasks were shaken during incubation for proper growth of inoculum.

3.1.5.6 Physico-chemical properties of soils used for different experimentation

The physico-chemical properties and texture of soils were got analysed by Department of Soil Science, CCS HAU, Hisar which are as follow:

Soil type	pH	E.C. ($dSm^{-1)}$	Organic carbon (%)	Availability (kg/ha)	
				Phosphorus	Potash
Sandy loam	7.4	1.38	0.71	18	302

3.1.5.7 Soil sterilization

Cleaned soil collected and brought to Nematology laboratory and sterilized in autoclave at 15 lbs pressure with 121±1^{O}C for one hour. Sterilized soil was tested to confirm that no living nematodes exist in it. The sterilized soil was then allowed to dry for one day and then filled in 15 cm diameter pots (two kg capacity). This sterilized sandy loam soil was used for all the experiments conducted in research programme.

3.1.5.8 Application of fertilizers

The recommended doses of fertilizers, *i.e.*, nitrogen (N), phosphorus (P), and Potash @ 14, 7 and 21 kg/ ha in the form of urea, single super phosphate and potash, respectively were incorporated in each pot at the time of sowing. The amounts of fertilizers to be applied were calculated on the basis of weight of soil/pot. Nitrogen was applied in two split doses, i.e.*;* half of dose of nitrogen and full dose of phosphorus and zinc were applied at the time of sowing. The other half of nitrogen dose was applied one month after sowing.

The plants were observed and watered daily depending upon the temperature and rainfall. Hand hoeing with a khurpa was done at desired intervals for removing weeds etc. To check the incidence of insect pests and diseases, chemical spray was done as and when required.

3.2 Survey for the incidence of guava decline and identification of root-knot nematode and fungi

3.2.1 Survey of the major guava orchards in western districts of Haryana

The systematic survey was conducted in four districts of western Haryana viz., Hisar, Jind, Sirsa and Fatehabad for the guava decline incidence. The list and address of old and newly established guava orchards were made available by the State Horticulture Department. Composite samples of soil (500 cc) and root (15g) were collected randomly from the rhizosphere of guava trees. Soil samples were collected in polythene bags, labelled, handled and refrigerated at 7-10^{0} C before processing. The number of wilted and dried guava trees or plants were counted in the orchard and disease incidence was expressed in per cent. The nematode population in soil and roots was identified and defined as the number of second-stage juveniles (j_2) nematode per 200 cc soil and 5 g of roots. 0-5 scale root-knot index was recorded according to the following scale: 0 = no galls or egg masses, 1=1-2 galls or egg

masses, 2=3-10, 3=11-30, 4 = 31-100, and 5=over 100 galls or egg masses (**Hartmant & Sasser, 1985).**

Observations;

1. Nematode populations per 200 cc soil.
2. Nematode populations per 5 g guava root.
3. Density range and per cent frequency of occurrence of the nematode with particular reference to root-knot nematode.
4. Isolation of fungi associated with the disease (Potato Dextrose Agar method)

$$\text{Per cent disease incidence} = \frac{\text{Number of plant infected}}{\text{Total number of Plants}} \times 100$$

3.2.2 Identification of *Meloidogyne* spp. and soil-borne fungi associated with guava decline

Females of root-knot nematode extracted from the galled roots and 4-5 perineal patterns were prepared from each sample collected during the survey. The identification of the species was made by comparing the observed characteristics of the perineal pattern with already documented descriptions (Chitwood 1949; Yang and Eisenback, 1983).

The fungi were isolated from infected guava plant parts and rhizospheric soil on potato dextrose agar medium. The pure culture of the fungus was obtained from Hyphal tip method and was maintained on PDA slants. The fungi were identified based on mycological observations such as mycelia growth, colour and sporulation (Booth, 1971; Ellis, 1971). The most predominant pathogens (root-knot nematode, *M. incognita* and soil-borne fungus *F. oxysporum* f. sp. *psidii*) were identified from infested samples and were further used for the pathogenicity test.

3.3 Pathogenicity studies of *M. incognita* and *F. oxysporum*

3.3.1 Pathogenicity studies of *M. incognita* on guava seedlings

The pure culture of second-stage juveniles (j_2) was obtained from egg masses collected from highly infected tomato plants by giving a waiting period of 24-48 hrs to egg masses at room temperature in water. The required numbers of j_2 were adjusted by adding water to the beaker and were inoculated in soil by making 3-4 holes around guava plant rhizosphere. The pathogenicity experiments included different inoculum levels of *M. incognita* at 0, 10, 100, 500, 1000, 2000 and 4000 j_2 per kg of soil (sterilized sandy loam soil). The experiment was conducted in the screen house, Department of Nematology, CCS HAU, Hisar. Observations on plants growth parameters *viz*., shoot length, fresh and dry shoot weight, fresh and dry root weight and nematode reproduction factors such as number of galls per plant, number of egg masses per plant, number of eggs per egg mass and final nematode population per 200 cc soil were recorded 60 days after inoculation.

- **Location** **:-** Screen house, Deptt. of Nematology
- **Crop** **:-** Guava (Hisar Safeda- 2 month old seedlings)
- **Replications** **:-** Four
- **Design** **:-** Complete randomized design (CRD**)**

3.3.2 Pathogenicity studies of *F. oxysporum* f.sp. *psidii*

The pathogenicity of the most prominent fungus (*F. oxysporum*) isolated from infected guava orchards was conducted under screen house conditions. The fungus at different inoculum levels *viz*., 2, 4, 6, 8, and 10 g mycelial weight (g/kg soil) was inoculated into the sterilized sandy loam soil and left for seven days for the pathogen to get established. The healthy seedlings of guava cultivar, Hisar Safeda (two months old) planted in each infected pot and four replications were maintained along with control. The wilting symptoms and re-isolation of *F. oxysporum* culture from inoculated plants were compared with originally noticed symptoms and conidial character. The pathogenicity, hence proved and the fungus was determined as *F. oxysporum* f.sp. *psidii*. Observations on plant growth parameters *viz*., shoot length, fresh and dry shoot weight, fresh and dry root weight were recorded 60 days after inoculation.

- **Location** **:-** Screen house, Deptt. of Nematology
- **Crop** **:-** Guava (Hisar Safeda- 2 month old seedlings)
- **Replications** **:-** Four
- **Design** **:-** Complete randomized design (CRD**)**

3.4 Studies on the interaction between *M. incognita* and *F. oxysporum* f.sp. *psidii* on guava seedlings

To study the effect of interaction between *M. incognita* and *F. oxysporum* f. sp. *psidii* on two months old guava seedlings (Hisar Safeda), the experiment was conducted in the screen house, Deptt. of Nematology, CCS HAU, Hisar. The experiment was designed according to a complete randomized design (CRD) with three replications each and non-inoculated control was maintained. Guava seedlings were raised in sterilized soil and then were transplanted in pots containing sterilized sandy loam soil. Fifteen days later (after the plants established in the pots), the pathogenic level of *M. incognita* (1000 j_2/kg soil) and *F. oxysporum* f. sp. *psidii* (6 g/kg soil) was inoculated alone or in combination in sequence as listed below:

- T1: Non inoculated check (no nematode, no fungus)
- T2: Inoculation with *M. incognita* alone
- T3: Inoculation with *F. oxysporum* f.sp. *psidii* alone
- T4: Inoculation with *M. incognita* 10 days prior to *F. oxysporum* f.sp. *psidii*
- T5: Inoculation with *M. incognita* 20 days prior to *F. oxysporum* f.sp. *psidii*
- T6: Inoculation with *F. oxysporum* f.sp. *psidii* 10 days prior to *M. incognita*

- T7: Inoculation with *F. oxysporum* f.sp. *psidii* 20 days prior to *M. incognita*
- T8: Simultaneous inoculation with *M. incognita* and *F. oxysporum* f.sp. *psidii*

Data on plant growth parameters *viz.*, shoot length, fresh and dry shoot weight, fresh and dry root weight and nematode reproduction factors *viz*., number of galls per plant, number of egg masses per plant, number of eggs per egg mass, final nematode population per 200 cc soil and per cent root rot were recorded 60 days after inoculation.

3.5 Management of guava decline by various management practices under screen house conditions

3.5.1 Effect of different oil cakes on guava inoculated with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

The screen house experiment was conducted in pots (15 cm size) to evaluate the effect of different organic amendments *viz.*, neem, mustard and castor cake against *M. incognita* and *F. oxysporum* f.sp. *psidii* infecting guava. The deoiled cakes at 20 g and 30g/kg soil were incorporated to sterilized sandy loam soil (as according to treatments) seven days before transplanting of guava seedlings (2 month old Hisar safeda). Fifteen days after transplanting, the pathogenic levels of *M. incognita* (1000 j_2/kg soil) and *F. oxysporum* f. sp. *psidii* (6g/kg soil) were inoculated alone or in combination. In this experiment, one set of plants was inoculated with nematode alone, the second set of plants was inoculated with fungus alone and the third set of plants inoculated with *M. incognita* 10 days prior to *F. oxysporum* f. sp. *psidii*. Untreated inoculated plants and untreated non-inoculated plants served as checks.

Location :- Screen house, Deptt. of Nematology

Crop :- Guava seedlings (Hisar Safeda- 2 month old seedlings)

Replications :- Three

Design :- Complete randomized design (CRD**)**

Pot size :- 15 cm (2 kg soil capacity)

Method :- Soil inoculation

Treatments of nematode and fungus:

- Inoculation with the root-knot nematode, *M. incognita* alone
- Inoculation with fungus, *F. oxysporum* f.sp. *psidii* alone
- Inoculation with *M. incognita* 10 days prior to *F. oxysporum* f. sp. *psidii*

Other treatments:

- T1: Soil application of neem cake @ 20 g/kg of soil
- T2: Soil application of neem cake @ 30 g/kg of soil
- T3: Soil application of mustard cake @ 20 g/kg of soil
- T4: Soil application of mustard cake @ 30 g/kg of soil
- T5: Soil application of castor cake @ 20 g/kg of soil
- T6: Soil application of castor cake @ 30 g/kg of soil
- T7: Carbofuran (Furadan) @ 0.1 g/ kg of soil

- T8: Carbendazim 50WP @ 2g/liter water
- T9: Untreated inoculated check (nematode or fungus alone or in combined inoculation)
- T10: Untreated non-inoculated check

Observations were recorded on plant growth parameters *viz*., shoot length, fresh and dry shoot weight, fresh and dry root weight, and nematode reproduction factors *viz*., number of galls per plant, number of egg masses per plant, number of eggs per egg mass and final nematode population per 200 cc soil, per cent root rot 60 days after inoculation.

3.5.2 Effect of different bio-agents on guava inoculated with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

The effect of bio-agents was evaluated under screen house conditions against *M. incognita* and *F. oxysporum* f. sp. *psidii* infecting guava. The bio-control agents *viz*., *T. viride*, *P. fluorescens, P. lilacinus* and their combined formulation received from TNAU, Coimbatore Tamil Nadu and IIHR, Benagluru, Karnataka were inoculated into pots containing sterilized sandy loam soil five days before the transplanting of guava seedlings. The moisture of the soil was maintained by adding sterilized water. The inoculum levels of nematode and fungus were taken based on the pathogenicity studies *i.e. M. incognita* (1000 j_2/kg soil) and *F. oxysporum* f. sp. *psidii* (6g/kg soil) and were inoculated alone or in combination. In this experiment, one set of plants was inoculated with nematode alone, the second set of plants inoculated with fungus alone and third set of plants inoculated was with *M. incognita* 10 days prior to *F. oxysporum* f. sp. *psidii*. Untreated inoculated plants and untreated non-inoculated plants served as checks.

Location **:-** Screen house, Deptt. of Nematology

Crop **:-** Guava seedlings (Hisar Safeda- 2 months old seedlings)

Replications **:-** Three

Design **:-** Complete randomized design (CRD**)**

Pot size :- 15 cm (2 kg capacity)

Method **:-** Soil inoculation

Treatements of nematode and fungus:

- Inoculation with nematode (*M. incognita*) alone
- Inoculation with fungus (*F. oxysporum* f.sp. *psidii*) alone
- Inoculation with *M. incognita* 10 days prior to *F. oxysporum* f.sp. *psidii*

Other treatments:

- T1: *Trichoderma viride* @ 10 g/kg of soil
- T2: *Pseudomonas fluorescens* @ 10g/kg of soil
- T3*: Purpureocillium lilacinum* @ 10g/kg of soil
- T4: combined formulation of these (*T. viride* + *P. fluorescens* + *P. lilacinum*) bio-agents @ 10 ml/ kg of soil
- T5: Carbofuran (Furadan) @ 0.1 g/ kg of soil
- T6:Carbendazim 50 WP @ 2g/liter water
- T7: Untreated inoculated check
- T8:Untreated non-inoculated check

Observations on plant growth parameters such as shoot length, fresh and dry shoot weight, fresh and dry root weight and nematode reproduction factors *viz*., number of galls per plant, number of egg masses per plant, number of eggs per egg mass and final nematode population per 200 cc soil, per cent root rot were recorded 60 days after inoculation.

CHAPTER- IV

RESULTS

The experiments were carried out on "Studies on the incidence and management of guava decline involving root-knot nematode and fungi" and the results pertaining to survey, pathogenicity, interaction of *Meloidogyne incognita* and *F. oxysporum* f.sp. *psidii* and effect of organic amendments and bio-agents on guava decline are presented in this chapter.

4.1 Survey for the incidence of guava decline caused by root-knot nematode and fungi

The survey conducted in guava orchards of Hisar, Jind, Sirsa and Fatehabad districts of Hayana revealed the prevalence of several plant parasitic nematodes viz., *Meloidogyne incognita, M. javanica, Rotylenchulus reniformis, Helicotylenchus* sp., *Hoplolaimus* sp., *Pratylenchus* sp., *Tylenchorhynchus* sp., *Xiphinema* sp., *Longidorus* sp. and many pathogenic fungi *viz*., *Fusarium oxysporum* f.sp. *psidii*, *Macrophomina phaseoli* and *Rhizoctonia solani* in guava orchards. Root-knot nematode species, *M. incognita* was more predominant to guava plants than *M. javanica* and *M. javanica* was mostly found in guava orchards having intercropping with vegetables.

Data collected (Table 1a to 1d) during the survey depicts information regarding guava cultivars, age of orchard, method of irrigation, gall index, soil population, root population, per cent incidence of guava decline and pathogens associated with it (Plate 1).

In Hisar district, the maximum disease incidence was recorded in Sainipura village (71.9%) followed by Mirpur (68.5%) and the least incidence was recorded in Adampur-1 (11.8%). Maximum gall index (5.0), soil population of *M. incognita* (710 j_2/200 cc soil) and root population (236/5 g of root) was observed in guava orchard in Sainipura village which was ten years old (Table 1a).

Survey of Jind district revealed that the guava decline incidence varied from 27.7 to 80.9 per cent. The maximum disease incidence was in Raichandwala (80.9%) followed by Nagura (68.2%) and least disease incidence of 27.7 per cent was in Dilluwala-1 village. The maximum number *M. incognita* (930 j_2/200 cc soil) was observed in Nagura village and gall index of 5.0 was reported in three orchards of Jind district (Table 1b).

It is indicated (Table 1c) from the results of Sirsa district that the density range of *M. incognita* was 425 to 940 j_2/200 cc soil and root population varied from 135 to 195/5 g of root. The maximum guava decline incidence was recorded in Dadbi-6 (100%) which was highly infested by *M. incognita* (835 j_2/200 cc soil) and root population of 195/5 g roots. The least disease incidence of 14.8 per cent was observed in Dadbi -2, in which *F. oxysporum* and *R. solani* pathogens were identified.

A survey was conducted in Fatehabad and the results (Table 1d) showed that the maximum disease incidence was in Ahlisadar village (63.4%) which was infected by *M. incognita*, *F. oxysporum* and *M. phaseoli* Nursery plants to the tune of 71.8 per cent were infected by *M. incognita* having soil population of 820j_2/200 cc soil and root population of 250/5g

Table:1a. Survey for the incidence of guava decline involved root-knot nematode and fungi in Hisar district

District	Villages	Guava varieties	Age of orchard (years)	Method of irrigation	GPS location	Species of root-knot nematode identified	Species of fungus Identified	Gall index	Soil population of RKN j_2/200cc soil	Root population of RKN /5g root	Per cent infection/ infestation in guava trees
Hisar	Siswal-1	Hisar Safeda	3	Flood irrigation	Lt. 29.207969 Lg.75.504356	*Meloidogyne incognita*	-	4	165	98	18.2
	Siswal-2	Allahabad Safeda	6	Flood irrigation	Lt. 29.230012 Lg.75.478131	-	*Fusarium oxysporum*	-	-	-	31.6
	Mohabatpura-1	Hisar Safeda	4	Flood irrigation	Lt. 29.258289 Lg.75.460182	*M. incognita* *M. javanica*	-	5	240	168	42.7
	Mohabatpura-2	Hisar Safeda	8	Flood irrigation	Lt. 29.287813 Lg.75.493572	*M. incognita*	*F. oxysporum*	5	330	215	65.5
	Peeranwali	Hisar Safeda	5	Flood irrigation	Lt. 29.191434 Lg.75.658085	-	*F. oxysporum* *M. phaseoli*	-	-	-	20.9
	Neolikalan	Allahabad Safeda	4	Flood irrigation	Lt. 29.194455 Lg.75.628572	-	*F. oxysporum* *M. phaseoli*	-	-	-	17.3
	Adampur-1	Hisar Safeda	3	Flood irrigation	Lt. 29.265262 Lg.75.477813	-	*F. oxysporum R. solani*	-	-	-	11.8
	Adampur-2	Hisar Safeda	2	Flood irrigation	Lt. 29.262275 Lg.75.476628	*M. incognita*	-	2	46	25	22.7
	Mirpur	Hisar Safeda	8	Flood irrigation	Lt. 29.329832 Lg.75.649556	*M. incognita*	*F. oxysporum* *M. phaseoli*	5	485	190	68.5
	Landhari	Hisar Safeda	5	Flood irrigation	Lt. 29.323403 Lg.75.666011	-	*F. oxysporum* *M. phaseoli*	-	-	-	23.4
	Kirmara-1	Allahabad Safeda	7	Flood irrigation	Lt. 29.381161 Lg.75.679978	*M. incognita*	*R. solani*	3	175	68	38.2
	Kirmara-2	Hisar Safeda	5	Flood irrigation	Lt. 29.381997 Lg.75.723503	-	*F. oxysporum*	-	-	-	24.4
	Kirmara-3	Hisar Safeda	3	Flood irrigation	Lt. 29.396574 Lg.75.803457	-	*F. oxysporum* *R. solani* *M. phaseoli*	-	-	-	32..7
	Barwala -1	Hisar Safeda	6	Flood irrigation	Lt. 29.384118 Lg.75.913174	*M. incognita*	*F. oxysporum*	5	220	140	46.4
	Barwala-2	Hisar Safeda	8	Flood irrigation	Lt. 29.394335 Lg.75.946477	*M. incognita*	*F. oxysporum* *M. phaseoli*	5	640	202	64.5
	Barwala-3	Hisar Safeda	5	Flood irrigation	Lt. 29.363541 Lg.75.891337	*M. incognita* *M. javanica*	-	5	260	173	48.2
	Sainipura	Hisar Safeda	10	Flood irrigation	Lt. 29.116309 Lg.75.965875	*M. incognita*	*F. oxysporum*	5	710	236	71.2

Table 1b: Survey for the incidence of guava decline involved root-knot nematode and fungi in Jind district

District	Villages	Guava Varieties	Age of orchard (years)	Method of irrigation	GPS Location	Species of root-knot nematode identified	Species of fungus identified	Gall Index	Soil population of RKN j_2/200cc soil	Root population of RKN /5g root	Per cent infection/ infestation in guava trees
Jind	Haibatpura	L-49	8	Flood irrigation	Lt. 29.32261 Lg.75.20569	*M.incognita* *M. javanica*	*F. oxysporum*	5	460	228	62.7
	Raichandwala	Hisar Safeda	5	Flood irrigation	Lt. 29.23961 Lg.75.25560	*M.incognita*	*F. oxysporum*	5	735	320	80.9
	Dilluwala-1	L-49	6	Flood irrigation	Lt. 29.24520 Lg.76.24853	*M.incognita*	*F. oxysporum* *M. phaseoli*	3	310	76	27.7
	Dilluwala-2	Allahabad Safeda	4	Flood irrigation	Lt. 29.25326 Lg.76.24974	-	*F. oxysporum* *R. solani*	-	-	-	38.6
	Nagura	L-49	5	Flood irrigation	Lt. 29.27329 Lg.76.22636	*M.incognita*	*F. oxysporum* *M. phaseoli*	5	930	265	68.2
	Palwan-1	Allahabad Safeda	7	Flood irrigation	Lt. 29.29463 Lg.76.10828	-	*F. oxysporum* *R. solani*	-	-	-	42.4
	Palwan-2	L-49	8	Flood irrigation	Lt. 29.29472 Lg.76.10858	*M.incognita* *M.javanica*	*F. oxysporum*	4	380	145	33.6
	Palwan-3	L-49	4	Flood irrigation	Lt. 29.30145 Lg.76.10917	-	*F. oxysporum* *M. phaseoli*	-	-	-	46.8
	Palwan-4	L-49	Nursery	Sprinkler	Lt. 29.29584 Lg.76.10938	*M.incognita* *M. javanica*	-	5	895	232	77.3
	Sangathpura	VNR-One Kg	5	Drip irrigation	Lt. 29.19763 Lg.76.15025	*M.incognita*	-	5	530	195	38.2

in Gillakhera. The least disease incidence was recorded in Karnoli-2 (16.4%) which was infected by *F. oxysporum* and *M. phaseoli*.

The frequency of occurrence of *M. incognita* among four districts was recorded maximum in Fatehabad (72.2%) followed by Hisar (63.2%), Jind (56.3%) and Sirsa district (53.3%). The maximum frequency of occurrence (66.0%) of *Helicotylenchus* spp. was in Sirsa district, whereas, high frequency of *Hoplolaimus* spp. (62.5%) was observed in Jind district. Among four districts, the occurrence of *Rotylenchulus reniformis* and *Pratylenchus* sp. varied from 24.8 to 47.4 and 31.6 to 42.1 per cent respectively (Table 1e).

The results from Table 1e summarizes the density range (j_2/200cc soil) of plant parasitic nematodes in four districts. The maximum density range of *M. incognita* was recorded in Sirsa (425-940) followed by Jind (310-930), Fatehabad (280-820) and Hisar district (46-710). Among four districts, the highest density range of *Helicotylenchus* spp. (80-425) was in Jind, *Rotylenchulus reniformis* (35-230) was in Fatehabad and *Pratylenchus* spp. (60-280) was observed in Sirsa district.

The maximum range (4-5) of gall index was recorded in Sirsa, while a 2-5 gall index range was recorded in Hisar, 3-5 gall index was recorded in Jind and Fathehabd districts. The maximum range of root population of *M. incognita* was recorded in Jind (76-265) followed by Fatehabad (62-250), Hisar (25-236) and Sirsa (135-195). The method of irrigation practice also influenced damage potential, reproduction and multiplication of *M incognita*. The highest soil and root population of *M. incognita* and compound galls were observed in orchards having drip irrigation than flood irrigated orchards (Table 1a-1d). The survey of four districts revealed that the maximum guava decline incidence was recorded in Dadbi-6 (100%), Sirsa district and the minimum was in Adampur-1 (11.8%), Hisar district (Plate 2). Among four districts surveyed, mean of guava decline incidence was maximum in Jind (51.6%) followed by Sirsa (49.4%), Hisar (40.4%) and least disease was observed in Fatehabad district (36.6%). The young orchards were less infected by guava decline when compared to old orchards. The field observations and survey results revealed that guava decline severity and incidence were prominent where guava plants were infected by *M. incognita* and *F. oxysporum* (Table 1f).

The individual and concomitant presence and incidence of *M. incognita* and *F. oxysporum* were observed on guava plants during the survey (Fig.1). Disease incidence and severity was low in orchards infected by *F. oxysporum* and *M. incognita* individually, whereas more disease severity was observed in orchards infected with both *M. incognita* and *F. oxysporum*. Among four districts, the incidence of *F. oxysporum* varied from 23.0 to 42.6% and *M. incognita* incidence varies from 33 to 57.7 per cent. The combined infection by *M. incognita* and *F. oxysporum* was recorded maximum in Sirsa (67.7%) followed by Jind (61.4%), Hisar (54.1%) and 49.1 per cent in Fatehabad district.

4.2 Pathogenicity studies of *M incognita* and *F. oxysporum* f.sp. *psidii* associated with guava decline.

The experiment on pathogenicity of *M. incognita* on guava seedlings (Hisar safeda) was studied under screen house conditions by inoculating different inoculum levels of 0, 10, 100, 500, 1000, 2000, and 4000 j_2/kg soil. Observations on the impact of *M. incognita* on plant growth parameters and nematode parameters *viz*., number of galls/plant, number of egg masses/plant, number of eggs/egg mass and final nematode population per 200 cc soil were recorded 60 days after inoculation and data are presented in hereunder (Plate 3).

Table 1c: Survey for the incidence of guava decline involved root-knot nematode and fungi in Sirsa district

District	Villages	Guava Varieties	Age of orchard (years)	Method of irrigation	Species of root-knot nematode identified	Species of fungus identified	Gall index	Soil population of RKN j_2/200cc soil	Root population of RKN /5g root	Per cent infection/ infestation in guava trees
Sirsa	Dadbi-1	Hisar Surka Hisar Safeda	9	Flood irrigation	*M.incognita*	*F. oxysporum*	5	525	173	57.3
	Dadbi-2	Hisar Safeda	2	Flood irrigation	-	*F. oxysporum* *R. solani*	-	-	-	14.8
	Dadbi-3	L-49	4	Flood irrigation	*M.incognita*	*F. oxysporum* *M. phaseoli*	4	425	135	23.3
	Dadbi-4	Allahabad Safeda & L-49		Flood irrigation	-	*F. oxysporum* *R. solani*	-	-	-	38.2
	Dadbi-5	Allahabad Safeda & L-49	12	Flood irrigation	*M.incognita*	*F. oxysporum*	5	940	240	90.9
	Dadbi-6	Allahabad Safeda & L-49	9	Flood irrigation	*M.incognita*	*F. oxysporum*	5	835	195	100.0
	Dadbi-7	Hisar Safeda	3	Flood irrigation	-	*F. oxysporum* *R. solani* *M. phaseoli*	-	-	-	21.2

Table.1d: Survey for the incidence of guava decline involved root knot nematode and fungi in Fatehabad district

District	Villages	Varieties	Age of orchard (years)	Method of irrigation	GPS location	Species of root-knot nematode identified	Species of fungus identified	Gall index	Soil population of RKN j_2/200cc soil	Root population of RKN /5g root	Per cent infection/ infestation in guava trees
Fatehabad	Govt. nursery Fatehabad	Hisar Safeda	Nursery	Manual/ Sprinkler	Lt. 29.19763 Lg.76.15025	Healthy	Healthy	-	-	-	00
	Gillakhera-1	Hisar Safeda	9	Flood irrigation	Lt. 29.31726 Lg.75.19578	-	*F. oxysporum* *R. solani*	-	-	-	35.5
	Gillakhera-2	Allahabad Safeda	6	Flood irrigation	Lt. 29.30295 Lg.75.17487	*M.incognita* *M.javanica*	-	3	430	62	46.4
	Gillakhera-3	L-49 & Hisar Safeda	Nursery	Sprinkler	Lt. 29.30296 Lg.75.17487	*M.incognita*	*R. solani*	5	820	250	71.8
	Karnoli-1	Hisar Safeda	10	Flood irrigation	Lt. 29.30298 Lg.75.17487	*M.incognita* *M.javanica*	*F. oxysporum* *R. solani* *M. phaseoli*	5	380	190	22.7
	Karnoli-2	Hisar Safeda	3	Flood irrigation	Lt. 29.31440 Lg.75.18837	-	*F. oxysporum* *M. phaseoli*	-	-	-	16.4
	Dariyapur	Allahabad Safeda	5	Flood irrigation	Lt. 29.32324 Lg.75.21250	*M.incognita*	*F. oxysporum*	4	470	168	39.1
	Hijarawan Kalan	L-49 & Hisar Safeda	4	Flood irrigation	Lt. 29.32519 Lg.75.20793	*M.incognita* *M.javanica*	-	3	280	80	34.3
	Ahlisadar	Allahabad Safeda & Hisar Safeda	6	Drip irrigation	Lt. 29.32259 Lg.75.20577	*M.incognita*	*F. oxysporum* *M. phaseoli*	5	690	175	63.4

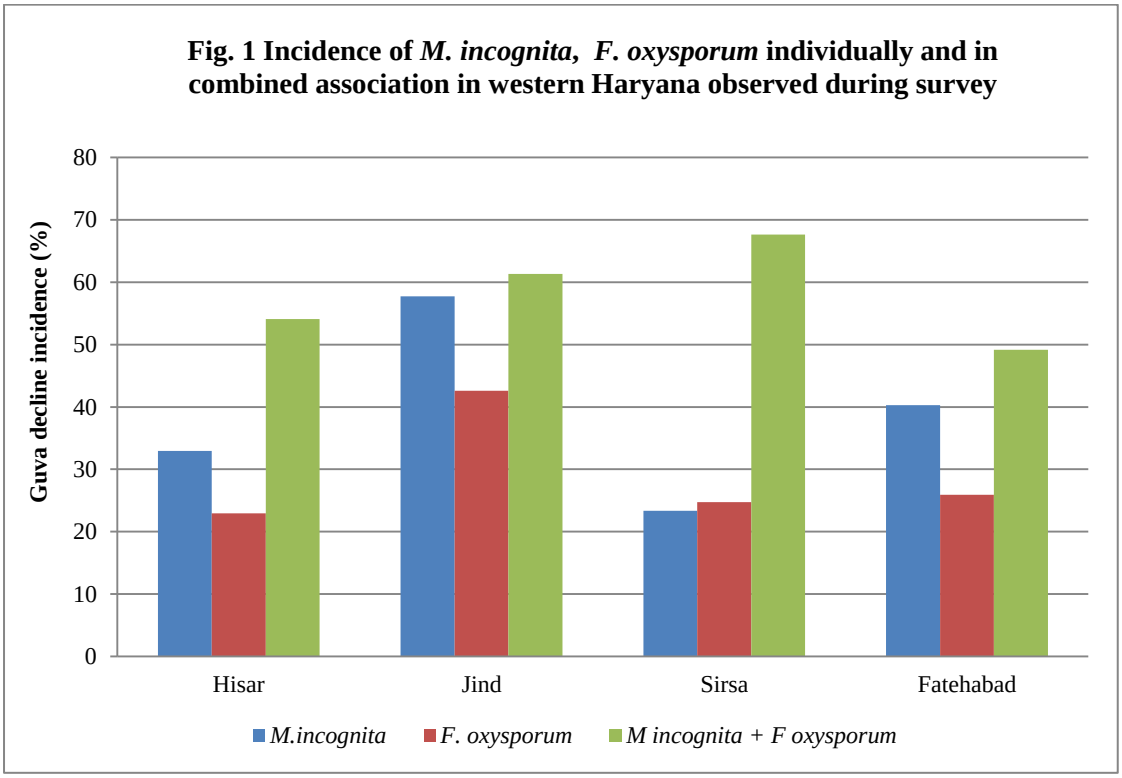
Table 1e: Relative abundance of *M. incognita* and other nematodes associated with guava in four districts of western Haryana

Districts	Important nematodes recorded	Frequency of occurrence (%)	Density Range (j_2/200 cc soil)	Other plant parasitic nematodes
Hisar	*M. incognita*	63.2	46-710	*Tylenchus* sp. *Longidorus* sp.
	Helicotylenchus spp.	50.0	25-350	
	Hoplolaimus spp.	36.8	20-200	
	Rotylenchulus reniformis	47.4	50-140	
	Pratylenchus spp.	31.6	30-100	
Jind	*M. incognita*	56.3	310-930	*Xiphinema* sp. *Longidorus* sp. *Tylenchorhynchus* sp.
	Helicotylenchus spp.	25.0	80-425	
	Hoplolaimus spp.	62.5	50-450	
	Rotylenchulus reniformis	32.7	45-80	
	Pratylenchus spp.	42.2	60-95	
Fatehabad	*M. incognita*	72.2	280-820	*Tylenchus* sp. *Xiphinema* sp. *Longidorus* sp.
	Helicotylenchus spp.	33.3	40-300	
	Hoplolaimus spp.	44.4	25-475	
	Rotylenchulus reniformis	28.3	35-230	
	Pratylenchus spp.	39.2	25-190	
Sirsa	*M. incognita*	53.3	425-940	*Longidorus* sp. *Tylenchorhynchus* sp.
	Helicotylenchus spp.	66.0	70-290	
	Hoplolaimus spp.	60.0	15-320	
	Rotylenchulus reniformis	24.7	40-170	
	Pratylenchus spp.	38.6	60-280	

Table:1f Occurrence of guava decline in four districts of western Haryana

District	Varieties	Age of orchards (years)	Range of Gall index	Range of RKN (j_2/200cc soil)	Range of root population (RKN /5g root)	Mean Per cent infection/ infestation in guava trees
Hisar	Hisar Safeda Allahabad Safeda	3-10	2-5	46-710	25-236	40.4
Jind	L-49 VNR-One Kg Hisar Safeda Allahabad Safeda	4-8	3-5	310-930	76-265	51.6
Fatehabad	Hisar Safeda Allahabad Safeda L-49	4-10	3-5	280-820	62-250	36.6
Sirsa	Hisar Surka Allahabad Safeda L-49	2-12	4-5	425-940	135-195	49.4

Fig. 1 Incidence of *M. incognita*, *F. oxysporum* individually and in combined association in western Haryana observed during survey



Yellowing of Plants

Completely wilted guava plants

Plate 1 Symptoms of guava decline caused by *M. incognita* and *F. oxysporum* f.sp. *psidii*

Galls on guava roots produced by root-knot nematode, *Meloidogyne incognita* observed in nursery during survey

Healthy Guava orchard

Plate 2 Highly infected guava orchard with root-knot nematode and fungus observed during survey (Village, Dadbi-6, Sirsa District)

4.2.1 Effect of different inoculum levels of *M. incognita* on growth parameters of guava

4.2.1.1 Shoot length

Data in Table 2 clearly indicated that shoot length was significantly lowest in the highest inoculum level (4000 J_2/kg soil). The maximum and significantly highest shoot length was observed in the non-inoculated check followed by the inoculum level of 10 and 100 j_2 /kg soil. Treatments of inoculum levels of 0 to 500 j_2/kg soil were significantly at par and the significant reduction in shoot length was observed at the inoculum level of 1000 juveniles onwards which was statistically at par with 2000 j_2 level. As nematode inoculum level increased from 10 to 4000 j_2/kg soil, shoot length decreased accordingly.

4.2.1.2 Fresh shoot weight

It was inferred from data in Table 2 that among the different inoculum levels, fresh shoot weight was significantly lowest in 4000 j_2 inoculum level. Significantly highest fresh shoot weight was obtained in non-inoculated check which was significantly at par with inoculum levels of 10, 100, 500 j_2**.** Fresh shoot weight was decreased as inoculum level increased from 10 to 4000 j_2/kg soil. The significant reduction in fresh shoot weight was observed at 1000 j_2 onwards and which was statistically at par with 2000 j_2 level.

4.2.1.3 Dry shoot weight

Perusal of data in Table 2 indicated that dry shoot weight was decreased as inoculum level increased from 10 to 4000 juveniles accordingly. Minimum and significantly lowest dry shoot weight was observed in 4000 juveniles. Dry shoot weight in 1000 j_2 was statistically different from inoculum level of 4000 j_2, but was statistically at par with 2000 j_2/kg soil. Maximum and significantly highest dry shoot weight was obtained in non-inoculated check which was at par with nematode densities of 10 and 100 juveniles.

4.2.1.4 Fresh root weight

Data in Table 2 depicted that maximum and significantly highest fresh root weight was observed in non-inoculated check which was at par with the inoculum levels of 10 and 100 j_2. Maximum reduction in fresh root weight was observed at 4000 juveniles. Fresh shoot weight was decreased as inoculum levels increased from 10 to 4000 j_2/kg soil. The significant reduction in fresh root weight was observed at 1000 j_2 onwards, but statistically at par with 2000 j_2 inoculum level.

4.2.1.5 Dry root weight

Data in Table 2 indicated that dry root weight was decreased as inoculum levels increased from 10 to 4000 j_2/kg soil. Significantly lowest and minimum dry root weight was however, observed in 4000 j_2 level of *M. incognita*. The maximum and significantly highest dry root weight was observed in non inoculated check followed by 10, 100, 500 j_2 and which were statistically at par with one other. The significant reduction in dry root weight was

observed at 1000 juveniles onwards which was par with 2000 j_2 inoculum level. A maximum reduction in dry root was observed at 4000 juveniles.

4.2.2 Effect of different inoculum levels on multiplication and reproduction of *M. incognita* on guava

4.2.2.1 Number of galls per plant

The number of galls was significantly increased as the inoculum level increased from 10 to 4000 juveniles, although the number of galls at inoculum levels of 1000 and 2000 j_2 were at par with each other. Minimum number of galls was recorded in the treatment level of 10 j_2/kg soil, while maximum and significantly highest galls were observed in the treatment level of 4000 juveniles. The significant increasing rate in the number of galls was observed from 10 to 500 juveniles, but the rate of increase in number of galls from 1000 to 4000 j_2/kg soil was lower (Table 3).

4.2.2.2 Number of egg masses per plant

Data from the Table 3 indicated that the number of egg masses per plant was significantly highest at inoculum level of 4000 j_2/kg soil. There was significant increase in number of egg masses as the inoculum levels increased from 10 to 4000 juveniles. The significantly minimum number of egg masses was recorded at the inoculum level of 10 j_2/kg soil. The number of egg masses at 1000 and 2000 juveniles was at par with each other.

4.2.2.3 Number of eggs per egg mass

Perusal of data in Table 3 clearly indicated that the highest number of eggs per egg mass was recorded in inoculum levels of 10 to 500 j_2, but decrease in number of eggs was recorded from the inoculum level of 1000 j_2 onwards. The number of eggs per egg mass from 10 to 1000 j_2 was significantly at par with each other. The significantly lowest number of eggs per egg mass was recorded in the inoculum level of 4000 j_2/kg soil.

4.2.2.4 Final nematode population

The data from Table 3 revealed that the final nematode population of *M. incognita* on guava seedlings was significantly different in all the treatments. The maximum and significantly highest nematode population was observed at 4000 j_2 followed by inoculum level of 2000 j_2/kg soil. As the inoculum level of *M. incognita* increased from 10 to 4000 j_2, final nematode population was increased significantly. The minimum and significantly lowest number of nematodes was observed at 10 j_2/kg soil.

4.3 Effect of different inoculum levels of *F. oxysporum* f.sp. *psidii* on growth parameters of guava

The pathogenicity of *F. oxysporum* f. sp. *psidii* at different inoculum levels *viz*., 2, 4, 6, 8, and 10 g mycelium/pot on guava seedlings was conducted in the screen house, Dept. of Nematology, CCS HAU Hisar. The Koch's postulates were proved by re-isolation from inoculated guava plants and compared with the mycological observations of originally

isolated *F. oxysporum* f.sp. *psidii* (Plate 4). Observations on the impact of *F. oxysporum* f.sp. *psidii* on plant growth parameters were recorded 60 days after inoculation and data are presented in Table 4.

4.3.1 Shoot length

Data in Table 4 depicted that the maximum and significantly highest shoot length was found in non-inoculated check (without fungus) which was statistically at par with inoculum levels of 2 and 4g mycelium per kg soil. The significant reduction in shoot length was observed at 6 g mycelium which was statistically different from 2, 4, 8 and 10 g mycelium. The minimum and significantly lowest shoot length was observed in 10 g mycelium followed by 8 g and 6 g mycelium which were significantly different from each other.

Table 2: Effect of different inoculum levels of *M. incognita* on growth parameters of guava

Sr. No.	Inoculum levels (j_2/ kg soil)	Shoot length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)
1	0	37.20	9.48	3.50	4.80	1.75
2	10	36.95	9.15	3.25	4.73	1.70
3	100	35.83	8.90	2.98	4.40	1.65
4	500	34.98	8.38	2.88	3.95	1.48
5	1000	25.70	6.23	2.23	3.13	1.20
6	2000	24.40	5.98	2.10	2.85	1.08
7	4000	20.28	4.05	1.33	1.90	0.70
	C.D. at 5 per cent	2.41	1.21	0.59	0.83	0.52

Mean of four replications

Table 3: Effect of different inoculum levels on multiplication and reproduction of *M. incognita* on guava

Sr. No.	Inoculum levels (j_2/ kg soil)	Number of galls/plant	Number of egg masses/plant	Number of eggs/egg mass	Final nematode population j_2/200 cc soil
1	10	4.50 (2.33)	3.50 (2.10)	284.00 (16.88)	20.50 (4.82)
2	100	36.50 (6.12)	24.75 (4.98)	280.25 (16.79)	144.25 (12.05)
3	500	92.00 (9.40)	46.52 (6.82)	280.00 (16.76)	196.32 (14.03)
4	1000	202.75(14.26)	96.50 (9.57)	272.50 (16.53)	342.50 (18.54)
5	2000	218.75(14.82)	107.25 (10.39)	256.25 (16.03)	441.25 (21.02)
6	4000	243.50(15.61)	142.00 (11.92)	238.75 (15.47)	501.00 (22.40)
	C.D. at 5 per cent	(0.75)	(0.82)	(0.44)	(1.02)

Mean of four replications

Figures in parentheses are $\sqrt{n}$ transformed value

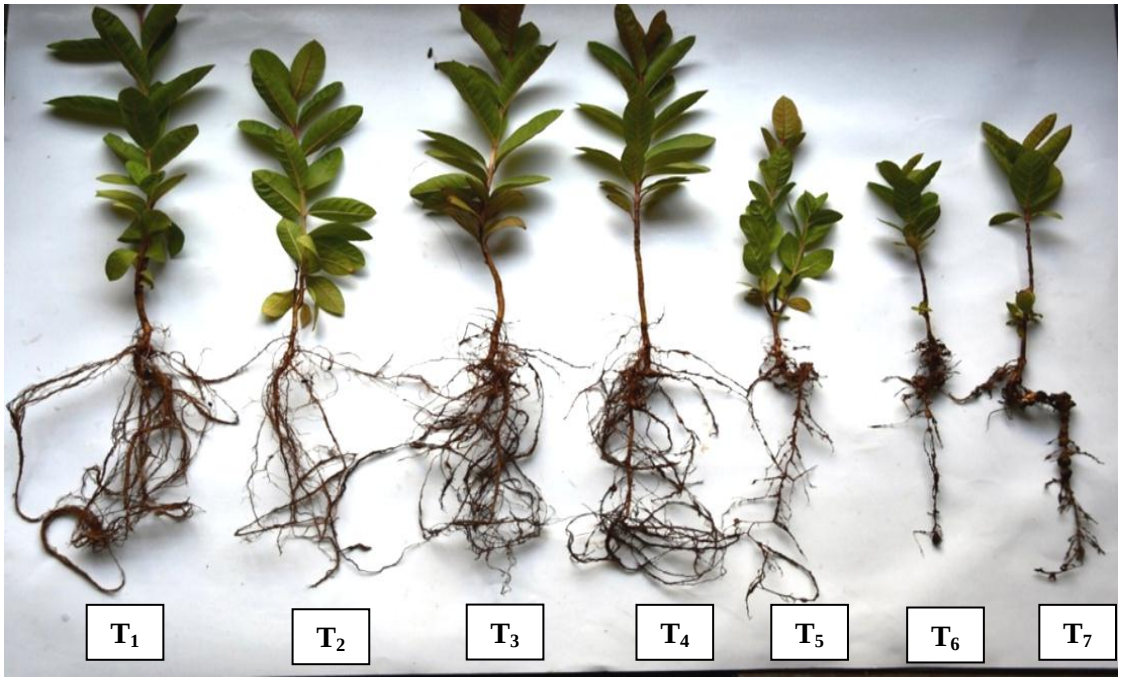


Plate 3 Effect of different inoculum levels of *M. incognita* on growth of guava

T1:0 (Non- inoculated check); T2: 10 j_2/ kg soil; T3: 100 j_2 /kg of soil; T4: 500 j_2 /kg of soil; T5:1000 j_2/kg of soil ; T6: 2000 j_2/kg of soil; T7: 4000 j_2 /kg of soi

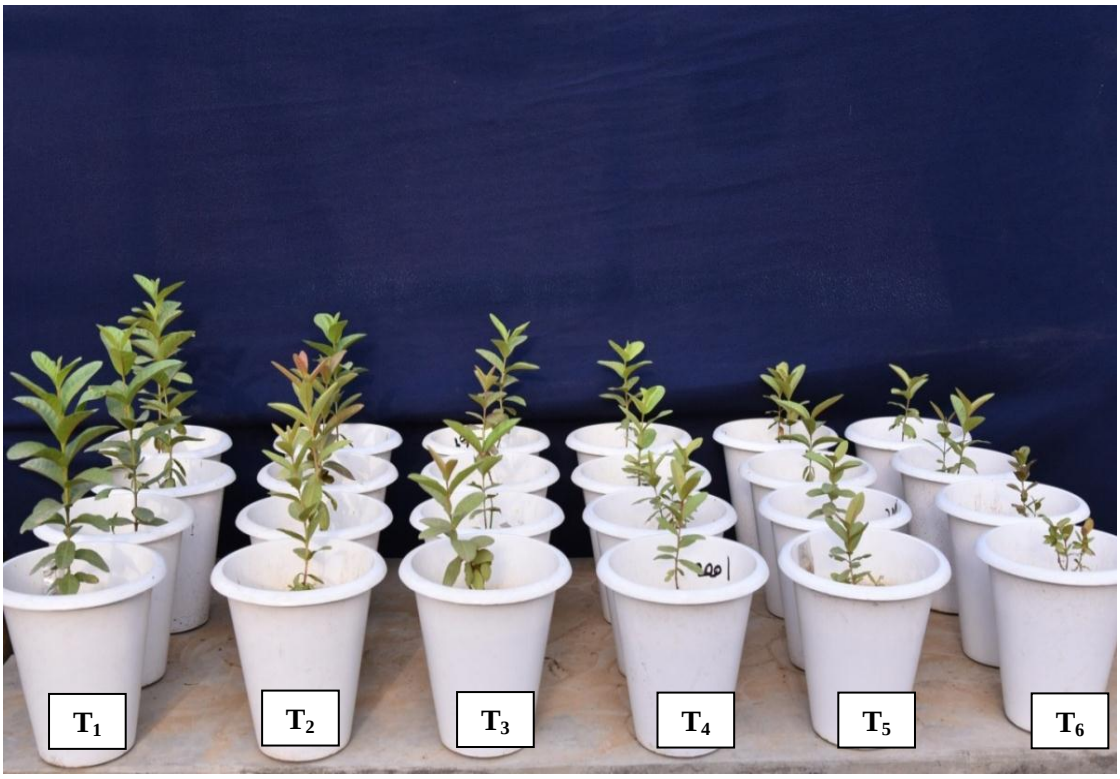


Plate 4 Effect of different inoculum levels of *F. oxysporum* on growth of guava

T1: Non- inoculated check; T2: 2g mycelium /kg of soil; T3: 4g mycelium /kg of soil; T4: 6g mycelium /kg of soil; T5: 8g mycelium /kg of soil; T6: 10g mycelium /kg of soil

Table 4: Effect of different inoculum levels of *F. oxysporum* f.sp. *psidii* on growth parameters of guava

Sr. No.	Inoculum levels	Shoot length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	Per cent root rot
1	Non- inoculated check	41.25	10.43	3.88	5.28	2.02	0.00 (0)
2	2g mycelium/kg soil	39.63	9.86	3.75	4.93	1.85	8.60 (16.91)
3	4g mycelium/kg soil	38.25	9.95	3.84	4.35	1.71	19.27 (26.01)
4	6g mycelium/kg soil	30.45	8.03	3.27	3.36	1.30	34.37 (35.82)
5	8g mycelium/kg soil	24.08	6.64	2.90	3.05	1.27	39.50 (38.65)
6	10g mycelium/kg soil	17.13	4.88	2.10	2.28	1.00	45.33 (42.31)
	C.D. at 5 per cent	3.27	1.50	0.47	0.70	0.36	(2.12)

Mean of four replications

4.3.2 Fresh shoot weight

The data in Table 4 indicated that fresh shoot weight was decreased as inoculum levels increased from 2 to 10 g mycelium/kg soil. Maximum and significantly highest fresh shoot weight was obtained at non-inoculated check which was statistically at par with 2 and 4g mycelium/kg soil. The significant reduction in fresh shoot weight was observed at 6g mycelium/kg soil. Maximum reduction in fresh shoot weight was recorded in plants inoculated with 10g of *F.oxysporum* f.sp. *psidii* followed by 8g mycelium /kg soil.

4.3.3 Dry shoot weight

It was inferred from data in Table 4 that the maximum and significantly highest dry shoot weight was observed in non-inoculated check which was at par with 2 and 4g mycelium/kg soil. The significant reduction in dry shoot weight was observed from 6g mycelium of *F.oxysporum* f.sp. *psidii* which was at par with inoculum level of 8 g mycelium per kg soil. The significantly lowest dry shoot weight was recorded at 10g mycelium/kg soil.

4.3.4 Fresh root weight

The perusal of the data in Table 4 indicated that the fresh root weight was significantly highest in non-inoculated check which was statistically at par with 2 g mycelium. The significant reduction in fresh root weight was observed at 4g mycelium onwards, but it was significantly different from inoculum level of 6g mycelium. The fresh root weight was significantly at par in treatments of 6 and 8g mycelium/kg soil and statistically lowest fresh root weight was recorded at 10g mycelium/kg soil.

4.3.5 Dry root weight

The data in Table 4 clearly exhibited that dry root weight decreased as inoculum levels increased from 2 to 10 g mycelium per kg soil. The maximum and significantly highest dry root was found in non-inoculated check which was statistically at par with inoculum level of 2 and 4 g mycelium per kg soil. The significant reduction of dry root weight was observed at 6g mycelium which was significantly at par with 8 and 10g mycelium and maximum reduction in dry root weight was observed at 10 g mycelium/kg soil.

4.3.6 Per cent root rot

The results in the Table 4 indicated that root rot increased as inoculum level increased from 2 to 10g mycelium per kg soil. Significantly highest root rot (45.33%) was observed in plants inoculated with inoculum level of 10g mycelium per kg soil followed by 8g mycelium per kg soil (39.50%) and 6g mycelium per kg soil (34.37%) and least was observed 2g mycelium per kg soil (8.60).

4.4 Effect of individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii* on growth parameters of guava

The interaction studies of *M. incognita* and *F. oxysporum* f.sp. *psidii* was conducted under screen house conditions by combined and sequential inoculation to evaluate their individual and combined effect on growth parameters of guava seedlings. The effect of individual or sequential inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii* on growth parameters was studied as described in 'Materials and Methods'. The significant decrease in growth parameters *viz*., shoot length, fresh and dry shoot and root weight of guava seedlings were observed in all the treatments in comparison to non-inoculated check (no fungus and no nematode) and data is presented in Table 5.

4.4.1 Shoot length

The significant reduction in shoot length was observed among the treatments when compared with non-inoculated check. Maximum and significantly highest shoot length was recorded in the non-inoculated check (35.26 cm) followed by fungus alone (28.2 cm). The statistically lowest shoot length (15.40 cm) was recorded in the treatment inoculating nematode 10 days prior to fungus which was at par with nematode 20 days prior to fungus (16.67 cm). There was significant reduction in shoot length where plants were inoculated with nematode and fungus simultaneously (23.80 cm) which was significantly at par with nematode alone (24.70 cm), fungus 10 days (25.63 cm) and 20 days (26.47 cm) prior to nematode (Table 5). Plants inoculated with nematode alone caused significant reduction in shoot length as compared to fungus alone.

4.4.2 Fresh shoot weight

The data in Table 5 exhibited that the highest fresh shoot weight was registered in the non-inoculated check (8.07 g) and which was statistically superior over rest of the treatments.

Minimum and significantly lowest fresh shoot weight was recorded with inoculation of nematode 10 days (3.84 g) and 20 days (4.16 g) prior to fungus which were significantly at par with simultaneous inoculation of nematode and fungus (4.82 g). There was reduction in fresh shoot weight where plants were inoculated with nematode alone, fungus 10 days and 20 days prior to nematode as compared to non-inoculated check and were significantly at par with each other.

4.4.3 Dry shoot weight

The results from the Table 5 depicted that minimum and significantly lowest dry shoot weight was registered in treatment having inoculation with nematode 10 days prior fungus (1.23 g) which was significantly at par with nematode 20 days prior to fungus (1.50 g) followed by simultaneous inoculation of nematode and fungus (1.90 g). The significantly highest dry shoot weight was recorded in non-inoculated check (3.23 g).

4.4.4 Fresh root weight

Data in the Table 5 indicated that maximum fresh root weight was recorded in non-inoculated check (4.13g) which was statistically highest and significantly different from other treatments. Minimum and significantly lowest fresh root weight was recorded in plants inoculated with nematode 10 days prior to fungus (2.40) which was at par with nematode 20 days prior to fungus (2.77 g) and simultaneous inoculation of nematode and fungus (2.80 g).

4.4.5 Dry root weight

The data on dry root weight revealed significant reduction of dry root weight in treatments inoculated with nematode alone and combined inoculation of nematode and fungus. However, minimum and statistically lowest dry root weight (0.80 g) was recorded in treatment having nematode 10 days prior to fungus. Significantly highest weight (1.63 g) was noticed in non-inoculated check (Table 5).

Thus, a considerable reduction in plant growth parameters was recorded in the treatments inoculated with *M. incognita* 10 days and 20 days prior to inoculation of *F. oxysporum* f.sp. *psidii* followed by simultaneous inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*. Therefore, combined infection by *M. incognita* and *F. oxysporum* f.sp. *psidii* caused maximum and significant reduction of plant growth parameters than that of individual effect. The results also revealed that nematode and fungus inoculated individually also caused a significant reduction of plant growth parameters in comparison to non-inoculated check. However, nematode inoculated individually caused significant reduction of plant growth parameters as compared to fungus alone.

Table 5: Effect of individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii* on growth parameters of guava

Sr. No.	Treatments	Shoot length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)
1	Nematode alone	24.70	5.25	2.10	2.90	1.10
2	Fungus alone	28.20	6.93	2.76	3.42	1.30
3	Nematode 10 days prior to fungus	15.40	3.84	1.23	2.40	0.80
4	Nematode 20 days prior to fungus	16.67	4.16	1.50	2.77	1.10
5	Fungus 10 days prior to nematode	25.63	5.80	2.23	3.20	1.17
6	Fungus 20 days prior to nematode	26.47	6.03	2.46	3.50	1.40
7	Nematode+fungus simultaneously	23.80	4.82	1.90	2.80	1.07
8	Non-inoculated check	35.26	8.07	3.23	4.13	1.63
	C.D. at 5 per cent	2.73	1.02	0.45	0.41	0.37

Mean of three replications

4.5 Effect of individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii* on multiplication and reproduction of *M. incognita*

The effect of individual, combined or sequential inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii* on multiplication and reproduction of nematode was studied and data is presented in Table 6. The reproduction of *M. incognita* was affected by combined or sequential inoculation of *F. oxysporum* f.sp. *psidii* as fungus affected the nematode infection (Plate 5).

Table 6: Effect of individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii* on multiplication and reproduction of *M. incognita*

Sr. No.	Treatments	Number of galls/plant	Number of egg masses /plant	Number of eggs/egg mass	Final nematode population j_2/200 cc soil	Per cent root rot
1	Nematode alone	175.00 (13.27)*	88.33 (9.42)*	282.00 (16.82)*	321.67 (17.95)*	5.62 (13.58)**
2	Fungus alone	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	31.00 (33.83)
3	Nematode 10 days prior to fungus	169.79 (13.06)	75.52 (8.71)	255.00 (16.00)	270.67 (16.46)	43.67 (41.36)
4	Nematode 20 days prior to fungus	172.34 (13.17)	79.69 (8.96)	269.67 (16.45)	290.33 (17.10)	39.34 (39.21)
5	Fungus 10 days prior to nematode	137.78 (11.75)	65.00 (8.12)	248.33 (15.79)	241.22 (15.51)	35.50 (36.57)
6	Fungus 20 days prior to nematode	123.56 (11.14)	56.67 (7.58)	231.67 (15.25)	233.65 (15.25)	33.17 (35.16)
7	Nematode + fungus simultaneously	145.67 (12.08)	61.00 (7.87)	253.33 (15.95)	260.00 (16.15)	38.00 (38.06)
	C.D. at 5 per cent	(0.32)	(0.53)	(0.38)	(0.62)	(1.67)

* Figures in parentheses are $\sqrt{n}$ +1 transformed values, ** Figures in parentheses Arc sine transformation

4.5.1 Number of galls per plant

The effect of combined and chronological inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii* indicated that there was reduction in number of galls in all the treatment combinations as compared to nematode alone (Table 6). The maximum and significantly highest number of galls was recorded in plants inoculated with nematode alone (175.00) followed by plants inoculated with nematode 20 days (172.34) prior to fungus inoculation. The significantly lowest number of galls (123.56) was recorded in plants inoculated with fungus 20 days prior to nematode which was significantly at par with fungus 10 days prior to nematode (137.78). However, significant reduction in number of galls was recorded in simultaneous inoculation of nematode and fungus (145.67) as compared to nematode alone.

4.5.2 Number of egg masses per plant

The significantly highest number of egg masses (88.33) was observed in plants inoculated with nematode alone followed by inoculation with nematode 20 days (79.96) prior to fungus inoculation which were statistically at par with each other. The significant reduction in egg mass formation was recorded in plants inoculated with fungus prior to nematode as well as in simultaneous inoculation of nematode and fungus (Table 6).

4.5.3 Number of eggs per egg mass

The highest and significant number of eggs per egg mass was recorded in treatment inoculated with nematode alone. The significant reduction in number of eggs per egg mass was observed in the treatments inoculated with combined and sequential inoculation of *M. incognita* and *F.oxysporum* f.sp. *psidii*. The significantly lowest number of eggs per egg mass was recorded on plants inoculated with fungus 20 days prior to nematode inoculation (Table 6).

4.5.4 Final nematode population

Data in Table 6 depicted that the considerable reduction in final nematode population was observed in combined and sequential inoculation of *M. incognita* and *F.oxysporum* f.sp. *psidii*. The final nematode population was significantly highest in plants inoculated with nematode alone, followed by plants inoculated with nematode 20 days prior to fungus which were statistically different from each other. The significantly lowest nematode population was recorded in plants inoculated with fungus 20 days prior to the nematode which was at par with fungus 10 days prior to nematode. The simultaneous inoculation of nematode and fungus also reduced the final nematode population significantly as compared to nematode alone.

The combined or sequential inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii* reduced the nematode reproduction factors *viz*., number of galls, number of egg mass, number of eggs per egg mass and final nematode population as compared to nematode alone.

4.5.5 Per cent root rot

Significantly highest root rot was observed in plants inoculated with *M. incognita* 10 days prior to inoculation of *F. oxysporum* f.sp. *psidii* (43.67%) followed by nematode 20 days prior to fungus (39.34%) and simultaneous inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii* (38.00 %). The inoculation of *F. oxysporum* f.sp. *psidii* prior to *M. incognita* also increased root rot in comparison to plants inoculated with fungus alone. Thus, the presence of both nematode and fungus caused more root rot in comparison to plants inoculated with nematode or fungus individually.

4.6 Management of guava decline by various management practices under screen house conditions

4.6.1 Effect of different oil cakes on plant growth parameters of guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

The addition of organic matter to the soil is very effective method for modifying the soil environment and also plant rhizosphere, which affects the life cycle of plant parasitic nematodes and soil borne pathogens. Some oil cakes have been reported effective against plant pathogens as they possess nematicidal and fungistatic properties and thereby suppress the soil borne pathogens. Thus, different deoiled cakes *viz*., neem, mustard and castor were used against *M. incognita* and *F. oxysporum* f.sp. *psidii* on guava seedlings under screen house conditions. The pot experiment was conducted as described in 'Materials and Methods' to find out the effect of different oil cakes against *M. incognita* and *F. oxysporum* f.sp. *psidii* on growth parameters, nematode reproduction factors and per cent root rot and results are presented in Table 7 to Table 11.

4.6.1.1 Shoot length

Perusal of data in Table 7 revealed that shoot length was significantly highest in non-inoculated check which was superior over other treatments. Among different oil cakes tested, the maximum shoot length was recorded in treatments having neem cake and mustard cake (30g /kg soil) irrespective of individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*. Although castor cake showed a significant effect on shoot length in comparison to untreated inoculated check (nematode or fungus alone or in combined inoculation) but was not as effective as neem and mustard cakes. The reduction of shoot length was more in plants inoculated with both nematode and fungus than that of individual inoculation; however, nematode alone reduced the shoot length more than the fungus alone.

In case of plants inoculated with fungus alone, the significantly highest shoot length was recorded in untreated non-inoculated check (47.30 cm) followed by carbendazim 50 WP at 0.2% (46.21 cm) and neem cake @ 30 g (45.33 cm) which were at par with each other. However, mustard cake @ 30 g recorded the shoot length of 44.30 cm and which was at par with neem cake @ 30 g/kg soil (Plate 6).

Fig. 2 Effect of individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii* on growth parameters of guava

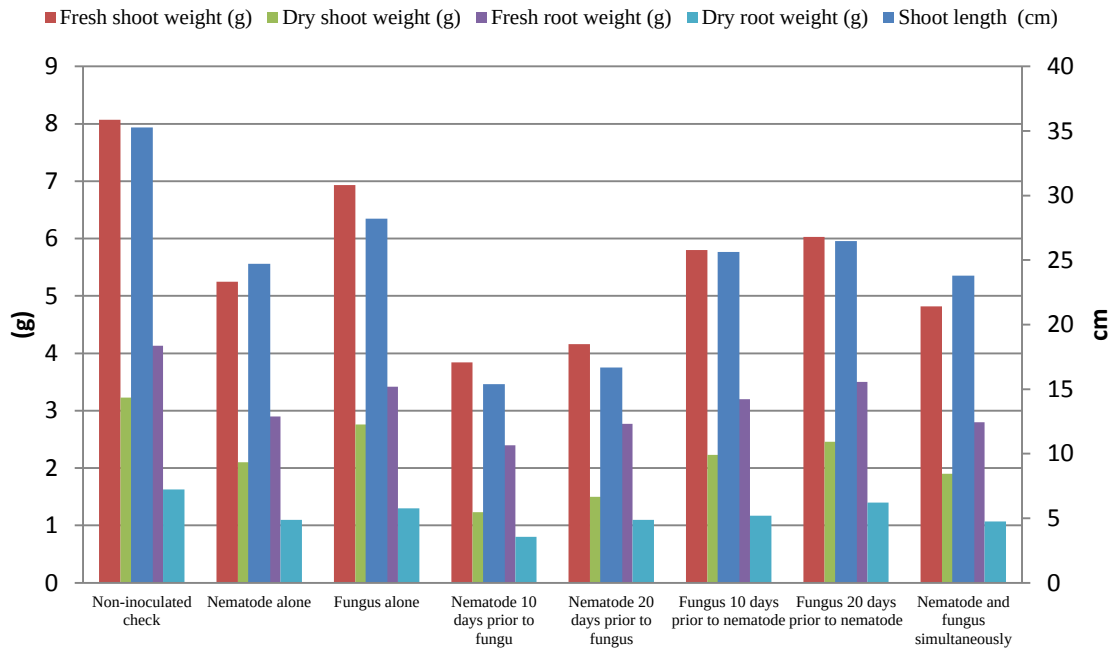
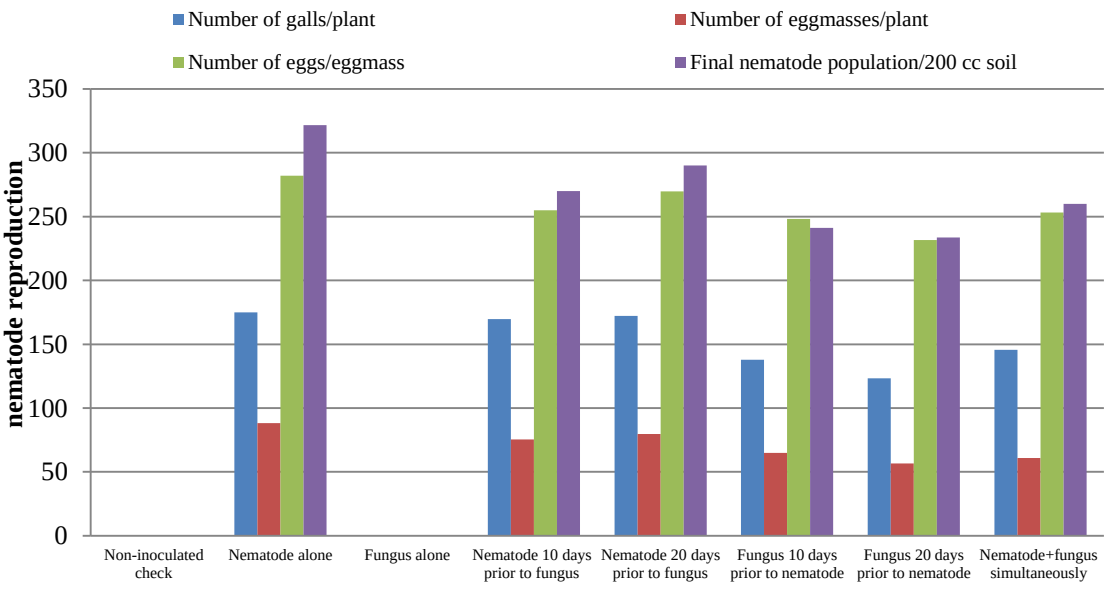


Fig.3 Effect of individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii* on multiplication and reproduction of *M. incognita*



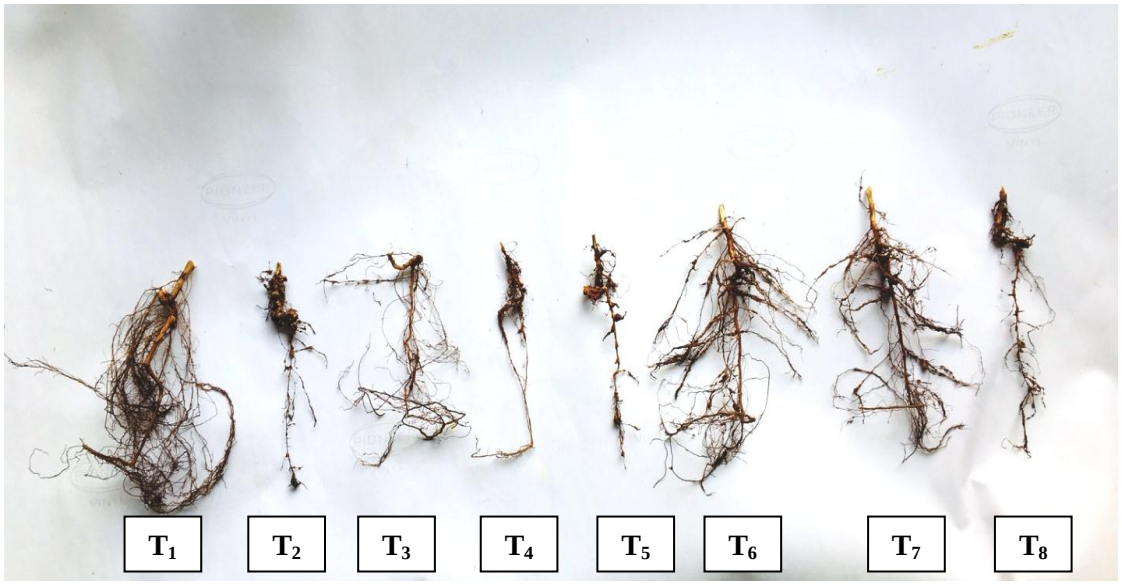


Plate 5 Effect of individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii* on growth parameters of guava

T1: Non- inoculated check

T2: Nematode alone

T3: Fungus alone

T4: Nematode 10 days prior to inoculation of fungus

T5: Nematode 20 days prior to inoculation of fungus

T6: Fungus 10 days prior to inoculation of Nematode

T7: Fungus 20 days prior to inoculation of Nematode

T8: Nematode + Fungus simultaneously

Table 7: Effect of different oil cakes on shoot length of guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

Sr. No	Treatments	Shoot length(cm)		
		Fungus alone	Nematode alone	Nematode +Fungus
1	Neem cake @ 20g /kg of soil	41.23	37.50	34.30
2	Neem cake @ 30g/kg of soil	45.33	41.77	39.13
3	Mustard cake @ 20g/kg of soil	38.30	32.37	33.57
4	Mustard cake @ 30g/kg of soil	44.30	39.83	38.77
5	Castor cake @ 20g/kg of soil	35.23	30.67	34.10
6	Castor cake @ 30g/kg of soil	37.77	34.33	36.24
7	Carbofuran @ 0.1/kg of soil	33.67	39.63	36.30
8	Carbendazim 50 WP @ 2g/lt of water	46.21	34.37	33.10
9	Untreated inoculated check	31.30	27.80	28.53
10	Untreated non-inoculated check	47.30	43.33	42.63
	C.D. at 5 per cent	2.72	3.22	2.61

Mean of three replications

Table 8: Effect of different oil cakes on fresh shoot weight of guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

Sr. No	Treatments	Fresh shoot weight (g)		
		Fungus alone	Nematode alone	Nematode +Fungus
1	Neem cake @ 20g /kg of soil	13.50	10.27	9.30
2	Neem cake @ 30g/kg of soil	16.17	14.07	12.77
3	Mustard cake @ 20g/kg of soil	11.57	9.33	9.07
4	Mustard cake @ 30g/kg of soil	14.73	12.20	11.60
5	Castor cake @ 20g/kg of soil	12.87	9.10	8.83
6	Castor cake @ 30g/kg of soil	13.60	11.00	10.97
7	Carbofuran @ 0.1g/kg of soil	14.23	11.93	12.17
8	Carbendazim 50 WP @ 2g/lt of water	17.43	9.50	11.33
9	Untreated inoculated check	10.80	8.63	7.77
10	Untreated non-inoculated check	18.83	16.67	14.13
	C.D. at 5 per cent	2.23	2.15	2.38

Mean of three replications

Table 9: Effect of different oil cakes on dry shoot weight of guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

Sr. No.	Treatments	Dry shoot weight (g)		
		Fungus alone	Nematode alone	Nematode + Fungus
1	Neem cake @ 20g /kg of soil	5.47	4.27	3.60
2	Neem cake @ 30g/kg of soil	6.23	5.93	5.17
3	Mustard cake @ 20g/kg of soil	4.57	3.90	3.63
4	Mustard cake @ 30g/kg of soil	5.43	4.83	4.57
5	Castor cake @ 20g/kg of soil	5.10	3.80	3.33
6	Castor cake @ 30g/kg of soil	5.30	4.57	4.47
7	Carbofuran @ 0.1g/kg of soil	5.87	4.80	4.80
8	Carbendazim 50 WP @ 2g/lt of water	6.63	3.97	4.37
9	Untreated inoculated check	4.13	3.73	3.10
10	Untreated non-inoculated check	7.50	6.43	5.83
	C.D. at 5 per cent	1.25	0.74	1.28

Mean of three replications

Table 10: Effect of different oil cakes on fresh root weight of guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

Sr. No.	Treatments	Fresh root weight (g)		
		Fungus alone	Nematode alone	Nematode + Fungus
1	Neem cake @20g /kg of soil	5.27	4.63	5.10
2	Neem cake @30g/kg of soil	6.84	5.93	5.77
3	Mustard cake @20g/kg of soil	5.12	4.47	4.00
4	Mustard cake @30g/kg of soil	5.94	5.30	4.63
5	Castor cake @20g/kg of soil	4.81	4.50	4.17
6	Castor cake @30g/kg of soil	5.50	4.90	4.30
7	Carbofuran @0.1g/kg of soil	4.63	5.53	5.43
8	Carbendazim 50 WP @2g/lt of water	7.02	4.40	5.10
9	Untreated inoculated check	4.33	4.07	3.40
10	Untreated non-inoculated check	7.27	6.53	6.27
	C.D. at 5 per cent	1.63	1.14	1.25

Mean of three replications

Fig. 4 Effect of different oil cakes on fresh shoot weight of guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

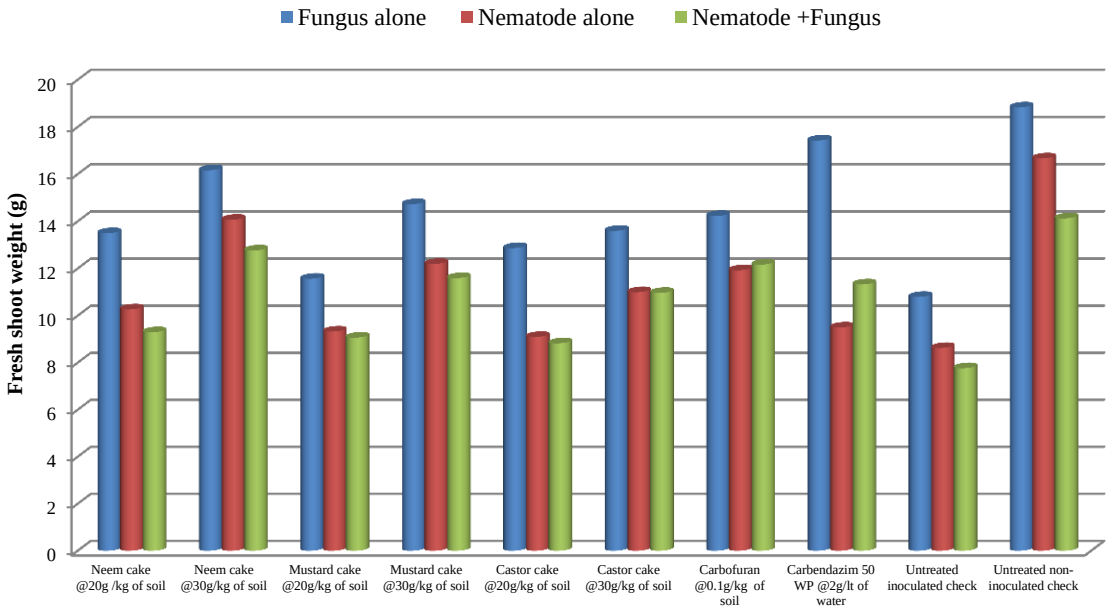
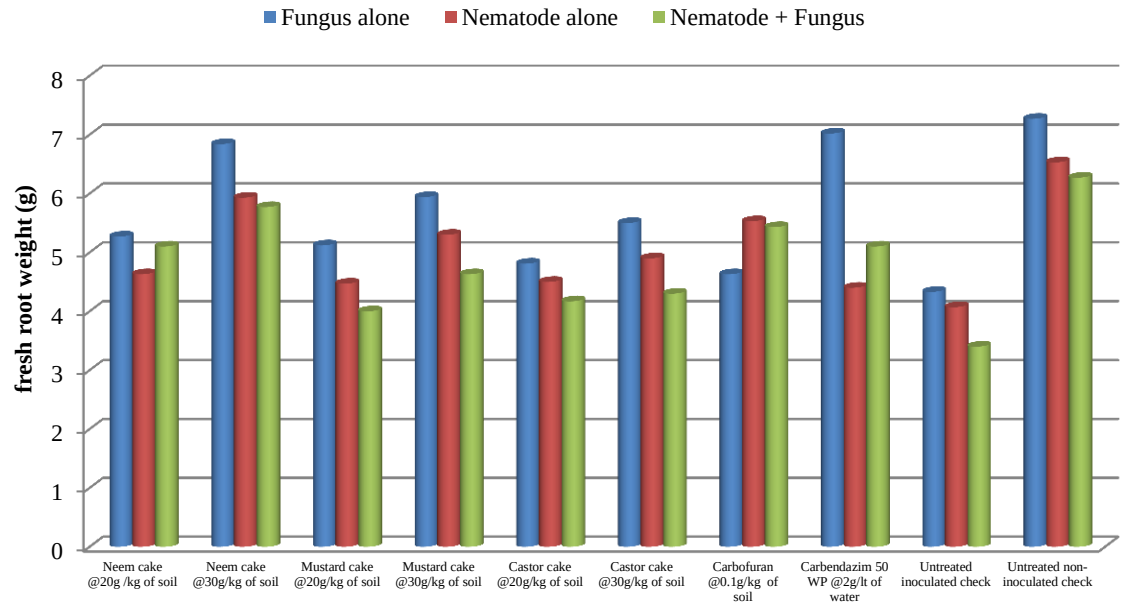


Fig.5 Effect of different oil cakes on fresh root weight of guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*



With respect to nematode alone, among oil cakes tested, the maximum shoot length was recorded in plants inoculated with neem cake @ 30 g (41.77 cm) followed by mustard cake @ 30 g (39.83 cm) and carbofuran (39.63 cm) which were significantly at par with each other (Plate 7).

In case of combined inoculation of nematode and fungus, significantly highest shoot length was recorded in untreated non-inoculated check (42.63 cm) followed by neem cake (39.13 cm) and mustard cake (38.77) at 30 g/kg soil. The plants receiving treatment of carbofuran (36.30 cm) and carbendazim 50 WP (33.10 cm) had lower shoot length than oil cakes, as they controlled either of the pathogens inoculated (Plate 8).

4.6.1.2 Fresh shoot weight

It was inferred from data in the Table 8 that there was a significant increase in fresh shoot weight in all the treatments in comparison to untreated inoculated check. Maximum reduction in fresh shoot weight was observed in plants inoculated with *M. incognita* and *F. oxysporum* f. sp. *psidii* followed by nematode alone while the minimum was in fungus alone. Among different oil cakes, the maximum fresh shoot weight was observed in neem and mustard cake at 30 g per kg soil irrespective of nematode alone or fungus alone or nematode and fungus in combined inoculation (Fig 4).

With respect to fungus alone, the significantly highest fresh shoot weight (18.83 g) was recorded in non-inoculated check which was at par with carbendazim 50 WP at 0.2% (17.43 g) followed by neem cake at 30g/kg soil (16.17g) and mustard cake at 30g/kg soil (14.73 g). Statistically, the lowest fresh shoot weight (10.80g) was recorded in untreated inoculated check.

In case of nematode alone, among oil cakes tested, the significantly highest fresh shoot weight was recorded in neem cake @ 30g (14.07 g) which was at par with mustard cake at 30g (12.20 g). Castor cake and carbofuran significantly increased the fresh shoot weight in comparison to untreated inoculated check, but were not as effective as neem cake or mustard cake. Significantly lowest fresh shoot weight was recorded in untreated inoculated check (8.63g) which was at par with carbendazim 50 WP (9.50 g).

In the combined inoculation of nematode and fungus, the reduction of fresh shoot weight was more when compared to nematode and fungus inoculated individually. The maximum and statistically highest fresh shoot weight was recorded in untreated non-inoculated check (14.13 g) followed by neem cake @ 30 g (12.77 g), mustard cake @ 30 g (11.60 g) and carbofuran (12.17 g) which were statistically at par with each other.

4.6.1.3 Dry shoot weight

Results in Table 9 revealed that among oil cakes tested, the dry shoot weight was significantly highest when plants received neem cake at 30 g/kg soil irrespective of

inoculation of nematode or fungus individually or in combination. In case of fungus alone, the significantly highest dry shoot weight was recovered in control (7.5 g) which was at par with carbendazim 50 WP (6.63g). With respect to nematode alone, dry shoot weight was statistically highest in neem cake at 30g (5.93 g). In case of combined inoculation of nematode and fungus, the dry shoot weight was significantly highest in control (5.83g) which was at par with neem cake (5.17 g), mustard cake (4.57 g) at 30g/kg soil and carbofuran (4.80 g). The significantly lowest dry shoot was recorded in untreated inoculated check whether nematode alone (4.13g), fungus alone (3.73g) and combined inoculation of nematode and fungus (3.10g).

Table 11: Effect of different oil cakes on dry root weight of guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

Sr. No.	Treatments	Dry root weight (g)		
		Fungus Alone	Nematode alone	Nematode + Fungus
1	Neem cake @ 20g /kg of soil	1.90	1.77	1.83
2	Neem cake @ 30g/kg of soil	2.60	2.30	2.07
3	Mustard cake @ 20g/kg of soil	1.83	1.63	1.63
4	Mustard cake @ 30g/kg of soil	2.10	1.87	1.73
5	Castor cake @ 20g/kg of soil	1.70	1.73	1.51
6	Castor cake @ 30g/kg of soil	2.07	1.93	1.68
7	Carbofuran @ 0.1g/kg of soil	1.80	2.07	2.20
8	Carbendazim 50 WP @ 2g/lt of water	2.59	1.60	1.86
9	Untreated inoculated check	1.66	1.47	1.43
10	Untreated non-inoculated check	2.63	2.37	2.27
	C.D. at 5 per cent	0.69	0.53	0.58

Mean of three replications

Table 12: Effect of different oil cakes on number of galls in guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

Sr. No.	Treatments	Number of galls	
		Nematode alone	Nematode + Fungus
1	Neem cake @20g /kg of soil	112.00 (10.63)	104.33(10.26)
2	Neem cake @30g/kg of soil	94.66 (9.77)	86.67 (9.36)
3	Mustard cake @20g/kg of soil	115.00 (10.78)	110.00 (10.52)
4	Mustard cake @30g/kg of soil	106.67 (10.37)	93.33 (9.69)
5	Castor cake @20g/kg of soil	140.00(11.87)	123.33 (11.15)
6	Castor cake @30g/kg of soil	121.66 (11.07)	115.00 (10.77)
7	Carbofuran @0.1g/kg of soil	107.33 (10.40)	91.67 (9.62)
8	Carbendazim 50 WP @2g/lt of water	171.66 (13.14)	165.00 (12.88)
9	Untreated inoculated check	186.00 (13.67)	154.00 (12.45)
10	Untreated non-inoculated check	0.00 (1.00)	0.00 (1.00)
	C.D. at 5 per cent	(0.68)	(0.74)

Mean of three replications

Figures in parentheses are $\sqrt{n}$ +1 transformed value

4.6.1.4 Fresh root weight

Among oil cakes tested, the highest fresh root weight was recorded in treatment where neem cake was incorporated at 30 g/kg soil (Table 10). Lowest fresh root weight was observed in untreated inoculated check where plants were inoculated with nematode and fungus individually or in combined inoculation (Fig. 5).

In case of fungus alone, maximum and significantly highest fresh root weight was recorded in untreated non-inoculated check (7.27g) which was at par with carbendazim 50 WP (7.02 g), neem cake (6.84 g) and mustard cake @ 30g (5.97 g). Plants inoculated with nematode alone, had significantly increased root weight with neem cake at 30 g/kg soil (5.93 g) which was at par with carbofuran 0.1g/kg soil (5.53 g). With respect to combined inoculation, fresh root weight was significantly increased where treatments were inoculated with neem cake at 30g, carbofuran at 0.1g and carbendazim 50 WP at 0.2% and were at par with each other.

4.6.1.5 Dry root weight

The results from Table 11 depicted that the significantly maximum shoot weight was recorded in untreated non-inoculated check. The dry root weight was increased significantly in all three cakes tested @ 30g and were at par with carbofuran and carbendazim irrespective of individual or combined inoculation of nematode and fungus.

4.7 Effect of different oil cakes on multiplication and reproduction of *M. incognita* on guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

4.7.1 Number of galls per plant

Perusal of data in Table 12 indicated that there was a significant reduction in number of galls in all the treatment when compared to untreated inoculated check (Fig. 6).

In case of nematode alone, the significantly maximum number of galls was observed in untreated inoculated check (186.00) which was at par with carbendazim 50 WP (171.00). Significantly lowest number of galls was observed in the treatment of neem cake at 30g/kg soil (94.66) which was at par with mustard cake at 30g (106.67) and carbofuran (107.33). However, the significant reduction in number of galls was registered where plants were inoculated with castor cake @ 30g (121.66) when compared to untreated inoculated check.

With respect to combined inoculation, the number of galls per plant was reduced due to combined inoculation of nematode and fungus, when compared with nematode alone conditions. The maximum number of galls was recorded in carbendazim 50 WP (165.00) as it suppressed the fungus and thus nematode infects the roots freely and caused maximum galling. The significantly lowest number of galls was recorded in treatment of neem cake at 30g (86.67) which was at par with carbofuran (91.67) and mustard cake at 30 g (93.33).

4.7.2 Number of egg masses per plant

The data in Table 13 revealed that there was significant decrease in number of egg masses when compared with the untreated inoculated check. In case of nematode alone, the maximum and significantly highest number of egg masses was observed in plants with untreated inoculated check (102.33). The significant reduction and lowest number of egg masses was recorded in neem cake @ 30g (49.00) which was at par with carbofuran, mustard cake @ 30g. However, castor cake

Table 13: Effect of different oil cakes on number of egg masses in guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

Sr. No.	Treatments	Number of egg masses/plant	
		Nematode alone	Nematode + Fungus
1	Neem cake @20g /kg of soil	60.00 (7.80)	48.33 (7.02)
2	Neem cake @30g/kg of soil	49.00 (7.06)	34.33 (5.93)
3	Mustard cake @20g/kg of soil	65.33 (8.13)	58.42 (7.73)
4	Mustard cake @30g/kg of soil	56.00 (7.54)	40.67 (6.45)
5	Castor cake @20g/kg of soil	71.33 (8.49)	54.67 (7.46)
6	Castor cake @30g/kg of soil	61.67 (7.90)	49.33 (7.09)
7	Carbofuran @0.1g/kg of soil	55.24 (7.51)	36.33 (6.10)
8	Carbendazim 50 WP @2g/lt of water	95.67 (9.81)	75.00 (8.71)
9	Untreated inoculated check	102.33 (10.15)	65.00 (8.11)
10	Untreated non-inoculated check	0.00 (1.00)	0.00 (1.00)
	C.D. at 5 per cent	(0.75)	(0.62)

Mean of three replications

Figures in parentheses are $\sqrt{n}$ +1 transformed value

Table 14: Effect of different oil cakes on number of eggs/egg mass in guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

Sr. No.	Treatments	Number of eggs/egg mass	
		Nematode alone	Nematode + Fungus
1	Neem cake @20g /kg of soil	275.00 (16.61)	258.33 (16.10)
2	Neem cake @30g/kg of soil	252.33 (15.90)	251.67 (15.89)
3	Mustard cake @20g/kg of soil	273.67 (16.57)	263.33 (16.25)
4	Mustard cake @30g/kg of soil	278.33 (16.71)	262.00 (15.84)
5	Castor cake @20g/kg of soil	275.00 (16.61)	260.00 (16.15)
6	Castor cake @30g/kg of soil	277.67 (16.69)	265.00 (16.30)
7	Carbofuran @0.1g/kg of soil	250.33 (15.00)	252.00 (15.90)
8	Carbendazim 50 WP @2g/lt of water	281.67 (16.81)	260.00 (16.15)
9	Untreated inoculated check	291.67 (17.11)	278.33 (16.71)
10	Untreated non-inoculated check	0.00 (1.00)	0.00 (1.00)
	C.D. at 5 per cent	(0.32)	(0.41)

Mean of three replications

Figures in parentheses are $\sqrt{n}$ +1 transformed value

@ 30g also reduced the number of egg masses when compared to untreated inoculated check which was at par with mustard cake @ 30g/kg soil.

In case of nematode and fungus inoculation, significantly lowest number of egg masses was recorded in neem cake @ 30g (34.33) and highest number of egg masses was recorded in carbendazim 50 WP (75.00) as it controls fungus, thus nematode infected roots without any competition. In combined inoculation of nematode and fungus, carbofuran caused more reduction in number of egg masses as compared to carbofuran in nematode alone.

4.7.3 Number of eggs per egg mass

Data in Table 14 revealed that the number of eggs per egg mass in all treatments was significantly different from untreated inoculated check. The significantly lowest number of eggs per egg mass was recorded in carbofuran and neem cake @ 30 g which were statistically at par. The number of eggs per egg mass was reduced in combined inoculation of nematode and fungus as compared to nematode alone. Similar trend was observed in combined inoculation treatments.

4.7.4 Final nematode population

Data in the Table 15 clearly indicated that different oil cakes had a pronounced effect on the final nematode population. Neem cake @ 30g significantly reduced final nematode population which was at par with carbofuran followed by mustard cake @ 30g. In case of combined inoculation, the nematode population was reduced due to the presence of fungus as it interfered with the nematode infection. The nematode population was maximum in treatment receiving carbendazim 50 WP. In combined inoculation also, neem cake @ 30 g had minimum final nematode population which was at par with carbofuran (Fig. 7).

4.7.5 Per cent root rot

Results from Table 16 revealed that maximum root rot was observed where plants were inoculated with nematode and fungus concomitantly than fungus alone. In case of fungus alone, significantly maximum root rot was observed in untreated inoculated check (39.20%) and least root rot was observed in carbendazim (9.33%) followed by neem cake and mustard cake @ 30g. Whereas in nematode and fungus having joint inoculation, the significantly highest root rot was observed in untreated inoculated check (48.33%). Significantly lowest root rot was noticed in plants treated with carbendazim as it suppressed root rot causing fungus.

4.8 Effect of bio-agents on plant growth parameters of guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

The bio-control agents, *Trichoderma viride*, *Pseudomonas fluorescens*, and *Purpureocillium lilacinu* were used against *M. incognita* and *F. oxysporum* f.sp. *psidii* on guava seedling under screen house conditions. The pot experiment was conducted to evaluate the effect of different bio-agents against *M. incognita* and *F. oxysporum* f.sp. *psidii* on growth

parameters, nematode reproduction factors and per cent root rot and results are presented in Table17-21 (Plate 9 and 10).

Table 15: Effect of different oil cakes on final nematode population in guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

Sr. No.	Treatments	Final nematode population (j_2)/200cc soil	
		Nematode alone	Nematode + Fungus
1	Neem cake @20g /kg of soil	241.67 (15.58)	194.33 (13.97)
2	Neem cake @30g/kg of soil	181.67 (13.51)	148.33 (12.21)
3	Mustard cake @20g/kg of soil	255.00 (16.00)	205.00 (14.35)
4	Mustard cake @30g/kg of soil	211.67 (14.58)	173.33 (13.20)
5	Castor cake @20g/kg of soil	270.00 (16.56)	241.67 (15.58)
6	Castor cake @30g/kg of soil	238.33 (15.47)	193.33 (13.94)
7	Carbofuran @0.1g/kg of soil	201.67(14.03)	160.00 (12.69)
8	Carbendazim 50 WP @2g/lt of water	319.00 (17.89)	311.67 (17.68)
9	Untreated inoculated check	368.33 (19.21)	291.67 (17.11)
10	Untreated non-inoculated check	0.00 (1.00)	0.00 (1.00)
	C.D. at 5 per cent	(0.61)	(0.86)

Mean of three replications

Figures in parentheses are $\sqrt{n}$ +1 transformed value

Table 16: Effect of different oil cakes on per cent root rot in guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

Sr. No.	Treatments	Per cent root rot	
		Fungus alone	Nematode + Fungus
1	Neem cake @ 20g /kg of soil	23.67 (29.11)	27.27 (31.47)
2	Neem cake @ 30g/kg of soil	20.90 (27.04)	24.37 (29.57)
3	Mustard cake @ 20g/kg of soil	27.33 (31.52)	30.30 (33.39)
4	Mustard cake @ 30g/kg of soil	23.80 (29.20)	26.07 (30.68)
5	Castor cake @ 20g/kg of soil	30.33 (33.42)	34.67 (36.07)
6	Castor cake @ 30g/kg of soil	28.23 (32.10)	32.13 (34.53)
7	Carbofuran @ 0.1g/kg of soil	36.33 (37.07)	29.00 (32.58)
8	Carbendazim 50 WP @ 2g/lt of water	9.33 (17.79)	20.67 (27.02)
9	Untreated inoculated check	39.20 (38.06)	48.33 (44.04)
10	Untreated non-inoculated check	0.00 (0.00)	0.00 (0.00)
	C.D. at 5 per cent	(2.13)	(2.04)

Mean of three replications

Figures in parentheses are Arc sin transformed values

4.8.1 Shoot length

Perusal of data in Table 17 revealed that shoot length was significantly highest in non-inoculated check under all conditions. Among different bio-agents tested, the treatments receiving combined formulation of *T. viride*, *P. fluorescens*

Fig.6 Effect of different oil cakes on number of galls in guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f. sp. *psidii*

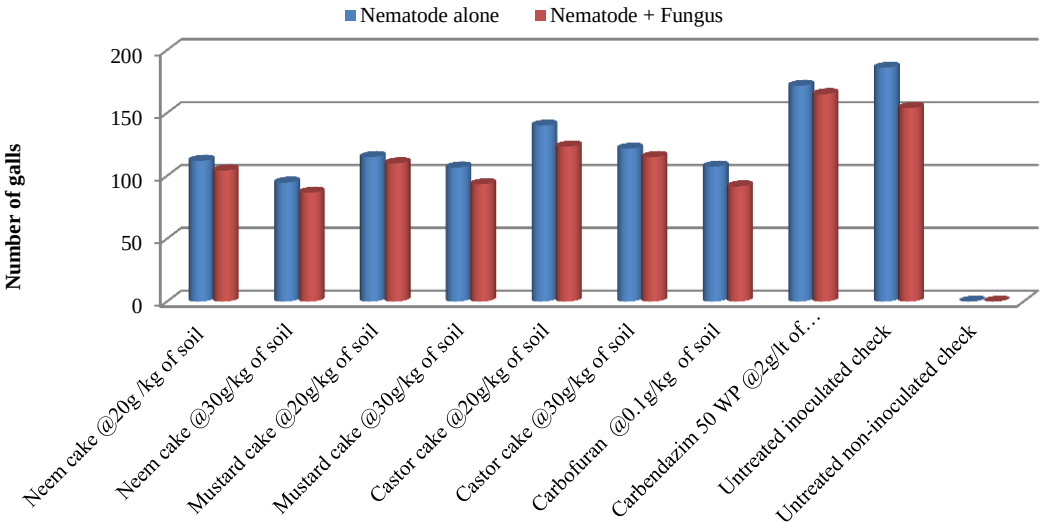
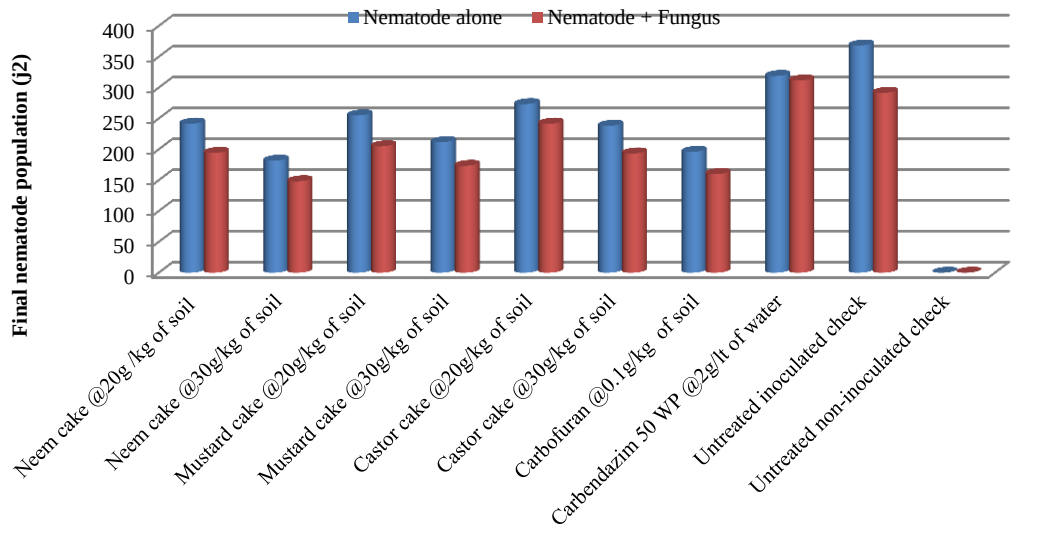


Fig.7 Effect of different oil cakes on final nematode population in guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*



and *P. lilacinum* @ 10ml /kg soil and *T. viride* alone @ 10g/kg soil recorded maximum shoot length irrespective of individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*. Although *P. fluorescens* and *P. lilacinum* when used individually, increased shoot length in comparison to untreated inoculated check but not as effective as combination of

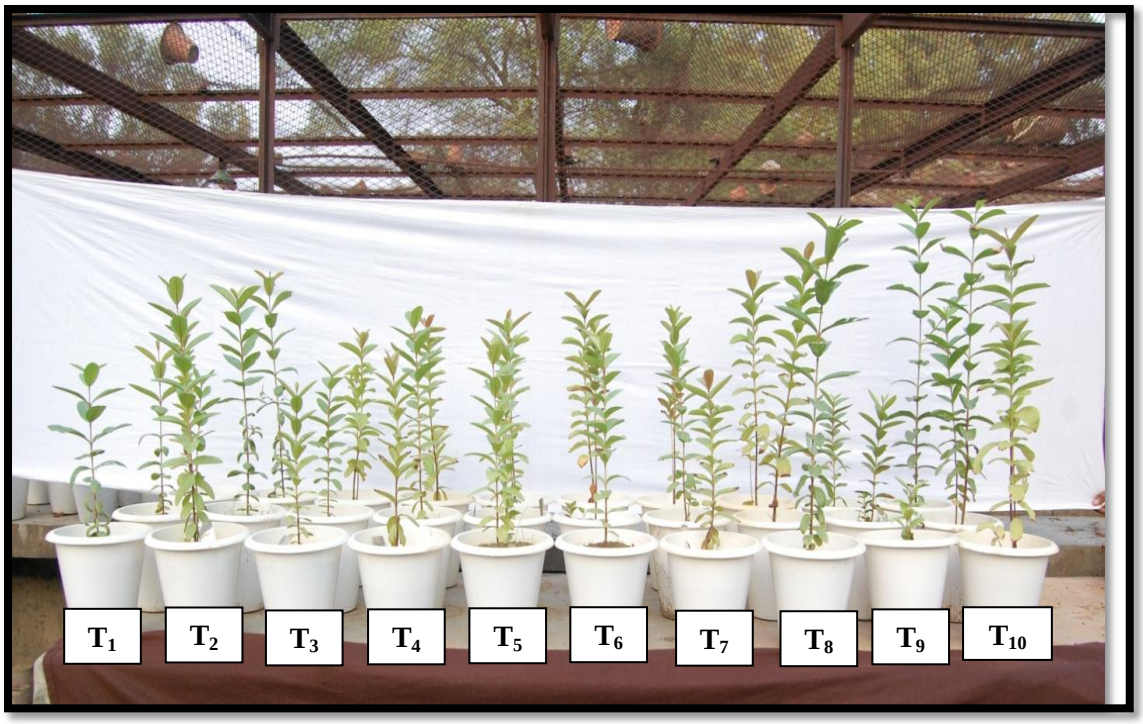


Plate 6 Effect of different oil cakes on growth of guava with inoculation of *F. oxysporum* f.sp. *psidii* alone

T1: Soil application of neem cake @ 20 g/kg of soil

T2: Soil application of neem cake @ 30 g/kg of soil

T3: Soil application of mustard cake @ 20 g/kg of soil

T4: Soil application of mustard cake @ 30 g/kg of soil

T5: Soil application of castor cake @ 20 g/kg of soil

T6: Soil application of castor cake @ 30g/kg of soil

T7: Carbofuran (Furadan) @ 0.1 g/ kg of soil

T8: Carbendazim 50WP @ 2g/liter water

T9: Untreated inoculated check (nematode or fungus alone or in combined inoculation)

T10: Untreated non-inoculated check

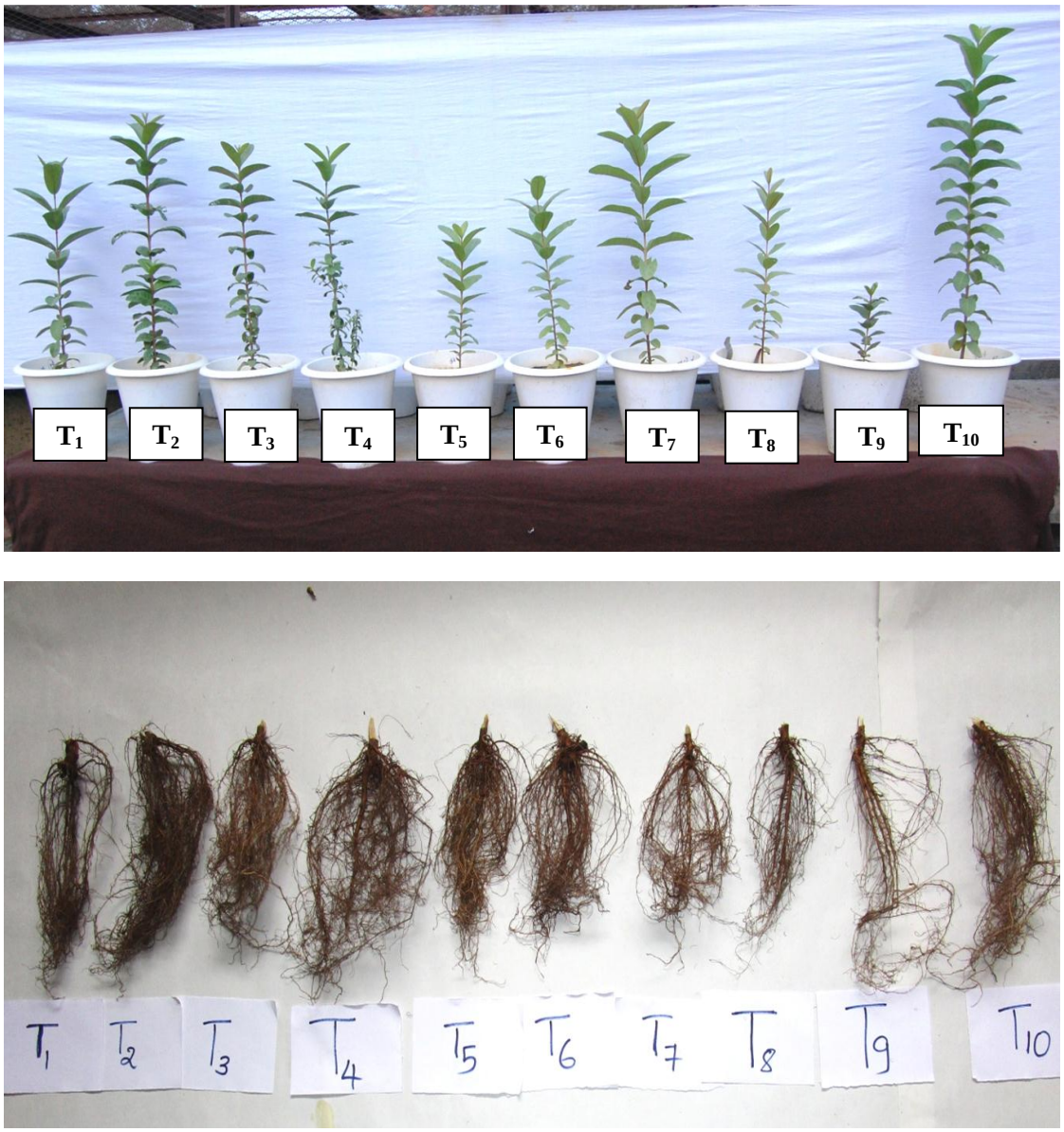


Plate.7 Effect of different oil cakes on growth of guava with inoculation of *M. incognita* alone

T1: Soil application of neem cake @ 20 g/kg of soil
T2: Soil application of neem cake @ 30 g/kg of soil
T3: Soil application of mustard cake @ 20 g/kg of soil
T4: Soil application of mustard cake @ 30 g/kg of soil
T5: Soil application of castor cake @ 20 g/kg of soil
T6: Soil application of castor cake @ 30g/kg of soil
T7: Carbofuran (Furadan) @ 0.1 g/ kg of soil
T8: Carbendazim 50WP @ 2g/liter water
T9: Untreated inoculated check (nematode or fungus alone or in combined inoculation)
T10: Untreated non-inoculated check

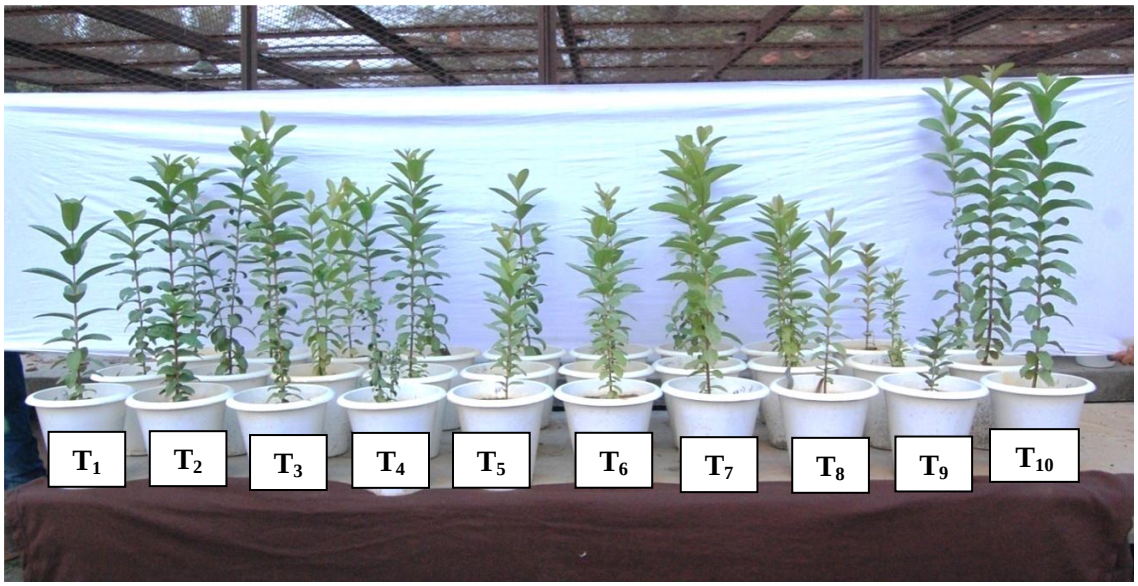


Plate 8 Effect of different oil cakes on growth of guava with combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

T1: Soil application of neem cake @ 20 g/kg of soil

T2: Soil application of neem cake @ 30 g/kg of soil

T3: Soil application of mustard cake @ 20 g/kg of soil

T4: Soil application of mustard cake @ 30 g/kg of soil

T5: Soil application of castor cake @ 20 g/kg of soil

T6: Soil application of castor cake @ 30g/kg of soil

T7: Carbofuran (Furadan) @ 0.1 g/kg of soil

T8: Carbendazim 50WP @ 2g/liter water

T9: Untreated inoculated check (nematode or fungus alone or in combined inoculation)

T10: Untreated non-inoculated check

three bio agents and *T. viride.* The reduction in shoot length was more in plants inoculated with both nematode and fungus than that of individual inoculation; however, nematode alone reduced the shoot length more than the fungus inoculated alone.

In case of plants inoculated with fungus alone, the significantly highest shoot length was recorded in untreated non-inoculated check (44.33 cm) which was at par with carbendazim 50 WP (42.83 cm) followed by combined formulation of *T. viride* + *P. fluorescens* + *P. lilacinu* @ 10ml (41.93 cm). However, among individual application, *T. viride* @ 10g (37.67cm) had highest shoot length.

In plants inoculated with nematode alone, the maximum shoot length was recorded in plants having combined formulation of *T. viride*+ *P. fluorescens* + *P. lilacinum* (38.33 cm) followed by *T. viride* 10g (33.20 cm). Carbofuran @ 0.1g recorded shoot length of 37.30 cm which was at par with combined formulation of bio-agents.

In case of combined inoculation of nematode and fungus, significantly highest shoot length was recorded in untreated non-inoculated check (41.27 cm) followed by combined formulation (37.33 cm). The plants receiving carbofuran (34.37 cm) and carbendazim 50 WP (28.60 cm) were not as effective as bio-agents tested, since they controlled either of pathogens inoculated.

4.8.2 Fresh shoot weight

Data in Table 18 revealed that there was significant increase in fresh shoot weight in all the treatments when compared to untreated inoculated check (Fig. 8). With respect to fungus alone, the significantly highest fresh shoot weight was recorded in untreated non-inoculated check (15.43 g) which was at par with carbendazim 50 WP (14.27 g) and combined formulation of *T. viride* + *P. fluorescens* + *P. lilacinum* (13.80 g) followed by *T. viride* (12.27g). Statistically lowest fresh shoot weight (10.03 g) was recorded in untreated inoculated check.

In case of nematode alone, the significantly highest fresh shoot weight was recorded in untreated non-inoculated check (14.20 g) which was at par with combined formulation of *T. viride* + *P. fluorescens* + *P. lilacinum* (13.20 g) and carbofuran (12.67 g). Significantly lowest fresh shoot weight was recorded in untreated inoculated check (7.63 g) which was at par with carbendazim 50 WP (8.73 g).

In combined inoculation of nematode and fungus, the reduction of fresh shoot weight was more when compared with nematode alone. The maximum and statistically highest fresh shoot weight was recorded in untreated non-inoculated check (12.17 g) which was at par with combined formulation of *T. viride* + *P. fluorescens* + *P. lilacinum* (11.27 g) followed by *T. viride* (9.77 g) and carbofuran (9.20 g) and significantly lowest fresh shoot weight was observed in untreated inoculated check (6.53 g)

Table 17: Effect of bio-agents on shoot length of guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

Sr. No.	Treatments	Shoot length(cm)		
		Fungus Alone	Nematode alone	Nematode +Fungus
1	*T. viride* @ 10g/kg of soil	37.67	33.20	31.40
2	*P. fluorescens* @ 10g/kg of soil	35.27	30.33	29.33
3	*P.lilacinum* @ 10g/kg of soil	32.43	28.83	25.33
4	*T. viride*+ *P. fluorescens* + *P.lilacinum* 10ml/kg of soil	41.93	38.33	37.33
5	Carbofuran @0.1g/kg of soil	33.17	37.30	34.37
6	Carbendazim 50 WP @2g/lt of water	42.83	30.83	28.60
7	Untreated inoculated check	29.20	28.17	24.67
8	Untreated non-inoculated check	44.33	40.50	41.27
	C.D. at 5 per cent	1.79	2.24	2.66

Mean of three replications

Table 18: Effect of bio-agents on fresh shoot weight of guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

Sr. No.	Treatments	Fresh shoot weight (g)		
		Fungus alone	Nematode alone	Nematode +Fungus
1	*T. viride* @ 10g/kg of soil	12.27	11.47	9.77
2	*P. fluorescens* @ 10g/kg of soil	11.57	10.23	8.40
3	*P.lilacinum* @ 10g/kg of soil	10.30	8.33	7.97
4	*T. viride*+ *P. fluorescens* + *P. lilacinum* 10ml/kg of soil	13.80	13.20	11.27
5	Carbofuran @ 0.1g/kg of soil	11.17	12.67	9.20
6	Carbendazim 50 WP @ 2g/lt of water	14.27	8.73	7.60
7	Untreated inoculated check	10.03	7.63	6.53
8	Untreated non-inoculated check	15.43	14.20	12.17
	C.D. at 5 per cent	1.76	2.10	1.54

Mean of three replications

Fig. 8 Effect of bio-agents on fresh shoot weight of guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

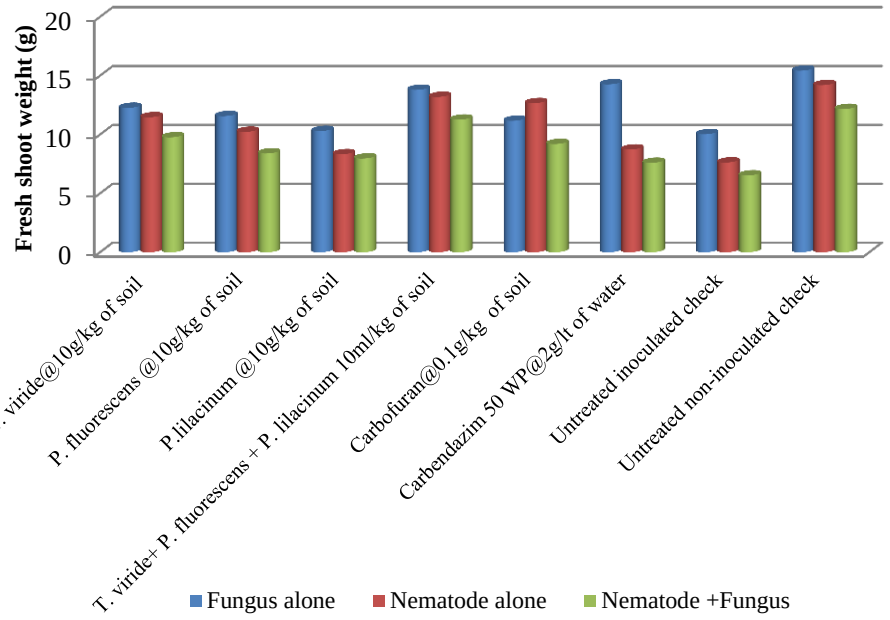
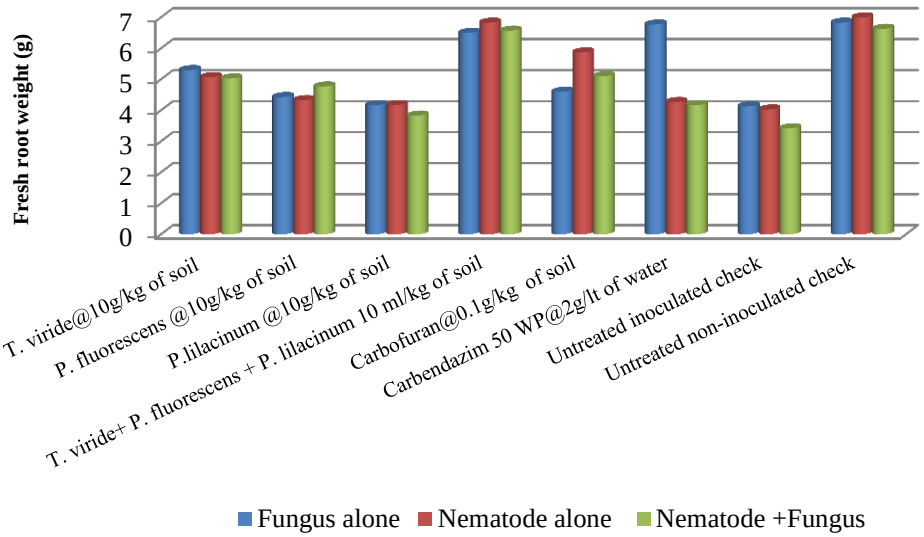


Fig. 9 Effect of bio-agents on fresh root weight of guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*



4.8.3 Dry shoot weight

Results in the Table 19 revealed that the dry shoot weight was increased when plants received combined formulation of *T. viride* + *P. fluorescens* + *P. lilacinum* irrespective whether inoculation of nematode or fungus individually and in combination.

In case of fungus alone, significantly highest dry shoot weight was recovered in untreated non-inoculated check (5.83 g) which was at par with combined formulation of *T. viride* + *P. fluorescens* + *P. lilacinum* (5.67g), carbendazim 50 WP (5.40 g) and *T. viride* (5.10 g) and lowest dry shoot was recorded in untreated inoculated check (3.90 g). With respect to nematode alone, dry shoot weight was statistically highest in untreated non-inoculated check (5.58 g) which was at par with carbofuran (5.03 g) and combined formulation (4.98 g). In case of nematode and fungus, the dry shoot weight was significantly lowest in untreated inoculated check (2.68 g) and significantly highest in untreated non-inoculated check (5.76 g). The combined formulation @ 10 ml recorded dry shoot weight of 4.70 g which was significantly different from carbofuran (3.53 g) and carbendazim 50 WP (3.09 g).

4.8.4 Fresh root weight

Data in the Table 20 indicated that application of combined formulation of *T. viride* + *P. fluorescens* + *P. lilacinum* significantly increased fresh root weight when compared to individual application of bio-agents (Fig. 9).

In case of fungus alone, significantly highest fresh root weight was observed in untreated non-inoculated check (6.83 g) which was at par with carbendazim 50 WP (6.77g). In case of nematode alone, maximum fresh root weight was recorded in untreated non-inoculated check (7.0 g) which was at par with combined formulation of three bio-agents (6.83 g) followed by carbofuran (5.87 g) and significantly lowest was recorded in untreated inoculated check (4.03 g).

With respect to nematode and fungus, significantly highest fresh root weight was recorded in untreated non-inoculated check (6.63 g) which was statistically at par with combined formulation of *T. viride* + *P. fluorescens* + *P. lilacinum* (6.57 g) and thus combined formulation of bio-agents was better than carbofuran (5.10 g) and carbendazim 50 WP (4.17g).

4.8.5 Dry root weight

It is clear from the data in Table 21 that dry root weight was significantly different from untreated inoculated check in all the treatments. Among bio-agents tested, combined formulation of three bio-agents recorded highest dry root weight, whereas significantly lowest dry root weight was recorded in untreated inoculated check.

In case of fungus alone, statistically highest dry root weight was recorded in untreated non-inoculated check (2.43 g) which was at par with carbendazim 50 WP (2.42 g), combined

formulation (2.37 g) and *T. viride* (2.07). Significantly lowest dry root weight was recorded in untreated inoculated check (1.50 g). In case of nematode alone, statistically highest dry root weight was recorded in untreated non-inoculated check (2.53 g) which was at par with carbofuran (2.50 g), combined formulation (2.41 g) and *T. viride* (1.83 g). With respect to nematode and fungus, significantly lowest dry root was recorded in untreated inoculated check (1.27 g). The combined formulation (2.36 g) and individual application of *T. viride* (1.80 g) and *P. fluorescens* (1.67 g) were effective than carbofuran (1.50 g) and carbendazim 50 WP (1.27 g).

Table 19: Effect of bio-agents on dry shoot weight of guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

Sr. No.	Treatments	Dry shoot weight (g)		
		Fungus alone	Nematode alone	Nematode +Fungus
1	*T. viride* @ 10g/kg of soil	5.10	4.73	3.84
2	*P. fluorescens* @ 10g/kg of soil	4.53	4.07	3.41
3	*P. lilacinum* @ 10g/kg of soil	4.20	3.43	3.25
4	*T. viride*+ *P. fluorescens*+*P.lilacinum* @ 10ml/kg of soil	5.67	4.98	4.70
5	Carbofuran @ 0.1g/kg of soil	4.23	5.03	3.53
6	Carbendazim 50 WP @ 2g/lt of water	5.40	3.50	3.09
7	Untreated inoculated check	3.90	3.07	2.68
8	Untreated non-inoculated check	5.83	5.58	5.76
	C.D. at 5 per cent	1.20	0.82	1.02

Mean of three replications

Table 20: Effect of bio-agents on fresh root weight of guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

Sr. No.	Treatments	Fresh root weight (g)		
		Fungus alone	Nematode alone	Nematode +Fungus
1	*T. viride* @ 10g/kg of soil	5.30	5.07	5.03
2	*P. fluorescens* @ 10g/kg of soil	4.43	4.33	4.77
3	*P.lilacinum* @ 10g/kg of soil	4.17	4.17	3.83
4	*T. viride*+ *P. fluorescens* +*P. lilacinum* @10 ml/kg of soil	6.50	6.83	6.57
5	Carbofuran @ 0.1g/kg of soil	4.60	5.87	5.10
6	Carbendazim 50 WP @ 2g/lt of water	6.77	4.27	4.17
7	Untreated inoculated check	4.13	4.03	3.42
8	Untreated non-inoculated check	6.83	7.00	6.63
	C.D. at 5 per cent	1.24	1.06	1.16

Mean of three replications

Table 21: Effect of bio-agents on dry root weight of guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

Sr. No.	Treatments	Dry root weight (g)		
		Fungus alone	Nematode alone	Nematode + Fungus
1	*T. viride* @ 10g/kg of soil	2.07	1.83	1.80
2	*P. fluorescens* @ 10g/kg of soil	1.67	1.52	1.67
3	*P.lilacinum* @10g/kg of soil	1.53	1.50	1.43
4	*T. viride*+ *P. fluorescens* + *P. lilacinum* @10 ml/kg of soil	2.37	2.41	2.36
5	Carbofuran@0.1g/kg of soil	1.63	2.50	1.60
6	Carbendazim 50 WP@2g/lt of water	2.43	1.57	1.50
7	Untreated inoculated check	1.50	1.44	1.27
8	Untreated non-inoculated check	2.42	2.53	2.40
	C.D. at 5 per cent	0.82	0.76	0.90

Mean of three replications

Table 22: Effect of bio-agents on number of galls in guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

Sr. No.	Treatments	Number of galls/plant	
		Nematode alone	Nematode + Fungus
1	*T. viride* @ 10g/kg of soil	130.67 (11.47)	110.67 (10.56)
2	*P. fluorescens* @10g/kg of soil	144.67(12.06)	126.67 (11.29)
3	*P.lilacinum* @ 10g/kg of soil	135.67 (11.68)	134.67 (11.644)
4	*T. viride*+ *P. fluorescens* + *P. lilacinum* 10ml/kg of soil	103.33 (10.21)	90.00 (9.53)
5	Carbofuran @ 0.1g/kg of soil	120.00 (10.99)	106.67 (10.35)
6	Carbendazim 50 WP @ 2g/lt of water	181.67 (13.51)	169.33 (13.04)
7	Untreated inoculated check	192.00(13.89)	158.33 (12.62)
8	Untreated non-inoculated check	0.00 (1.00)	0.00 (1.00)
	C.D. at 5 per cent	(0.76)	(0.89)

Mean of three replications
Figures in parentheses are $\sqrt{n}$ +1 transformed value

4.9 Effect of bio-agents on reproduction and multiplication of *M. incognita* in guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

4.9.1 Number of galls per plant

Results from Table 22 indicated that significantly highest number of galls was observed in untreated inoculated check. Application of combined formulation of *T. viride* + *P.*

fluorescens + *P. lilacinum* significantly reduced number of galls irrespective of nematode alone or nematode and fungus inoculation in combination (Fig. 10).

In case of nematode alone, significantly minimum number of galls was observed in combined formulation (103.33) followed by carbofuran (120.00) and *T. viride* (130.67). Significantly maximum number of galls was observed in untreated inoculated check (192.00) which was at par with carbendazim (181.67). With respect to combined inoculation of nematode and fungus, the significantly maximum number of galls was recorded in carbendazim (169.33) as it suppressed the fungus and thus nematode freely infected roots and caused maximum galling. The significantly lowest number of galls was recorded in treatment receiving combined formulation (90.00) which was at par with carbofuran (106.67)

4.9.2 Number of egg masses per plant

It was inferred from data in Table 23 that combined formulation of three bio-agents significantly reduced number of egg masses per plant as compared to other treatments.

Plants inoculated with nematode alone, maximum and significantly highest number of egg masses was observed in untreated inoculated check (105.67) and significant reduction in number of egg masses was observed in all the treatments except carbendazim (100.33) which was at par with untreated inoculated check. The significantly minimum number of egg masses was recorded in combined formulation (46.00) which was at par with carbofuran (55.00) followed by *T. viride* (65.67) and *P. fluorescens* (72.33). The application of *P. lilacinum* also reduced number of egg masses significantly when compared to untreated inoculated check, but not as effective as that of *T. viride* and *P. fluorescens.*

In case of nematode and fungus inoculation, significantly minimum number of egg masses was recorded in combined formulation (40.67) which was at par with carbofuran (48.33) and highest number of egg masses was recorded in Carbendazim 50 WP (95.00).

4.9.3 Number of eggs per egg mass

In the case of nematode alone and in nematode and fungus combined inoculation, the significantly lowest number of eggs was recorded in combined formulation followed by *T. viride* alone and carbofuran and were at par with each other (Table 24). Although, application of *P. fluorescens* and *P. lilacinum* reduced the number of eggs when compared to untreated inoculated check.

4.9.4 Final nematode population

The results from Table 25 clearly indicated that the final nematode population was significantly maximum in untreated inoculated check which was at par with carbendazim 50 WP. A combination of three bio-agents significantly reduced final nematode population which was statistically effective than their individual inoculation (Fig. 11).

Table 23: Effect of bio-agents on number of egg mass in guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

Sr. No.	Treatments	Number of egg mass/plant	
		Nematode alone	Nematode + Fungus
1	*T. viride* @ 10g/kg of soil	65.67 (8.16)	53.33 (7.35)
2	*P. fluorescens* @ 10g/kg of soil	72.33 (8.55)	72.67 (8.58)
3	*P. lilacinum* @ 10g/kg of soil	81.67 (9.08)	79.33 (8.94)
4	*T. viride*+ *P. fluorescens* + *P. lilacinum* @ 10 ml/kg of soil	46.00 (6.85)	40.67 (6.45)
5	Carbofuran @ 0.1g/kg of soil	55.00 (7.48)	48.33 (7.02)
6	Carbendazim 50 WP @ 2g/lt of water	100.33 (10.06)	95.00 (9.80)
7	Untreated inoculated check	105.67 (10.32)	90.00 (9.54)
8	Untreated non-inoculated check	0.00 (1.00)	0.00 (1.00)
	C.D. at 5 per cent	(0.84)	(0.77)

Mean of three replications
Figures in parentheses are $\sqrt{n}$ +1 transformed value

Table 24: Effect of bio-agents on number of eggs per egg mass in guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

Sr. No.	Treatments	Number of eggs per egg mass	
		Nematode alone	Nematode + Fungus
1	*T. viride* @ 10g/kg of soil	265.00(16.30)	258.33 (16.10)
2	*P. fluorescens* @ 10g/kg of soil	276.67 (16.66)	275.92 (16.63)
3	*P. lilacinum* @ 10g/kg of soil	279.67 (16.75)	276.17 (16.61)
4	*T. viride*+ *P. fluorescens* + *P. lilacinum* @ 10ml/kg of soil	260.67(16.17)	252.33 (15.91)
5	Carbofuran @ 0.1g/kg of soil	267.56(16.38)	256.67 (16.05)
6	Carbendazim 50 WP @ 2g/lt of water	284.33 (16.89)	277.83 (16.63)
7	Untreated inoculated check	288.00 (17.00)	283.25 (16.81)
8	Untreated non-inoculated check	0.00 (1.00)	0.00 (1.00)
	C.D. at 5 per cent	(0.42)	(0.46)

Mean of three replications
Figures in parentheses are $\sqrt{n}$ +1 transformed value

Table 25: Effect of bio-agents on final nematode population in guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

Sr. No.	Treatments	Final nematode population /200 cc soil	
		Nematode alone	Nematode + Fungus
1	*T. viride* @ 10g/kg of soil	238.33 (15.47)	190.00 (13.82)
2	*P. fluorescens* @ 10g/kg of soil	261.67 (16.21)	233.33 (15.28)
3	*P. lilacinum* @ 10g/kg of soil	273.33 (16.56)	251.67 (15.89)
4	*T. viride*+ *P. fluorescens* + *P. lilacinum* @ 10 ml/kg of soil	173.00 (13.19)	150.00 (12.29)
5	Carbofuran @ 0.1g/kg of soil	210.00 (14.52)	170.00 (13.07)
6	Carbendazim 50 WP @ 2g/lt of water	362.67 (19.07)	331.67 (18.24)
7	Untreated inoculated check	380.00 (19.51)	340.00 (18.47)
8	Untreated non-inoculated check	0.00 (1.00)	0.00 (1.00)
	C.D. at 5 per cent	(0.47)	(0.54)

Mean of three replications
Figures in parentheses are $\sqrt{n}$ +1 transformed value

Table 26: Effect of bio-agents on per cent root rot in guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

Sr. No.	Treatments	Per cent root rot	
		Fungus Alone	Nematode + Fungus
1	*T. viride* @ 10g/kg of soil	22.67 (28.43)	20.33 (26.14)
2	*P. fluorescens* @ 10g/kg of soil	24.50 (29.67)	23.00 (28.66)
3	*P.lilacinum* @10g/kg of soil	31.67 (34.24)	27.20 (31.31)
4	*T. viride*+ *P. fluorescens* + *P. lilacinum* @ 10 ml/kg of soil	19.20 (25.99)	17.33 (25.10)
5	Carbofuran @ 0.1g/kg of soil	29.67 (33.00)	29.00 (32.58)
6	Carbendazim 50 WP @ 2g/lt of water	10.33 (18.75)	22.33 (27.97)
7	Untreated inoculated check	35.67 (36.67)	47.33 (43.28)
8	Untreated non-inoculated check	0.00 (0)	0.00 (0.00)
	C.D. at 5 per cent	(2.26)	(2.10)

Mean of three replications
Figures in parentheses are Arc sine transformed values

Fig. 10 Effect of bio-agents on number of galls in guava withindividual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

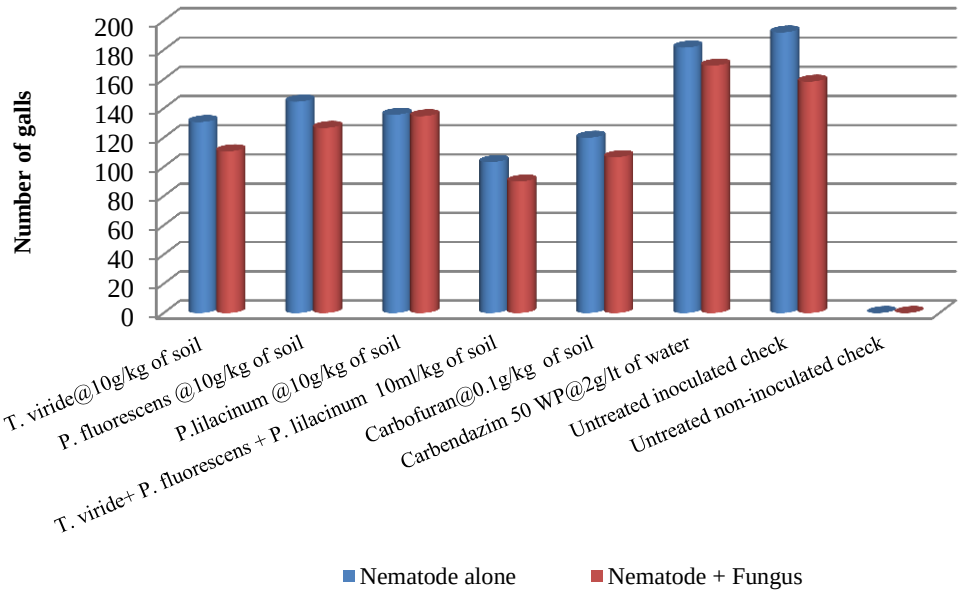


Fig. 11 Effect of bio-agents on final nematode population in guava with individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*

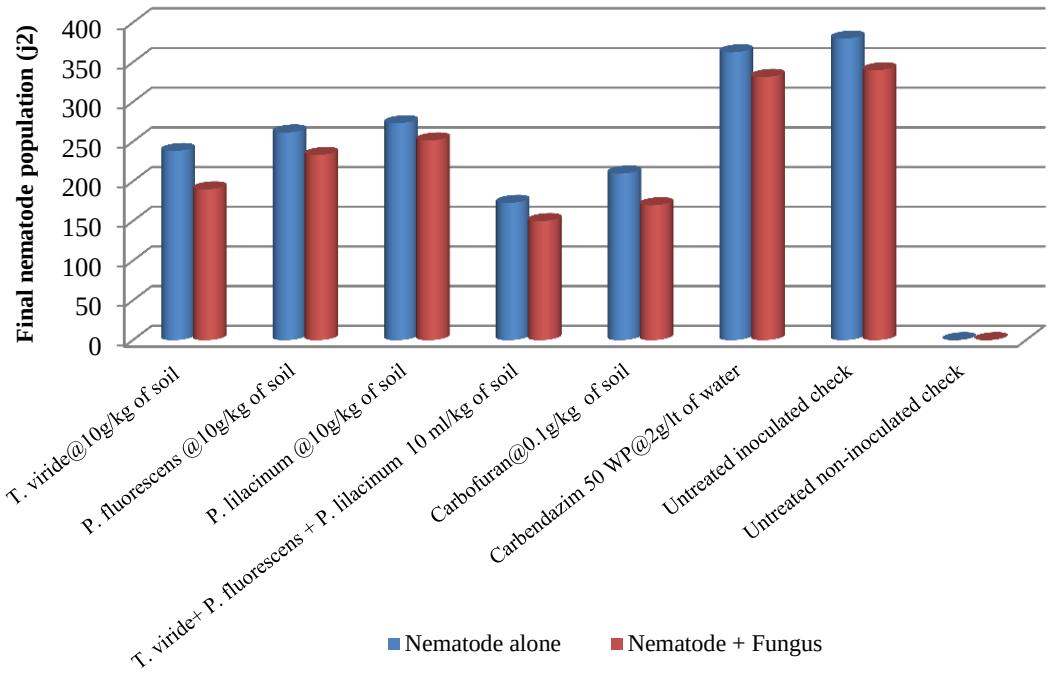


Plate 9 Effect of bio-agents on growth of guava with inoculation of *F. oxysporum* f. sp. *psidii*

T1: *Trichoderma viride* @ 10 g/kg of soil. T2: *Pseudomonas fluorescens* @ 10 g/kg of soil.

T3*: Purpureocillium lilacinum* @ 10 g/kg of soil.

T4: Combined formulation of these (*T. viride* + *P. fluorescens* + *P. lilacinum*) bio-agents @ 10 ml/ kg of soil.

T5: Carbofuran (Furadan) @ 0.1 g/ kg of soil T6: Carbendazim 50 WP @ 2g/liter water

T7: Untreated inoculated check T8: Untreated non-inoculated check

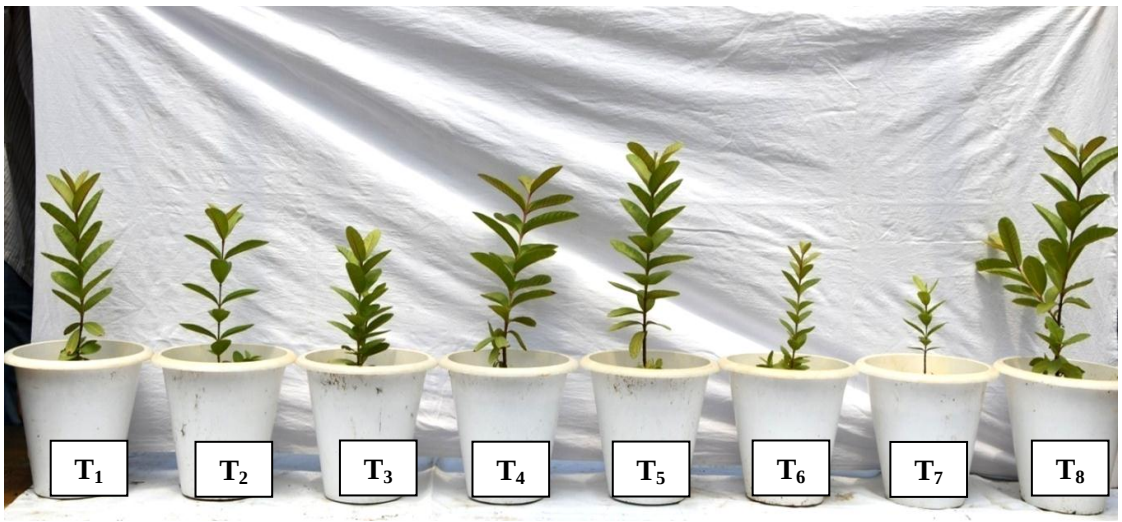


Plate 10 Effect of bio-agents on growth of guava with combined inoculation of *M. incognita* and *F. oxysporum* f. sp. *psidii*

T1: *Trichoderma viride* @ 10 g/kg of soil. T2: *Pseudomonas fluorescens* @ 10g/kg of soil.

T3*: Purpureocillium lilacinum* @ 10g/kg of soil.

T4: Combined formulation of these (*T. viride* + *P. fluorescens* + *P. lilacinum*) bio-agents @ 10 ml/ kg of soil.

T5: Carbofuran (Furadan) @ 0.1 g/ kg of soil T6: Carbendazim 50 WP @ 2g/liter water

T7: Untreated inoculated check T8: Untreated non-inoculated check

In case of nematode alone, the final nematode population of *M. incognita* was significantly reduced where plants were treated with combined formulation (173.00) followed by carbofuran (210.00). Statistically highest nematode population was recorded in untreated inoculated check (380.00) which was at par with carbendazim 50 WP (362.67).

With respect to combined inoculation of nematode and fungus, the final nematode population was affected due to *F. oxysporum* f.sp *psidii.* The combined formulation reduced the final nematode population significantly (150.00) followed by carbofuran (170.00) and *T. viride* (190.00).

4.9.5 Per cent root rot

The data in Table 26 depicted that maximum root rot was observed in plants inoculated with combination of *M. incognita* and *F. oxysporum* f.sp *psidii* than fungus alone.

In case of plants inoculated with fungus alone, significantly maximum root rot (35.67%) was observed in untreated inoculated check and minimum root rot was observed in treatment receiving carbendazim 50 WP (10.33%) followed by combined formulation (19.20%). The individual application of *T. viride* (22.67%) and *P. fluorescens* (24.50 %) were also effective when compared to untreated inoculated check. Whereas in the combined inoculation of nematode and fungus, the significantly maximum root rot was observed in untreated check (47.33%) and minimum root rot was noticed in plants receiving treatment of combined formulation (17.33%) which was at par with *T. viride* (20.33 %). Thus, combined formulation of *T. viride, P. fluorescens,* and *P. lilacinum* was more effective than individual application.

CHAPTER- V

DISCUSSION

Root-knot nematodes are obligate endoparasites of economic importance and distributed predominantly throughout the world, especially in tropical and subtropical countries. Root-knot nematodes parasitization causes damage to roots due to gall formation and deprives the plants of nutrients. These also act as predisposing agents for secondary pathogens *viz*., fungi, bacteria and viruses. In guava orchards, root-knot nematode is becoming an important co-factor in guava wilt as it facilitates the entry of fungus *F. oxysporum* f.sp. *psidii* by causing physical injuries to roots which leads to the severe cause of 'guava decline'. Hence, the present investigation entitled **"Studies on the incidence and management of guava decline involving root-knot nematode and fungi"** was planned to study the guava decline and its incidence.

The comprehensive research on the interaction between *M. incognita* and *F. oxysporum* f.sp. *psidii* is the need of the hour to develop appropriate management practices. The use of chemical pesticides to manage the complex disease is expensive, has harmful effects on the environment and pathogens chance to develop resistance to chemicals. Therefore, the use of soil organic amendments and bio-agents are cost-effective, non-hazardous and eco-friendly. Most promising bio-agents, *Trichoderma viride*, *Pseudomonas fluorescens* and organic amendments such as mustard and neem cake have shown broad-spectrum pesticidal effect on both *M. incognita* and *F. oxysporum* f.sp. *psidii* and thus reduced the severity of the complex diseases.

The results obtained from the survey for the guava decline incidence, experiments on the effect of different inoculum levels of *M. incognita* and *F. oxysporum* f.sp. *psidii* on guava, interaction studies between them and effect of deoiled cakes and bio-agents on guava, are discussed hereunder.

5.1 Survey for the incidence of guava decline caused by root-knot nematode and fungi

The intensive survey was conducted in guava orchards of Hisar, Jind, Sirsa and Fatehabad districts of western Haryana. The results from the survey of four districts revealed the occurrence of ten plant-parasitic nematode genera *viz*., *Meloidogyne* sp., (*M. incognita, M. javanica*) *Rotylenchulus reniformis, Helicotylenchus* sp., *Hoplolaimus* sp., *Pratylenchus* sp., *Tylenchorhynchus* sp., *Tylenchus* sp., *Xiphinema* sp., and *Longidorus* sp. in surveyed guava orchards. The population densities of *M. incognita, Rotylenchulus reniformis, Helicotylenchus* spp., *Hoplolaimus* sp., and *Pratylenchus* sp. were recorded from all four districts and were having a parasitic association with guava orchards showing symptoms of guava decline. The results are confirmed with those of Khan *et al.* (2007) who observed the presence of *M.*

incognita, M. graminicola, M. javanica, Pratylenchus coffeae, P. brachyurus, R. reniformis, Hoplolaimus indicus, Helicotylenchus goodi, H. indicus, H. abunamai, Tylenchorhynchus mashhoodi, T. nudus and *Aphelenchus avenae* from guava rhizosphere of West Bengal, India. Similar results are reported by Khan *et al.* (2001); Ruchi *et al.* (2002) who observed the role of plant-parasitic nematodes as co-factor in guava decline. Among plant-parasitic nematodes, root-knot nematode caused much damage and was highly pathogenic to guava plants as are the findings of the present studies. Root-knot nematode species, *M. incognita* was more predominant to guava plants than *M. javanica* and *M. javanica* was mostly found in guava orchards having intercropping with vegetables. The guava plants parasitized by *M. incognita* have been reported from Brazil (Moura *et al*., 1989) and South Africa (Willers and Welgemoed, 1993). Ansari and Khan (2012) also reported that *M. incognita* was highly pathogenic to guava orchards of Aligarh district, Uttar Pradesh and which caused symptoms of stunted growth, chlorosis, dieback, patchy growth and numerous small to big size galls.

The isolation and identification of fungal pathogens *viz*., *Fusarium oxysporum*, *Macrophomina phaseoli* and *Rhizoctonia solani* was done from infected guava orchards in surveyed districts of Haryana. These results are confirmed with Suhag (1976), who reported the isolation of two fungi namely *Rhizoctonia* and *Fusarium* spp. from guava decline orchards of Haryana. Dwivedi *et al.* (1990) also reported that *F. oxysporum, F. solani* were primary colonizers followed by *Macrophomina phaseoli* and *Rhizoctonia solani* from wilted guava orchards.

The survey of four districts revealed that the maximum guava decline incidence was recorded in Dadbi-6 (100%), Sirsa district and the minimum was in Adampur-1 (11.8%), Hisar district. More incidence of guava decline in Sirsa district may be due to light soil (coarse textured soils) having more percentage of sand. The light soils having higher amount of sand and more pore spaces which favours the development of root-knot nematode (Razak and Lim, 1987). Mehta (1987) also observed that the incidence of guava wilt was more in sandy loam and clay loam soils than heavy soil types. The present results were confirmed with findings of Ruchi *et al.* (2002) who observed that 50-60 per cent guava plants were drying due to infestation of *Tylenchorhynchus brassicae, Longidorus* sp., *Xiphinema* sp., *Hoplolaimus indicus, Helicotylenchus indicus, Hemicriconemoides* spp., and wilt causing fungi *viz*., *F. oxysporum, F. solani, F. equiseti, F.moniliforme, F. accuminatum.*

Among four districts surveyed, mean of guava decline incidence was maximum in Jind (51.6%) followed by Sirsa (49.4%), Hisar (40.4%) and least disease was observed in Fatehabad district (36.6%). The severe incidence of guava decline might be attributed to soil pH, imbalanced application of fertilizers, soil texture, soil moisture, age of the plants and environmental conditions. The soil pH range of 4-8 is optimum for multiplication, development and infestation of *M. incognita* and *F. oxysporum* f. sp. *psidii*. According to

earlier reports, *F. oxysporum* f. sp. *psidii* incidence was more in alkaline soil with pH range of 7.5 to 9.0 (Mehta, 1951) whereas Sen and Verma (1954) reported severe incidence of guava wilt in laterite soils with pH 6.5. The growing season also influences the disease incidence, guava plants infected by *F. oxysporum* f. sp. *psidii* shows symptoms initiation at the onset of monsoon *i.e.* during July to September and maximum disease severity was observed in September to October (Das Gupta and Rai, 1947; Edward, 1960; Suhag, 1976). The wilt causing fungus survives in association with the root bit during summer months (adverse environmental conditions), whereas in winter and rainy seasons, fungus survive on roots (Dwivedi *et al.,* 1990). Misra and Pandey (1999a, 1999b, 2000) studied the development of natural wilting of guava plants in different seasons and they found that maximum wilting of plants was during October and some plants were recovered from December onwards. They analyzed the weather data and found that heavy rainfall, maximum temperature (31.3 to 33.5^0C), minimum temperature (23 to 25^0C) and humidity (76%) from July to September. The above environmental conditions are very congenial to *M. incognita* and *F. oxysporum* f. sp. *psidii* for multiplication and infection to guava plants. Khan *et al.* (2001) also observed a high population of *Meloidogyne* spp., *Helicotylenchus dihystera, Rotylenchulus reniformis, Hoplalaimus* spp., *Pratylenchus* spp., other tylenchids and dorylaimids during July and October in guava orchards of northeastern states, India. In our investigations, the survey was conducted during September and October 2017 which recorded a high nematode population and more guava decline incidence. Therefore the present survey results were confirmed with earlier findings. These environmental conditions may be reasons for maximum guava decline incidence. The maximum disease incidence could be attributed to the use of high nitrogenous fertilizers which makes plants susceptible to pathogens and also favors the multiplication of plant-parasitic nematodes and *Fusarium* spp. The application of high nitrogen fertilizers causes the development of new succulent vegetative growth and delays the maturity and pathogens attacks such plant organs easily (Chattopadyay and Bhattacharya, 1968; Ruchi *et al.*, 2002).

The data presented in Table 1e indicated that the density range (j_2/200cc soil) of *M. incognita* was observed maximum in Sirsa (425-940) followed by Jind (310-930), Fatehabad (280-820) and Hisar district (46-710). Among four districts, the highest density range of *Helicotylenchus* spp. (80-425) in Jind, *Rotylenchulus reniformis* (35-230) in Fatehabad and *Pratylenchus* spp. (60-280) observed in Sirsa district. The highest soil and root population of *M. incognita* and compound galls were observed in orchards having drip irrigation than flood irrigated orchards. The continuous availability of moisture in the soil by drip irrigation method favors the nematode multiplication and reproduction than the flood irrigation method. The gap of 15-20 days between two irrigations in flood irrigation and also irrigation at a specific time interval affects the nematode population. The results are in conformity with

those of Ansari and Khan (2012) who conducted an extensive survey of guava orchards in Aligarh district and reported that the highest absolute density, relative density were recorded in *Meloidogyne* spp. (density range 95-3234) followed by *Hoplolaimus* spp. The results inferred that *Meloidogyne* spp., *Hoplolaimus* spp., *Rotylenchulus* spp., and *Helicotylenchus* spp. are the most frequently occurring and highly pathogenic to guava plants. Khan *et al.* (2007) also reported the high population density of *M. incognita*, *R. reniformis* and *Helicotylenchus* from guava rhizosphere of West Bengal. Ansari and Ahmad (2000) analyzed the nematode density of guava orchards of Aligarh, India and found that *Hoplolaimus indicus* had the highest frequency, density and prominence value but not biomass.

The variation in nematode populations and non-uniform distribution could be due to different method of cultivation, intercropping with vegetables, resistance or susceptible cultivar, physical and chemical properties of soil and environmental factors. The highest nematode density could be attributed to the more susceptible cultivars, the use of nematode infested seedlings and unweeded orchards. Interestingly, our survey results also observed the guava nurseries of Palwan, (Jind district) and Gillakhera (Fatehabad district) were severely infected by root-knot nematodes. Khan *et al.* (2001) observed the high nematode population in unweeded guava orchards.

The frequency of occurrence of *M. incognita* was recorded maximum in Fatehabad (72.2%) followed by Hisar (63.2%), Jind (56.3%) and Sirsa district (53.3%). The maximum frequency of occurrence (66.0%) of *Helicotylenchus* spp. was in Sirsa district, whereas, high frequency of *Hoplolaimus* spp. (62.5%) was observed in Jind district. Among four districts, the occurrence of *R. reniformis* and *Pratylenchus* spp. varied from 24.8 to 47.4 and 31.6 to 42.1 per cent respectively. The results are in agreement with those of Ansari and Khan (2012), who reported the absolute frequency of *M. incognita* (78.0%), *R. reniformis* (68.9%), *Helicotylenchus* sp. (90.8%), *Hoplolaimus* sp. (100%), and *Pratylenchus* sp. (48.7%). The results also indicated that guava is a good host of *M. incognita, R. reniformis, Helicotylenchus* spp., *Hoplolaimus* spp., and *Pratylenchus* spp., based on occurrence and population density of plant-parasitic nematodes infecting guava.

The individual incidence of *F. oxysporum* f.sp. *psidii* varied from 23.0 to 42.6 per cent and *M. incognita* incidence varied from 33.0 to 57.7 per cent, whereas combined infection by *M. incognita* and *F. oxysporum* f. sp. *psidii* was varied from 49.1 to 67.7 per cent. The present results are in agreement with those of Poornima *et al*. (2016) who observed that the root-knot nematode alone caused chlorosis, stunted growth whereas sudden yellowing, shedding of leave (wilting symptoms) and complete death of guava plants must be attributed to the association of wilt causing fungus. The presence of *Meloidogyne* spp. in the rhizosphere acts as co-factor through direct or indirect involvement in the disease development (Khan *et al.*, 2001). Suarez *et al.* (1999) observed that the simultaneous presence

of *M. incognita, F. oxysporum* and *M. phaseolina* caused a greater detrimental effect than that of each pathogen alone on guava plants. Gomes *et al.* (2014) observed that the parasitization by *M. enterolobii,* breakdown of the resistance of guava plants to *F. solani*, makes morpho-physiological changes in the root system of guava plants and galled tissues, the modified chemical composition of root exudates which favoured colonization by the fungus.

The incidence and severity of guava decline was observed to be more in old orchards than young orchards. The low incidence of guava decline in young orchards might be attributed to low inoculum potential of *M. incognita* and *F. oxysporum*, the high metabolic activity of plants during the initial growing period and hardy nature of the plant. Whereas in old orchards, the high inoculum potential and density of both the pathogens due to the continuous availability of host, physical injuries by agronomical practices and pest and disease incidence make plants to disease-prone which causes the more diseases incidence and finally death of plants. As obligate sedentary endoparasitic nature and deep rooting of perennial crops, root-knot nematodes are well protected in host root and thus, they are difficult to eradicate once they are in an orchard. Misra and Shukla (2002) also reported that guava plants above the age of five years were more susceptible to the disease. However, guava seedlings are more susceptible to *Macrophomina phasolina* as well as *F. solani* than the older plants of 3 years age. The field observations and survey results revealed that guava decline, severity and incidence was prominent where guava plants were infected by *M. incognita* and *F. oxysporum* f.sp. *psidii* in combination.

5.2 Pathogenicity studies of *M incognita* and *F. oxysporum* associated with guava decline

The experiment on pathogenicity of *M. incognita* on guava was studied under screen house conditions by inoculating different inoculum levels of 0, 10, 100, 500, 1000, 2000, and 4000 j_2. The maximum and significantly highest plant growth parameters such as shoot length, fresh and dry shoot and root weight were observed in the non-inoculated check, which was at par with inoculum levels of 10 and 100 j_2/kg soil. As nematode density increased from 10 to 4000 j_2, plant growth parameters decreased accordingly. The considerable reduction of plant variables started at 500 j_2, however, a significant reduction of plant variables was observed at the inoculum levels of 1000 j_2 onwards and which was considered to be pathogenic level to guava plants. Significantly lowest plant growth parameters were observed in the highest inoculum level of 4000 j_2/kg soil. The reduction of plant variables may be due to the intervention of *M. incognita* with the normal physiological process of the plant such as nutrient and water uptake, changes in phytohormones, chlorophyll synthesis and photosynthesis and which leads to poor growth of the plants. At the initial inoculum levels, plants were compensating the loss caused by nematodes infestation, but at higher nematode densities, plants were unable to compensate the loss and thus led to poor growth of the plants.

The lower nematode density failed to reduce plant growth but they established host-parasite relationship by infestation and causing galls. Higher nematode densities disrupted the root system, which affects nutrient uptake and translocation of photosynthates and finally, plants fail to perform the normal functions. The decrease in fresh and dry root weight at higher nematode densities was due to deterioration of the root system. The present results are in conformity with those of Babatola and Oyedunmade (1992) who reported the significant reduction in dry matter of guava at increasing inoculum level of *M. incognita* and also considerable reduction of plant growth observed in plants inoculated with *M. incognita* as compared to non-inoculated plants. The above results are in conformity with results of Raut 1981, Sharma *et al.,* 1999 and Ganaie *et al.,* 2011 who reported that the nematode density of 1000 j_2 per plant significantly reduced the plant variables.

The nematode reproduction factors *viz*., number of galls, number of egg masses, and final nematode population were increased significantly as the inoculum levels increased from 10 to 4000 j_2/kg soil. The highest number of galls, number of egg masses and the final nematode population were observed at 4000 j_2 and lowest were recorded at 10 j_2/kg soil. The increasing rate in the number of galls, number of egg masses and eggs per egg mass was recorded from the inoculum levels of 10 to 1000 j_2, but the rate of multiplication was inversely proportional to initial inoculum levels. The number of galls and egg masses per plant at 1000 and 2000 j_2 were at par with each other; however number of eggs per egg mass decreased at 1000 j_2 onwards. The decrease in number of eggs per egg mass might be due to a higher nematode population per plant, which leads to competition for food and space. On the other hand, the final nematode population was significantly increased from nematode densities of 10 to 4000 j_2/kg soil. Plant variables at inoculum levels of 0 to 500 j_2 were significantly at par and the significant reduction was observed at 1000 juveniles onwards and the nematode reproduction factors were directly proportional to inoculum levels from 10 to 1000 j_2 and decreased rate of nematode multiplication *i.e.* decreasing in number of galls, egg masses, eggs per egg mass due to spatial and nutritional competition indicated the pathogenic level or damaging threshold level of *M. incognita* on guava seedlings (Hisar Safeda). The results are in conformity with those of Babtola and Oyedunmade (1992) who reported the increase in nematode factors at increased inoculum levels of *M. incognita* on guava cultivars. Singh and Nath (1996) observed the pathogenic level of *M. incognita* by inoculating at 0, 10, 100, 1000 and 10000 j_2/ 500g of soil on papaya (cv. Ranchi) under glasshouse conditions and results revealed that an inoculum level of *M. incognita* at 1000 j_2/500g of soil was pathogenic level for papaya plant; and they also observed that with increasing inoculum level of the nematode, there was a gradual reduction in plant variables except at 10 j_2. Better plant growth at 10 j_2 can be attributed to the production of more roots and light infection of the plant (was much below the damaging threshold level).

The present investigation included an objective of pathogenicity of *F. oxysporum* f.sp. *psidii* on guava seedlings. The Koch's postulates were proved by soil inoculation method using different inoculum levels *viz.,* 2, 4, 6, 8, and 10 g mycelium of *F. oxysporum* f.sp. *psidii*. The isolation from inoculated guava plants and compared them with mycological observation of originally isolated *F. oxysporum* f.sp. *psidii* from field soil during the survey. The results revealed that plant growth parameters were decreased with the increase in inoculum levels. The significantly lowest plant variables were observed in 10 g mycelium followed by 8 g and 6 g mycelium which was significantly different from each other. The *F. oxysporum* f.sp. *psidii* infecting guava plants showed the symptoms of yellowing of leaves and stunted growth 45 days after inoculation. As increase in inoculum levels, there was a decrease in plant growth gradually and least plant variables at maximum inoculum level *i.e.* 10g mycelium/kg soil. The significant reduction in shoot length, fresh and dry shoot weight was observed at 6 g mycelium and hgher doses, so 6g was considered to be pathogenic level to guava plants. Since, *F. oxysporum* f.sp. *psidii* infects vascular tissues which affect the absorption and translocation of nutrients and water through the root system, thus plant variables reduced due to the fungal infection and colonization in roots. The present results are in conformity with findings of Edward and Srivastava (1957), Pandy and Dwivedi (1985) and Dwivedi *et al.* (1988) who conducted the experiments on pathogenicity test of causal organism of guava wilt *(F. oxysporum* f.sp. *psidii).* They observed the symptoms of chlorosis, wilting of plant and presence of mycelium or hyphae in root xylem vessels by histopathological studies. Misra and Pandey (2000) developed the stem inoculation techniques for the pathogenicity test in guava wilt in which plants show the symptoms of wilting quickly, whereas in soil or root inoculation take more time to produce wilting symptoms.

5.3 Effect of individual or combined inoculation of *M. incognita* and *F. oxysporum* f. sp. *psidii* on growth parameters of guava

The interaction studies of *M. incognita* and *F. oxysporum* f.sp. *psidii* were conducted to evaluate their individual and combined effects on the growth of guava. The results revealed that significant reduction in growth parameters of guava seedlings was noticed in all the treatments in comparison to non-inoculated check. The statistically lowest shoot length, fresh and dry shoot weight, fresh and dry root weight were recorded in nematode inoculated 10 and 20 days prior to fungus inoculation. The simultaneous inoculation of nematode and fungus reduced plant variables more when compared with their individual inoculation. The results also revealed that the individual inoculation of nematode and fungus caused a significant reduction of plant growth parameters in comparison to non-inoculated check. However, nematode alone caused a significant reduction of plant growth parameters as compared to fungus alone. Thus fungus took a prolonged penetration period, since *F. oxysporum* f.sp. *psidii* had to germinate and produce appressoria and germ tubes to penetrate the tissue. The

significant decrease of plant variables in combined inoculation might be attributed to the synergistic interaction between nematode and fungus. When the plants were inoculated with nematode prior to fungus, nematode predisposes roots to fungus through wound formation and root exudates of nematode infestation trigger the fungus invasion process. As *F. oxysporum* f. sp. *psidii* took more time to penetrate and also a weak fungus cannot penetrate easily, so due to injuries by *M. incoginta*, it can easily enter the plants directly to vascular tissue where nematodes formed their permanent feeding sites. The specialized feeding cells of *M. incognita* act as a nutrient source to fungus which helps to colonize rapidly. Nematode parasitization also causes the biochemical changes, destruction of antibiotic substrates and break down of resistance mechanism to fungus and thus fungus infection increased. Finally, the plant fails to compensate loss induced by nematode and fungus which resulted in considerable reduction of plant variables. Therefore, sequential inoculation of *M. incognita* 10 and 20 days prior to *F. oxysporum* f. sp. *psidii* caused statistically highest reduction of plant variables than individual inoculation. The present results are in agreement with that of Suarez *et al.* (1999) who conducted the experiment on the synergistic effect of *F. oxysporum* and *M. phaseolina* individually or in combination with *Meloidogyne* spp. (*M. incognita* and *M. arenaria*) on guava and they reported that there was no significant difference in plant growth parameters, however the simultaneous presence of fungi and nematode caused a greater determental effect than each pathogen alone. Gomes *et al.* (2014) also reported the mechanisms involved in guava decline caused by *M. enterolobii* and *F. solani* and the results concluded that the nematode infestation alters root physiology, the chemical composition of root exudates and breakdown of resistance to fungus. Thus, fungus *F. solani* infection was increased and caused severe guava decline. The present results are in conformity with findings of Sonyal (2015) who observed that the combined or sequential inoculations of *M. incognita*, *Ceratocystis fimbriata* and *F. oxysporum* increased the wilt incidence and considerably reduced the shoot and root length, fresh shoot and root weight of pomegranate. Ansari and Ahmad 2000, Avelar Mejia *et al.*, 2001 and Khan *et al.*, 2001 reported that the interaction of *Meloidogyne* spp. and other soil-borne fungi cause high incidence of guava decline.

5.4 Effect of individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii* on multiplication and reproduction of *M. incognita*

In the present investigation, the nematode reproduction factors were affected by the combined and chronological inoculation (fungus 10 and 20 days prior to nematodes) of *M. incognita* and *F. oxysporum* f.sp. *psidii* and results indicated the significant reduction in number of galls, number of egg masses, eggs per egg mass and final nematode population in combined treatments as compared to nematode alone. Nematode reproduction factors were significantly highest in plants inoculated with nematode alone followed by nematode 20 and

10 days prior to fungus and which were significantly at par with each other. The highest nematode reproduction factors observed in plants inoculated with nematode alone may be due to the absence of *F. oxysporum* f.sp. *psidii*, hence rhizosphere and rhizoplane being conducive to nematode reproduction. The significantly lowest number of galls, number of egg masses, eggs per egg mass and final nematode population were recorded in plants inoculated with fungus 20 days prior to nematodes inoculation, followed by fungus 10 days prior to the nematode. The nematode factors didn't affect much when plants were inoculated with nematode prior to fungus, but reduction of nematode reproduction factors were observed in the treatment of fungus prior to nematode and also in simultaneous inoculation of nematode and fungus. The reduction in nematode factors might be due to competition for food and space, toxic metabolites produced by the fungus which caused adverse effects on nematode infestation (Fattah and Webster, 1989). The fungus might have interfered with nematode parasitization; hence nematode was unable to penetrate the roots freely in the presence of *F. oxysporum* f.sp. *psidii.* The decrease in nematode reproduction factors on guava might be due to the reduction of the root system (Akhtar *et al.*, 2007). Soumya (2015) reported the effect of *M. incognita* and *F. solani* interaction on nematode factors in black pepper. Nematode reproduction factors were reduced significantly in concomitant inoculation of nematode and fungus. Present results are also correlated with the studies of Ashok (2017) who recorded the number of galls, egg masses and final nematode population to be significantly decreased in plants inoculated with *M. incognita* seven days after fungus, *F. oxysporum*.

The results revealed that the maximum root rot was observed in plants inoculated with *M. incognita* 10 and 20 days prior to inoculation of *F. oxysporum* f.sp. *psidii* followed by simultaneous inoculation of nematode and fungus. The inoculation of fungus prior to nematode also increases rot root in comparison to plants inoculated with fungus alone. Thus, the presence of both nematode and fungus caused more root rotting in comparison to plants inoculated with nematode and fungus individually. The interaction between *M. incognita* and *F. oxysporum* f.sp. *psidii*, in which nematode predisposes roots to fungus and thereby roots were decayed by the fungus. The present results are in agreement with those of Gomes *et al*. (2011) who reported that the maximum root rot and effect on growth parameters was observed in the guava seedlings inoculated with *M. mayaguensis* 21 days prior to *F. solani* isolates. The experimental results also revealed that the complex nature of guava decline, synergistic interaction between organisms is the main cause of guava decline. Mejia *et al.* (2002) observed that combined inoculation of *Meloidogyne* spp., *F. solani, V. dahliae, P. aphanidermatum* and *T. roseum* caused the disease syndrome and root-knot nematode species caused the physical injuries to roots and disease severity is due to the presence of fungi which cause root rot in guava.

5.5 Management of guava decline by various management practices

The effect of different deoiled cakes *viz*., neem, mustard and castor were evaluated against *M. incognita* and *F. oxysporum* f.sp. *psidii* on guava seedlings under screen house conditions and the experimental results on growth parameters, nematode reproduction factors and per cent root rot are discussed hereunder.

The present investigation revealed that shoot length, fresh and dry shoot and root weight was increased where soil was amended with different deoiled cakes in comparison with the untreated inoculated check. Among different oil cakes tested, the maximum plant growth parameters were observed in treatments having neem cake @ 30g followed by mustard cake @ 30g irrespective of individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii.* Although castor cake increased the plant variables in comparison to untreated inoculated check (nematode or fungus alone or in combined inoculation) but was not as effective as neem cake or mustard cake. Carbendazim and carbofuran were effective when the plants were inoculated with fungus and nematode individually but were not effective in combined inoculation, as they controlled either of pathogens inoculated. Therefore, our results indicated that the application of organic amendments *viz*., neem and mustard cakes @ 30g improved the plant variables and were effective than chemicals tested in plants inoculated with nematode and fungus simultaneously.

The increase in plant growth parameters of guava might be attributed to plant nutrients and novel nematicidal compounds (Khan *et al.*, 1974; Ellenby, 1951 and Rich *et al.*, 1989) present in oil cakes. Neem cake contains higher concentration of nitrogen (6%), phosphorus (2%), potassium (3%), sodium, calcium and magnesium (Abbasi *et al.*, 2005) than mustard cake and castor cake and thus improved plant growth parameters by providing these nutrients. The application of oilcakes increases organic matter in soil which increases microbial activity (bacteria, fungi, actinomycetes, algae) leads to increase in the enzymatic activity. This microbial activity in the amended soil releases wide variety of chemical substances which are toxic to *M. incognita* and *F. oxysporum* f.sp. *psidii.* The incorporation of organic amendments improves the soil fertility, water holding capacity and release of toxic compounds such as propionic acid, acetic and butyric acid during decomposition (Khattak and Khattak 2011). These improvements in soil enhance the root system which increases the nutrient uptake capacity and plants become more vigorous, thus plants are capable of defending against many diseases. The application of organic amendments increased the levels of microbial biomass, soil organic matter, basal respiration and some enzyme activities related to the C and N cycles (Margarita *et al.,* 2003). The plant growth increased due to a decrease in *M. incognita* and *F. oxysporum* f.sp. *psidii* population, which otherwise destruct the root system and affect the nutrient and water absorption. Incorporation of organic amendments to soil improves plant growth parameters through the supply of micro and macronutrients and

through which increases the photosynthetic activity due to which more accumulation of carbohydrates and metabolites, resulting in more biomass (Rehman *et al.,* 2014).

A significant reduction in nematode reproduction factors in the treatments having different deoiled cakes and carbofuran was observed when compared to untreated inoculated check. The significantly lowest number of galls was recorded in treatments having neem cake 30g, carbofuran and mustard cake 30g in comparison to untreated inoculated check. With respect to combined inoculation, the maximum number of galls was recorded in carbendazim as it suppressed the fungus and thus nematode infects the roots freely and caused maximum galling. The significant reduction in the final nematode population was also observed in treatments having organic amendments irrespective of the fact whether nematode alone or combined inoculation of nematode and fungus. Neem cake @ 30g significantly reduced the final nematode population, which was at par with carbofuran followed by mustard cake @ 30g. In case of combined inoculation, the final nematode population was maximum where plants received carbendazim 50 WP. An increase in nematode population may be due to the fungicidal effect of carbendazim on *F. oxysporum* f. sp. *psidii* as it controlled fungus and thereby nematodes penetrated freely. A similar trend was observed in the number of egg mass and eggs per egg mass. Results revealed that the significantly lowest root rot was observed in carbendazim followed by neem cake and mustard cake @ 30g irrespective of fungus alone or combined inoculation of fungus and nematode. Maximum root rot was observed where plants were inoculated with nematode and fungus concomitantly than fungus alone.

The reduction of plant-parasitic pathogens and an increase in plant growth might be due to presence of nematicidal and fungicidal substrates in neem (Azadirachtin), mustard (allyl isothiocynate) and castor cake (ricin) (Ellenby, 1951; Khan *et al.,* 1974 and Rich *et al.,* 1989). The decrease in infection by *M. incognita* and *F. oxysporum* f. sp. *psidii* may also be through modifying the chemical composition of the rhizosphere. The incorporation of organic amendments modifies the physical and chemical properties of the soil and increases soil organic matter which increases activity of antagonistic microorganisms (Singh and Singh 2015). The ammonia toxicity is another possible mechanism involved in reducing the *M. incognita* population and *F. oxysporum* infestation. When organic amendments are applied to the soil, degradation of high N amendments tends to release ammonium which is converted to ammonia at high pH levels (Tenuta and Lazarovits, 2002). The decomposition of organic amendments in the soil releases organic acids (butyric and propionic acids), fatty acids, tannins, nematotoxic polyphenols which are toxic to nematodes and fungi (Hollis and Rodriguez-Kabana, 1966; Taylor and Murant, 1966; El-Naggar *et al*., 1993)

The results of the present investigation are in agreement with those of Logani *et al.* (2002) who reported that neem cake integrated with botanicals prevented the plant from infection by wilt causing fungi and also increased plant growth parameters of guava by

providing nutrients. Singh and Singh (2015) reported that neem and mahua cake (4.0 kg/tree) significantly reduced the wilt causing fungi *F. oxysporum* f.sp. *psidii* and also showed an adverse effect on plant-parasitic nematodes. The results of the present investigation are in accordance with the earlier reports. Abbasi (2005) reported the decrease of soil-borne pathogens viz., *Meloidogyne hapla, Pratylenchus penetrans, Verticillium dahliae, Rhizoctonia solani* and *Pythium aphanidermatum* due to the release of ammonia during decomposition of neem cake. Ganie *et al.* (2011) also reported that the incorporation of different oil cakes decreased nematode reproduction factors of *M. incognita* and increase in plant growth parameters. Ashok (2017) evaluated different deoiled cakes against *M. incognita* and *F. oxysporum* f.sp. *cucumerinum* (alone or in combination) infesting cucumber under polyhouse conditions. The results revealed that maximum plant growth parameters and the number of galls, number of egg mass and final nematode population were minimum in treatment applied with neem and mustard cake each @ 30 g per kg of soil. Sayed *et al.* (2007) also observed that the application of organic amendments significantly suppressed the reproduction and multiplication of *M. incognita* on grapes under greenhouse conditions and they reported the possible mechanisms such as toxic compounds released during decomposition and ammonia toxicity. Rahman and Somers (2005) recorded the applications of mustard as green manure or seed meal in the inter-row or vine row suppressed nematode population densities of *M. javanica* second stage juveniles in vineyards.

The experiment was conducted to evaluate the effect of different bio-agents such as *Trichoderma viride, Pseudomonas fluorescens,* and *Purpureocillium lilacinum* against *M. incognita* and *F. oxysporum* f.sp. *psidii* on growth parameters, nematode reproduction factors and per cent root rot and results are discussed here.

Among different bio-agents tested, the treatments receiving combined formulation of *T. viride, P. fluorescens* and *P. lilacinum* @ 10ml /kg soil and *T. viride* alone @ 10g/kg soil recorded maximum plant growth parameter irrespective of whether individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii.* Although *P. fluorescens* and *P. lilacinum* when used individually, also increased plant growth parameters in comparison to untreated inoculated check but not as effective as combination of three bio-agents and *T. viride.* In case of fungus alone, highest plant growth parameters were recorded in untreated non-inoculated check followed by carbendazim, combined formulation of three bio-agents and *T. viride.* In case of nematode alone, highest plant growth parameters were recorded in untreated non-inoculated check followed by carbofuran, combined formulation. Carbendazim and carbofuran were effective when the plants were inoculated with fungus and nematode individually but were not effective in the presence of both nematode and fungus, as they controlled either of pathogens inoculated. Therefore, the combined formulation of *T. viride,*

P. fluorescens and *P. lilacinum* was effective than carbofuran and carbendazim in the presence of both *M. incognita* and *F. oxysporum* f.sp. *psidii*.

The results of the present studies revealed that application of combined formulation of *T. viride, P. fluorescens* and *P. lilacinum* significantly reduced the number of galls and final nematode population irrespective of nematode alone or combined inoculation of nematode and fungus. In case of nematode alone, significant reduction of number of galls, egg masses and final nematode population were observed in combined formulation followed by carbofuran and *T. viride* alone. Significantly maximum number of galls, egg masses and final nematode population were noticed in untreated inoculated check. With respect to nematode and fungus combined inoculation, the significantly maximum number of galls, egg masses and final nematode were recorded in carbendazim as it suppressed the fungus and thus nematode freely infected roots and caused maximum galling. The significantly lowest number of galls and final nematode population were recorded in treatment receiving combined formulation followed by carbofuran. In case of combined inoculation of nematode and fungus, the final nematode population was affected by inoculation of *F. oxysporum* f. sp. *psidii* and thus the final nematode population was less compared to nematode alone.

The present investigation indicated that combined formulation of three bio-agents significantly reduced nematode reproduction factors (number of galls, egg masses, eggs/egg mass and final nematode population) as compared to other treatments. Maximum and significantly highest nematode reproduction factors were observed in untreated inoculated check. These factors were reduced statistically where plants were treated with the combined formulation of bio-agents which was at par with carbofuran followed by *T. viride* and *P. fluorescens* individually. The application of *P. lilacinum* also reduced nematode reproduction factors significantly when compared to untreated inoculated check, but not as effective as that of *T. viride* and *P. fluorescens.*

The results indicated that maximum root rot was observed in plants inoculated with combination of *M. incognita* and *F. oxysporum* f. sp. *psidii* than fungus alone. In case of plants inoculated with fungus alone, significantly maximum root rot was observed in untreated inoculated check and minimum root rot was observed in treatment receiving carbendazim followed by combined formulation. The individual application of *T. viride* and *P. fluorescens* was also effective when compared to untreated inoculated check. Whereas in the combined inoculation of nematode and fungus, the significantly maximum root rot was observed in untreated check and minimum root rot was noticed in plants receiving treatment of combined formulation of three bioagents which was at par with *T. viride*. Thus, the combined formulation was more effective than individual applications.

The reduction of nematode multiplication and reproduction could be attributed to various mechanisms of bio-agents which reduced the infestation by *M. incognita* and *F.*

oxysporum f.sp. *psidii*, and thus plant growth was improved. The most promising bio-agent, *T. viride* reduced the severity and incidence of *M. inocgnita* and *F. oxysporum* f.sp. *psidii* through various mechanisms such as competition for food and space, antibiosis through the production of hydrolytic enzymes and toxic compounds, hyperparasitism, and induced disease resistance. Production of hydrolytic enzymes, toxic compounds (peptides, trichodermin, chitinase, glucanse) involved in cell wall lysis of pathogenic fungi and immobilize the nematodes by proteolytic activity (Howell, 2003). *Trichoderma viride* also produces antibiotics such as dermadin, trichoviridin, and sesquiterpene heptalic acid which are involved in the suppression of nematodes. Pseudomonads inhibit both *M. incognita* and *F. oxysporum* f.sp. *psidii* by producing high-affinity iron-chelators, that creates competition for iron with soil-borne pathogens having less potent siderophores (Kloepper *et al.,* 1980). Secondary metabolites of fluorescent pseudomonads such as phenazine, pyocyanine, pyrrolnitrin, acetylphloroglucinols and cyanide inhibit soil borne pathogens and also affect egg hatching and all juvenile stages (Davison, 1988; Defago and Hass, 1990). *Pseudomonas* spp. inoculation enhances the plant growth parameters by increasing the activity of peroxidase and phenylalanine ammonia-lyase in chilli and tomato plants (Sharma *et al.,* 2007). The inhibition in *M. incognita* density may be due to the parasitic activity of *P. lilacinum* on eggs and all stages of nematodes by producing peptidal antibiotics such as paecilo, leucinostatin, lilacin toxin which has nematicidal effect. *P. lilacinum* also produces enzymes *viz*., protease and chitinase and protease causes eggshell degradation and inhibits hatching. Whereas, chitinase breaks down the eggshell making route for the fungus and the decomposition of chitin releases ammonia, which kill the root-knot nematode juveniles (Elgawad and Askary, 2018). The fungus penetrates the egg and develops profusely inside and over the eggs, completely inhibiting juvenile development. However, *P. lilacinum* reduced nematode reproduction factors, but plant variables were not affected. Therefore plants treated with *T. viride* and *P. fluorescens* individually and in combined formulation of three bio-agents had increased growth parameters. The antibiotics, secondary metabolites, toxic compounds and enzymes produced by three bio-agents are effective against both *M. incognita* and *F. oxysporum* f.sp. *psidii* through their nematicidal and fungicidal property and thus, these bio-agents decrease diseases incidence and enhanced the plant growth. The synergistic effects of bio-agents *T. viride, P. fluorescens* and *P. lilacinum* in combined formulation clearly indicate their potential in enhacing guava seedlings growth and reduction of both *M. incognita* and *F. oxysporum* f.sp. *psidii* population.

The results of Gupta and Misra (2009) showed that the bio-agents, *T. virens* and *T. viride* inhibited the growth of *Fusarium oxysporum* f. sp. *psidii* and *Fusarium solani* under *in-vitro* through toxic metabolites released by them in culture filtrate or action of volatile

compounds. Under field conditions, *Trichoderma* and *Aspergillus* isolates completely suppressed guava wilt and also enhanced the growth of the guava plants

In the present investigation, results are in agreement with the findings of Jindapunnapat *et al.,* (2013) who evaluated commercially available fungal bio-agent, *Trichoderma harzianum* against *M. incognita* and *M. enterolobii* on guava orchards in Thailand. It was found that inoculation of *T. harzianum* reduced nematode population through producing toxic compounds, induced resistance and stimulation of the adventitious root growth. Almeida *et al.* (2011) isolated 120 rhizobacterial isolates from guava orchards infected by *Meloidogyne enterolobii*, of which, 44 isolates were tested on guava plants and all 44 rhizobacteria reduced the number of galls, number of egg mass and final nematode population. Singh and Singh (2015) assessed the effect of non-host crops intercropping, bio-agents and oil cakes on population dynamics of *F. oxysporum* f. sp. *psidii* and wilt of guava. Among oil cakes tested, neem cake significantly reduced population of *F. oxysporum* f. sp. *psidii*, followed by mahua cake, over control. The combination of neem cake, *T. harzianum* and garlic reduced the fungus population significantly followed by the combination of neem cake, *T. harzianum* and marigold. Rao (2007) reported the bio-agent colonized seedlings of papaya by *P. fluorescens* and *T. harzianum*, effectively reduced the root-knot nematode in both nursery and field as well.

Zaitoun *et al.* (2015) evaluated bio-agents, *Bacillus subtilis, P. fluorescens* and *T. harzianum* against guava decline causing pathogens viz., *Fusarium oxysporum*, *Botryodiplodia theobromae* and *Rhizoctonia solani*. *T. harzianum* isolate-T4 at different densities (50, 100 and 150 ml, 10^7 spores/ ml) decreased the disease severity and increased plant height, dry weight of shoots and roots and total pigments in guava trees in comparison with untreated trees.

Our results indicated that the combined formulation of *T. viride, P. fluorescens*, and *P. lilacinus* was more effective against *M. incognita* and *F. oxysporum* f. sp. *psidii* and also enhanced the plant growth. Similar results were observed by Sonyal (2015) who conducted the experiment to evaluate different bio-control agents and their combination with chemicals for managing pomegranate wilt complex caused by *M. incognita, C. fimbriata* and *F. oxysporum.* The combination of *P. fluorescens, T. harzianum, P. lilacinum* and difenconazole was significantly superior over all the treatments in reducing the wilting of branches, plants and managing the *M. incognita* population. The integrated management practices such use of nematode free guava seedling, removal and destroying of nematode infested plants, application of 100 kg of farm yard manure, 250 g of neem cake and 25 g of *P. lilacinum* and carbofuran @ 60g per tree at early stage of nematode infestation were effective against *Meloidogyne enterolobii* in guava orchards and to enhance fruit yield in guava (Anonymous, 2015).

Chormule *et al.* (2017) also observed that reduction of *M. incognita* population in grapevine inoculated with bio-agents *viz.*, *P. fluorescence, P. lilacinum, Trichoderma* plus, *T. viride* and *Pochonia chlamydosporia* @ 20 kg/ha and organic amendment, neem cake @ 2 t/ha and were effective in reducing number of root galls and egg masses and increasing the yield and among bio-agents *P. fluorescence* was found to be most effective in reducing nematode population (38.6%).

Dawabah *et al*. (2019) also observed that the combination of bio-agents along with organic amendments (*P. lilacinum* + *P. penetrans* + urea and *T. harzianum* + *P. penetrans* + poultry manure) reduced the nematode densities of *M. javanica, Tylenchorhynchus mediterraneus, Hoplolaimus seinhorsti, Longidorus latocephalus,* and *Xiphinema elongatum* on guava and fig trees in Saudi Arabia.

Since the use of chemical pesticides can be hazardous and causes environmental pollution, the use of bio-pesticides (*T. viride, P. fluorescens,* and *P. lilacinum*) are alternative for the chemical pesticides and eco-friendly for production of healthy crop yield.

CHAPTER- VI

SUMMARY AND CONCLUSIONS

Guava (*Psidium guajava* L.) is an important fruit crop of tropical and subtropical countries and plays an important role in Indian farmers' economy by exporting 1229.75 MT that earns 553.26 lakh rupees every year. India ranks first in the production and shares 44.4% of world guava production (National Horticulture Board, 2018). Many biotic and abiotic factors are affecting the production and productivity of guava in India; among biotic factors 'Guava decline' a complex infestation of *Meloidogyne* spp. and *F. oxysporum* f. sp. *psidii* causes severe losses in terms of quality and quantity of fruits. The present investigation was conducted with objectives of the survey for the incidence of guava decline in four districts of Haryana, identification of pathogens involved, the interaction of *M. incognita* and *F. oxysporum* f.sp. *psidii*, and management of guava decline by organic amendments and bio-agents.

The results from the survey of Hisar, Jind, Sirsa and Fatehabad districts revealed the occurrence of nine plant-parasitic nematode genera *viz*., *Meloidogyne* sp., (*M. incognita, M. javanica*) *Rotylenchulus reniformis, Helicotylenchus* sp., *Hoplolaimus* sp., *Pratylenchus* sp., *Tylenchorhynchus* sp., *Tylenchus* sp., *Xiphinema* sp., and *Longidorus* sp. and fungal pathogens *viz*., *F. oxysporum, Macrophomina phaseoli* and *Rhizoctonia solani* from infected guava orchards. Among plant-parasitic nematodes, root-knot nematode, *M. incognita* was predominant, caused much damage and was highly pathogenic to guava plants. The frequency of occurrence of *M. incognita* was recorded maximum in Fatehabad (72.2%) followed by Hisar (63.2%), Jind (56.3%) and Sirsa district (53.3%). The maximum density range (j_2/200cc soil) of *M. incognita* was observed in Sirsa (425-940), *Helicotylenchus* spp. (80-425) in Jind, *Rotylenchulus reniformis* (35-230) in Fatehabad and *Pratylenchus* spp. (60-280) in Sirsa district. The individual incidence of *F. oxysporum* f. sp. *psidii* varied from 23.0 to 42.6 per cent and *M. incognita* incidence varied from 33.0 to 57.7 per cent, whereas combined infection by *M. incognita* and *F. oxysporum* f. sp. *psidii* varied from 49.1 to 67.7 per cent.

The experiment on pathogenicity of *M. incognita* on guava was studied by inoculating different inoculum levels *viz*., 0, 10, 100, 500, 1000, 2000, and 4000 j_2/kg soil. The experimental results revealed that the significant reduction of plant variables were observed at the inoculum level of 1000 j_2 and which was considered to be pathogenic level to guava plants. As nematode density increased from 10 to 4000 j_2, plant growth parameters decreased accordingly. The significantly lowest plant growth parameters were observed in the highest inoculum level of 4000 j_2/kg soil. The nematode reproduction factors were increased significantly as the inoculum levels increased from 10 to 4000 j_2. The highest nematode factors were observed at 4000 j_2 and the lowest was recorded at 10 j_2/kg soil.

The pathogenicity of *F. oxysporum* f. sp. *psidii* was also studied at different inoculum levels *viz*., 2, 4, 6, 8, and 10g mycelium/kg soil on guava seedlings under screen house conditions. The wilting symptoms and isolation of *F. oxysporum* from inoculated plants were compared with originally observed symptoms and conidial character. Hence pathogenicity was proved and the fungus was determined as *F. oxysporum* f.sp. *psidii*. The significant reduction in shoot length, fresh and dry shoot weight was observed at 6 g mycelium and which was considered to be pathogenic level to guava plants. As increase in inoculum levels, there was a decrease in plant growth gradually and least plant growth parameters were observed at inoculums level of 10g mycelium/kg soil.

The interaction of *M. incognita* and *F. oxysporum* f. sp. *psidii* revealed that statistically lowest plant growth parameters were recorded in plants inoculated with *M. incognita* 10 and 20 days prior to *F. oxysporum* f.sp. *psidii* inoculation. Nematode reproduction factors were significantly highest in plants inoculated with nematode alone which was significantly at par with nematode 20 and 10 days prior to fungus. The nematode reproduction factors were affected by the combined and chronological inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii.* The significantly lowest number of galls, egg masses, eggs per egg mass and final nematode population were recorded in plants inoculated with fungus 20 days prior to nematodes inoculation, followed by fungus 10 days prior to the nematode. The maximum root rot was observed in plants inoculated with *M. incognita* 10 (43.6%) days and 20 days (39.34%) prior to inoculation of *F. oxysporum* f.sp. *psidii* followed by simultaneous inoculation of nematode and fungus (38.00%). Thus, the presence of both nematode and fungus caused more root rotting comparison to plants inoculated with nematode and fungus individually.

The effects of different deoiled cakes *viz*., neem, mustard and castor were evaluated against *M. incognita* and *F. oxysporum* f.sp. *psidii* individually and in combination on guava seedlings under screen house conditions. Among different oil cakes evaluated, the maximum plant growth parameters were observed in treatments having neem cake @ 30g followed by mustard cake @ 30g irrespective of individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*. Carbendazim and carbofuran were effective when the plants were inoculated with fungus and nematode individually but were not effective in combined inoculation, as they controlled either of pathogens inoculated. The application of organic amendments *viz*., neem and mustard cakes @ 30g improved the plant variables and were effective than chemicals tested in plants inoculated with nematode and fungus simultaneously.

The incorporation of deoiled cakes reduced the nematode reproduction factors. In case of nematode alone, the significantly lowest number of galls was recorded in treatments having neem cake @ 30g (94.66), carbofuran (107.33) and mustard cake @ 30g (106.67) in comparison to untreated inoculated check (186). With respect to combined inoculation, the

significantly lowest number of galls was recorded in treatment of neem cake @ 30g (86.67) which was at par with carbofuran (91.67) and mustard cake @ 30g (93.33). The significant reduction in the nematode reproduction factors viz., number of egg masses, eggs per egg mass and final nematode population were also observed in treatments having neem cake @ 30g/kg soil irrespective of whether nematode alone or combined inoculation of nematode and fungus.

The effect of different bio-agents such as *Trichoderma viride*, *Pseudomonas fluorescens*, and *Purpureocillium lilacinum* were evaluated against *M. incognita* and *F. oxysporum* f.sp. *psidii*. Among different bio-agents tested, the treatments receiving combined formulation of *T. viride*, *P. fluorescens* and *P. lilacinum* @ 10ml/kg soil recorded maximum plant growth parameter irrespective of whether individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii.*

The combined formulation of three bio-agents @ 10ml significantly reduced nematode reproduction factors (number of galls, egg masses, eggs/egg mass and final nematode population). In case of nematode alone, significantly minimum number of galls was observed in combined formulation of bio-agents (103.33) followed by carbofuran (120.00) and *T. viride* (130.67). With respect to combined inoculation of nematode and fungus, the significantly lowest number of galls was recorded in treatment receiving combined formulation (90.00) which was at par with carbofuran (106.67). In case of plants inoculated with fungus alone, significantly minimum root rot was observed in treatment receiving carbendazim followed by combined formulation of *T. viride, P. fluorescens* and *P. lilacinum* @ 10ml/kg soil. In the combined inoculation of nematode and fungus, the significantly minimum root rot was observed in plants receiving combined formulation of bio-agents @ 10 ml/kg soil. Thus, the combined formulation of *T. viride, P. fluorescens,* and *P. lilacinum* was more effective than individual applications of bio-agents.

The following conclusions were drawn based on the present investigation results;

- The survey results of Hisar, Jind, Sirsa and Fatehabad districts revealed the occurrence *M. incognita, M. javanica* and other plant-parasitic nematodes; and fungal pathogens *viz.*, *Fusarium oxysporum, Macrophomina phaseoli* and *Rhizoctonia solani* from infected guava orchards.
- Among plant-parasitic nematodes, *M. incognita* was predominant, caused much damage and was highly pathogenic to guava plants.
- The guava decline severity and incidence was prominent where plants were infected by both *M. incognita* and *F. oxysporum* f.sp. *psidii* under field conditions.
- The experiment on pathogenicity of *M. incognita* on guava revealed that the significant reduction of plant variables were observed in inoculum level of 1000 j_2/kg soil and which was considered to be pathogenic level to guava plants.

- The pathogenicity of *F. oxysporum* f. sp. *psidii* on guava seedlings was proved and inoculum level of 6g mycelium/kg soil was considered to be pathogenic level to guava plants.
- The interaction of *M. incognita* and *F. oxysporum* f. sp. *psidii* revealed that statistically lowest plant growth parameters and maximum root rot were recorded in plants inoculated with *M. incognita* 10 and 20 days prior to *F. oxysporum* f. sp. *psidii* inoculation.
- Soil application of neem and mustard cake @ 30g/kg soil reduced the nematode reproduction, fungus infection and enhanced plant growth parameters irrespective of individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*.
- Among different bio-agents tested, the treatments receiving combined formulation of *T. viride*, *P. fluorescens* and *P. lilacinum* @ 10ml/kg soil recorded maximum plant growth parameters, minimum root rot and significantly reduced nematode reproduction factors irrespective of whether individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*.
- The combined formulation of *T. viride*, *P. fluorescens* and *P. lilacinum* @ 10 ml/kg soil was effective than carbofuran and carbendazim in the presence of both *M. incognita* and *F. oxysporum* f.sp. *psidii*.

BIBLIOGRAPHY

Abbasi, P. A., Riga, E., Conn, K. L. and Lazarovits, G. (2005). Effect of neem cake soil amendment on reduction of damping-off severity and population densities of plant-parasitic nematodes and soilborne plant pathogens. *Canadian Journal of Plant Pathology*, **27**(1): 38-45.

Abd-elgawad, M. M., and Askary, T. H. (2018). Fungal and bacterial nematicides in integrated nematode management strategies. *Egyptian Journal of Biological Pest Control*, **28**(1): 74.

Akhtar, H., Amin A. and Sharma A. (2007). Disease complex in *Pisum sativum* involving *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *pisi. Annals of Plant Protection Sciences.,* **15**(1): 189-194.

Almeida, A. M., Gomes, V. M. and Souza, R. M. (2011). Greenhouse and field assessment of rhizobacteria to control guava decline. *Bragantia*, **70**(4): 837-842.

Almeida, A. M., Souza, R. M., Gomes, V. M. and Miranda, G. B. (2012). Greenhouse and field assessment of different organic compounds against guava-parasitic *Meloidogyne enterolobii. Bragantia*, **71:** 67-74.

Anonymous (2015). Technical report, Tamil Nadu Agricultural University, Coimbatore, India.

Anonymous (2018). Horticultural Statistics at a Glance, Department of Agriculture, Cooperation and Farmers' Welfare, Horticulture Statistics Division**,** Ministry of Agriculture and Farmers' Welfare**,** Government of India. 458 pp.

Ansari, M. A. and Ahmad, W. (2000). Community analysis of plant parasitic and predatory nematodes in Guava (*Psidium guajava*) orchard. Proc. 52nd International Symposium on Crop Protection, Gent, Belgium, 9 May, Part II. Mededelingen Faculteit Land bouwkundige en Toegepaste Biologische Wetenschappen, Universiteit Gent, **65**: 557-562.

Ansari, R. A. and Khan, T. A. (2012). Parasitic association of root-knot nematode, *Meloidogyne incognita* on guava. *Journal of Science and Technology*, **5**: 65-67.

Arredondo, J. A. R. (1992). Effect of some plant, soil and management factors on nematode populations in grape vineyards and pathogenicity of *Meloidogyne incognita* on cv. Carignane. *Revista Mexicana de Fitopatologia*, **10**(1): 49-53.

Ashok, J. P. (2017). Studies on incidence, interaction and management of root-knot nematode, *Meloidogyne incognita* and soil borne fungus infesting cucumber in polyhouse. *Ph.D. Thesis*. CCS HAU, Hisar, Haryana, India.

Avelar Mejia, J. J., Teliz Ortiz, D. and Zavaleta Mejia, E. 2001. Pathogens associated to guava decline. *Revista Mexicana de Fitopatologia*, **19**: 223-229.

Babatola, J. O. and Oyedunmade, E. E. A. (1992). Host-parasite relationships of *Psidium guajava* cultivars and *Meloidogyne incognita*. *Nematologia Mediterranea*, **20**(2): 233-235.

Bokhari, A. A. (2009). Studies on guava decline and disease management, *Ph.D. Thesis*, University of Agriculture, Faisalabad, Pakistan.

Bokhari, A. A., Sahi, S. T., Khan, M. A., Ahmad, R., and Din, I. U. (2008). *In vivo* studies on the biological and chemical control of guava decline caused by different soil borne pathogens. *Pakistan Journal Agricultural Sciences,* **45**(1): 54-56.

Booth, C. (1971). The genus *Fusarium*. Common Wealth Mycolgocial Institute Surrey England, 237.

Burla, R. S., Souza, R. M., Gomes, V. M. and Corre, F. M. (2010). Comparison between inoculum levels, evaluation times and variables for selection of *Psidium* spp. targeting resistance to *Meloidogyne enterolobii*. *Nematologia Brasileira*, **34** (2): 82-90.

Carneiro, R. M., Cirotto, P. A., Quintanilha, A. P., Silva, D. B. and Carneiro, R. G. (2007). Resistance to *Meloidogyne mayaguensis* in *Psidium* spp. accessions and their grafting compatibility with *P. guajava* cv. Paluma. *Fitopatologia Brasileira*, **32**(4): 281-284.

Chattopadhyay, S. B. and Bhattacharya, S. K. (1968). Investigations on the wilt disease of guava (*Psidium guajava* L.) in West Bengal. *Indian Journal of Agricultural Science*, **38**: 176-183.

Chattopadhyay, S. B. and Sengupta, S. K. (1955). Studies on wilt of *Psidium guajava* L. in West Bengal. *Indian Journal of Horticulture*,**12**: 76-79.

Chaudhary, K. K. and Kaul, R. K. (2013). Efficacy of *Pasteuria penetrans* and various oil seed cakes in management of *Meloidogyne incognita* in chilli pepper (*Capsicum annuum L.). Journal of Agricultural Science and Technology*, **15:** 617-626.

Chitwood, B. G. (1949). Root-knot nematode Part-1. A revision of the genus *Meloidogyne* Goeldi, *Proceedings of the Helminthological Society of Washington*,**16**: 90-104.

Chormule A. J., Mhase, N. L. and Gurve, S.S. (2017). Management of root-knot nematode, *Meloidogyne incognita* infesting grape under field conditions. *Annals of Plant Protection Science*, **25** (1) : 181-185.

Cobb, N. A. (1918). Estimating the nematode population of soil. *U.S. Dep. Agr. Bur. plant Ind. Agriculture technical circular*, **1:**1-48.

Crozzoli, R. and Casassa, A. M. (1998). *Meloidogyne* species and races in the cultivation of guava in Venezuela. *Journal of the Faculty of Agronomy*, Universidad del Zulia, **15**: 107-108 (in Spanish).

Das Gupta, S. N. and Rai, J. N. (1947). Wilt disease of guava (*P. guajava*). *Current Science*,**16**: 256-258.

Davison, J. (1988). Plant beneficial bacteria. *Biotechnology*, **6**(3): 282.

Dawabah, A. A., Al-Yahya, F. A., and Lafi, H. A. (2019). Integrated management of plant-parasitic nematodes on guava and fig trees under tropical field conditions. *Egyptian Journal of Biological Pest Control*, ***29***(1): 29.

De Moura, R. M. and De Moura, A.M. (1989). Meloidogyninose da goiabeira: doenca de alta severidade no estado de Pernambuco, Brasil. *Nematologia Brasileira*, **13**: 13–19 (in Portuguese).

De Siqueira, M. S. K., Freitas, V. M., Almeida, M. R. A., dos Sar, M. F. A., Cares J. A., Tigano, M. S. and Carneiro, R. M. D. G. (2009). Detection of *Meloidogyne mayaguensis* on guava and papaya in Goias State using molecular markers. *Tropical Plant Pathology*, **34:** 1-8.

Defago, G. and Haas, D. (1990). *Pseudomonads* as antagonists of soil borne plant pathogens: modes of action and genetic analysis. *Soil Biochemistry*, **6**: 249–291

Dhingra, O. D. and Sinclair, J. B. (1995). Basic plant pathology methods. Boca Raton, Florida, USA, *Chemical Rubber Company Press*.

Dinesh, B. M. (2014). Investigations on the burrowing nematode *(Radopholus similis)* and fungal wilt (*Fusarium oxysporum* f.sp. *cubense*) complex in Banana, *Ph.D. Thesis.* University of Agricultural Sciences, Bengaluru, India. 248 pp.

Dwivedi, B. K., Malhotra, S. K. and Misra, S. L. (1987). Interrelationship of population distribution of *Hoplolaimus indicus* around root zones of *Psidium guajava* with season and soil temperature at Allahabad. *Indian Journal of Nematology*, **17**(1): 49 – 53.

Dwivedi, S .K. (1990). Guava wilt incited by *Macrophomina phaseolina*, *National Academic Science Letter*, India, **13** (8): 302-303.

Dwivedi, S .K.and Dwivedi, P. (1999). Wilt disease of Guava a national problem, *Journal of Applied Horticulture*, **1**(2): 151-154.

Dwivedi, S. K., Dwivedi, R. S. and Tiwari, V.P. (1990). Studies on pathogenic fungi inciting guava wilt in Varanasi. *Indian Phytopathology*, **43**:116-117.

Dwivedi, S. K., Misra, R. C. and Dwivedi, R. S. (1988). Incidence of wilt disease of guava (*Psidium guajava* L.) in Varanasi, India, *International Journal of Tropical Plant Diseases*, **6(**2): 213-216.

Edward, J. C. (1960). Wilt disease of guava. *The Allahabad Farmer*, **34**: 289-293.

Edward, J. C. and Srivastava, R. N. (1957). Studies on guava wilt. *Allahabad Farmer*, **31**: 144-146.

Ellenby, C. (1951). Mustard oil and control of potato root eelworm, *Heterodera rostochinensis* Wollenweber: Further field experiments. *Annals of Applied Biology*, **1**: 287-288.

Ellis, M. B. 1971. Dematiaceous Hyphomycetes. Kew, UK: CABI Publishing.

El-Naggar, H.I., H.H. Hendy, S.H. Abdel-Hameed, A.A. Farahat and A.A. Osman, (1993). The role of dry ground leaves of some plants in controlling the reniform nematode, *Rotylenchulus reniformis* infecting sunflower. *Bulletin of Faculty of Agriculture*, Cairo University, **44**: 205-216.

Fattah, F. A., and Webster, J. M. (1989). Ultrastructural modifications of *Meloidogyne javanica* induced giant cells caused by fungal culture filtrates. *Revue de Nématologie*, **12**: 197-210.

Fernandez, D. and Silveira, M. (1975). El *Psidium friedrichsthalianum* como patron para guayabo, resistente a los nematodos del genero *Meloidogyne*. *Revista de Agricultura Cuba*, **3**: 80–85.

Ganaie, M. A., Azhar, A., Rather and Mansoor, A. (2011). Pathogenicity of root knot nematode, *Meloidogyne incognita* on okra and its management through botanicals. *Archives of Phytopathology and Plant Protection*, **44**(17): 1683–1688.

Gomes, V. M., Souza, R. M., Almeida, A. M., and Dolinski, C. (2014). Relationships between *M. enterolobii* and *F. solani*: spatial and temporal dynamics in the occurrence of guava decline. *Nematoda*, **1**(1): e01014.

Gomes, V. M., Souza, R. M., Corrêa, F. M. and Dolinski, C. (2010). Management of *Meloidogyne mayaguensis* in commercial guava orchards with chemical fertilization and organic amendments. *Nematologia Brasileira*, **34:** 23-30.

Gomes, V. M., Souza, R. M., Da Silveira, S. F. and Almeida, A. M. (2013). Guava decline: effect of root exudates from *Meloidogyne enterolobii*-parasitized plants on *Fusarium solani* in vitro and on growth and development of guava seedlings under controlled conditions. *European Journal of Plant Pathology*, **137**(2): 393-401.

Gomes, V. M., Souza, R. M., Midorikawa, G., Miller, R. and Almeida, A. M. (2012). Guava decline: evidence of nationwide incidence in Brazil. *Nematropica*, **42**:153-162.

Gomes, V. M., Souza, R. M., Mussi Dias, V., Silveira, S. F. and Dolinski, C. (2011). Guava decline: a complex disease involving *Meloidogyne mayaguensis* and *Fusarium solani*. *Journal of Phytopathology*, **159**: 45-50.

Gupta, P., Dubey, V., David, A. and John, D. (2003). *Verticillium albo-atrum* a new pathogen associated with guava wilt. *Indian Phytopathology*, **56**(4): 503-504.

Gupta, V. K. and Misra, A. K. (2012). *Fusarium chlamydosporum*, causing wilt disease of guava (*Psidium guajava* L.) in India. *Archives of Phytopathology and Plant Protection*, **45**(20): 2425-2428.

Gupta, V. K., and Misra, A. K. (2009). Efficacy of bioagents against *Fusarium* wilt of guava. *Journal of Mycology and Plant Pathology*, **39**(1): 101-106.

Gupta, V. K., Misra, A. K., Gaur, R. K., Jain, P. K., Gaur, D. and Sharma, S. (2010). Current status of *Fusarium* wilt disease of guava (*Psidium guajava* L.) in India. *Biotechnology*,**9**: 176–192.

Hamiduzzaman, M. M., Meah, M. B., and Ahmad, M. U. (1997). Effect of *Fusarium oxysporum* and nematode interaction on guava wilt. *Bangladesh Journal of Plant Patho*logy, ***13***(1): 9-11.

Hollis, J. P. and Rodriguez-Kabana, R. (1966). Rapid kill of nematodes in flooded soil. *Phytopathology*, **56**: 1015-1019.

Howell, C. R. (2003). Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. *Plant Disease*, **87**(1): 4-10.

Jhooty, S., Chand, N. and Krishnamurthy, V. (1984). Report: Guava decline in Punjab and Haryana. In :*Consolidated Report of Multilocational Project on Guava Decline*, 34-41.

Jindapunnapat, K, Chinnari, B. and Kwankuae, S. (2013). Biological control of root-knot nematodes (*Meloidogyne enterolobii*) in guava by the fungus *Trichoderma harzianum. Journal of Developments in Sustainable Agriculture,* **8:**110-118.

Jindapunnapat, K. (2012). Development of the molecular markers for species identification of root-knot nematode infesting guava in Thailand. Master's thesis, Kasetsart University, Bangkhen.

Jonathan, E. I., Gajendran, G. and Manuel, W.W. (2000). Management of *Meloidogyne incognita* and *Helicotylenchus multicinctus* in banana with organic amendments. *Nematologia Mediterranea,* **28**: 103-105.

Karssen, G, Liao J. L., Kan, Z., Heese, E. and Nijs, L. (2012). On the species status of the root-knot nematode *Meloidogyne mayaguensis* Rammah and Hirschmann, 1988. *ZooKeys*, **181:**67–77.

Khan, A. H., Haseeb, A., Rehman, R., Saxena, S. K. and Khan, A. M. (1980). Population fluctuation of some nematodes around roots. *Geobios,* **7**: 55-57.

Khan, M, R., Kumar, S. and Reddy, P. P. (2001). Role of plant parasitic nematode(s) and fungi in guava wilt. *Pest Management Horticulture Ecosystem,* **7** : 152-16.

Khan, M. R., Hassan, A., Ghosh, B., Das, B., Ghosh, S. and Ray, S. K. (2007). Diversity and community analysis of soil nematodes associated with guava from West Bengal, India. *Acta Horticulture,***735**: 483-488.

Khan, M. W., Alam, M. M., Khan, A. M., and Saxena, S. K. (1974). Effect of water soluble fractions of oil-cakes and bitter principles of neem on some fungi and nematodes. *Acta Botanica Indica,* **2**: 120-128.

Khattak, S and Khattak, B. (2011). Management of root-knot nematode with *Trichoderma harzianum* and spent mushroom compost. In: proceedings of "*46th Croatia and 6th international Symposium on Agriculture*" held at *Optija, Croatia,* 14-18 February 2011.pp.157-160.

Kloepper, J. W., Leong, J., Teintze, M. and Schroth, M. N. (1980). *Pseudomonas* siderophores: a mechanism explaining disease-suppressive soils. *Current Microbiology*, **4**: 317–320.

Kumar, S. and Rawat, S. (2018). First report on the root-knot nematode *Meloidogyne enterolobii* (Yang and Eisenback, 1988) infecting guava (*Psidium guajava*) in Udham Singh Nagar of Uttarakhand, India. *International Journal of Current Microbiology and Applied Science*, **7(**4): 1720-1724.

Logani, R., Pandey, R. C., Dwivedi, B. K. and Singh, S. P. (2002). An eco-friendly management of wilt disease of guava by wilt nema (a botanical fungicide). *Bioved*, **13**:47–50.

Mahapatra, S. (1995). Etiology of interaction between *Meloidogyne incognita* and *Fusarium oxysporum* on blackgram, *Vigna mungo* (L.) Hepper. *Ph.D thesis,* The Utkal University, Bhubaneswar, India.113 pp.

Mani, A. and Sethi, C. L. (1987). Interaction of root-knot nematode, *Meloidogyne incognita* with *Fusarium oxysporium* f. sp. *ciceri* and *Fusarium solani* in chickpea. *Indian Journal of Nematology*, **17**: 1-6.

Marais, M. (2014). Identification job sheet N03630: Dataset from South African Plant-Parasitic Nematode Survey (SAPPNS) database. Pretoria: Nematology Unit, Biosystematics Division, Agricultural Research Council - Plant Protection Research Institute, Pretoria.

Margarita, R., Maria, T., Hernandez. and Carlos, G. (2003). Soil microbial activity after restoration of a semi arid soil by organic amendments. *Soil Biology and Biochemistry*, **35**:463–469.

Martin, L. S. S., Musser, R. D. S., Souza, A. D. G., Resende, L. V. and Maluf, W. R. (2013). Parasitism of *Meloidogyne enterolobii* in myrtaceae species. *Revista Brasileira de Fruticultura*, **35**(2): 477-484.

Mehta, N. (1987). Distribution of guava wilt in relation to age, soil type, management practices and varieties grown in Haryana. *Plant Disease Research*, ***2***:116-119.

Mehta, P.R. (1951). Observations on new and known diseases of crop plants in Uttar Pradesh. *Plant Proto Bull.,* **3**: 712.

Mejia, R. S., Jain, S. S. and Fravel, D. (2002). The synergistic effect of fungi and nematodes causing guava decline. Proceedings of the National Academy of Sciences, India. **20**(2): 65-71.

Mervat, A. A., Shawky, S. M., and Shaker, G. S. (2012). Comparative efficacy of some bioagents, plant oil and plant aqueous extracts in controlling *Meloidogyne incognita* on growth and yield of grapevines. *Annals of Agricultural Sciences*, **57**(1): 7-18.

Milan, A. R. (2005). Breeding of *Psidium* species for root knot nematode resistance in Malaysia. In *International Guava Symposium,* **735**: 61-69.

Miranda, G. B., Souza, R. M. and Viana, A.P. (2010). Assessment of methods and criteria for screening *Psidium* spp. for resistance to *Meloidogyne enterolobii*. *Nematologia Brasileira*, **34**(4)**:** 211-219**.**

Mishra, R. K., Pandey, B. K., Muthukumar, M., Pathak, N. and Zeeshan, M. (2013). Detection of *Fusarium* wilt pathogens of *Psidium guajava* L. in soil using culture independent PCR (ciPCR). *Saudi Journal of Biological Sciences*, **20**(1): 51-56.

Misra, A. K. and Pandey, B. K. (1999a). Natural wilting of guava plants during different months. 51st Annual Meeting and National Symposium on Seed health care and Phyto-sanitation for Sustainable Agriculture. 17-19 Feb. 1999, IISR, Lucknow. pp. 64-65.

Misra A. K. and Pandey, B. K. (1999b). Natural wilting of guava plants during different months. *Indian Phytopathology*, **52**: 312.

Misra, A. K. and Gupta, V. K. (2010). Relative pathogenicity of *Fusarium* wilt isolates to *Psidium guajava*. *Journal of Mycology and Plant Pathology*, **40(**1): 72.

Misra, A. K. and Pandey, B. K. (1992). Wilt of guava and associated pathogen. *Indian Journal of Mycology and Plant Pathology*, **22**:85-86.

Misra, A. K. and Pandey, B. K. (2000). Progressive natural wilting of guava plants during different months. *Indian Phytopathology*, **53**: 423-427.

Misra, A. K. and Shukla, S. K. (2002). Assessment of loss due to Guava wilt around Lucknow. Proceedings of the National Seminar on Production and Post-Harvest Technology of Guava, Jan. 9-10, Dept. Hort. CSAUA & T, Kanpur, 34-35.

Misra, A. K., Prasad, D., Prasad, B. and Shukla, S. K. (2004). Effective management of wilt disease of guava. Proceedings of the National Symposium on Crop Surveillance, Disease Forecasting and Management, Division of Plant Pathology, IARI, New Delhi, 92-93.

Moura, R. M de, Moura, A. M de, Marinho de Moura, R. and Marinho, de Moura, A. 1989. Root-knot on guava: a severe disease in Pernambuco State, Brazil. *Nematologia Brasileira*, **13** : 13-19.

Onkendi, E. M. and Moleleki, L. N. (2013). Detection of *Meloidogyne enterolobii* in potatoes in South Africa and phylogenetic analysis based on intergenic region and the mitochondrial DNA sequences. *European Journal of Plant Pathology* ,**136**: 1-5.

Pandey, R. R. and Dwivedi, R. S. (1985). *Fusarium oxysporum* f. sp. *psidii* as a pathogen causing Wilt of Guava in Varanasi District India. *Journal of Phytopatology*,**114**: 243-248.

Pathak, K. N., Roy, S., Ojha, K. L. and Jha, M. M. (1999). Influence of *Meloidogyne incognita* on the fungal and bacterial wilt complex of banana. *Indian Journal of Nematology*, **29**(1): 39-43.

Pereira, F. M., Souza, R. M., Souza, P. M., Dolinski, C. and Santos, G. K. (2009). Estimativa do Impacto Econômico e Social Direto de *Meloidogyne mayaguensisna* Cultura da Goiaba no Brasil *Nematologia Brasileira*, **33**: 176–181.

Poornima, K., Suresh, P., Kalaiarasan, P., Subramanian, S. and Ramaraju, K. (2016). Root-knot nematode, *Meloidogyne enterolobii* in guava (*Psidium guajava* L.) a new record from India. *Madras Agricultural Journal*, **103**: 359-365.

Prasad, N., Mehta, P. R. and Lal, S. B. (1952). *Fusarium* wilt of guava (*Psidium guajava*) in Uttar Pradesh, India. *Nature*, **169**: 753.

Raguchander, T., Shanmugam, V. and Samiyappan, R. (2001). Biological control of Panama wilt disease of banana. *Madras Agricultural Journal*, **87**(4/6): 320-321.

Rahman, L. and Somers, T. (2005). Suppression of root knot nematode (*Meloidogyne javanica*) after incorporation of Indian mustard cv. Nemfix as green manure and seed meal in vineyards. *Australasian Plant Pathology*, **34:** 77‑83.

Rao, M. S. (2007). Papaya seedlings colonized by the bioagents, *Trichoderma harzianum* and *Pseudomonas fluorescens* to control root-knot nematodes. *Nematologia Mediterranea*, **35**: 199-203.

Rao, M. S. (2010). Effect of combinations of bio-pesticides on the management of nematodes on *Carica papaya* L. *Acta Horticulture*, **851**: 459-464.

Raut, S. P. (1981). Effect of initial inoculums levels of *Meloidogyne incognita* on plant growth and rhizobial nodulation of mungbean. *Indian Phytopathology*, **33**: 351-353.

Razak, A. R. and Lim, T. K. (1987). Occurrence of the root-knot nematode *Meloidogyne incognita* on guava in Malaysia, *Pertanika*, **10**(3): 265-270.

Reddy, P. P., Nagesh, M. and Devappa, V. (1997). Effect of integration of *Pasteuria penetrans*, *Paecilomyces lilacinus* and neem cake for the management of root-knot nematodes infecting tomato. *Pest Management in Horticultural Ecosystems*, **3:** 100-104.

Rehman, B., Ganai, M. A., Parihar, K., Asif, M. and Siddiqui, M. A. (2014). Biopotency of oilcakes against *Meloidogyne incognita* affecting *Vigna mungo*. *European Journal of Applied Sciences*, **6**(3): 57-63.

Rich, J. R., Rahi, G. S., Opperman, C. H., and Davis, E. L. (1989). Influence of the castor bean (*Ricinus communis*) lectin (ricin) on motility of *Meloidogyne incognita*. *Nematropica*, **19**(1): 99-103.

Ruchi, L., Dwivedi, B. K., Dwivedi, B. P. and Shukla, D. N. (2002). Nematode and wilt problems of guava in and around Allahabad region. *Current Nematology*, **13**(2): 23-26.

Safdar, A, Nazir Javed, Sajid Aleem Khan, Hina Safdar, Imran Ul Haq, Huma Abbas and Zia Ullah (2013). Synergistic effect of a fungus, *Fusarium semitectum*, and a nematode, *Tylenchulus semipenetrans*, on citrus decline *Pakistan Journal Zoology*, **45**(3): 643-651.

Sayed, A., Kheir, A. M., El-Naggar, H. I. and Kesba, H. H. (2007). Organic management of *Meloidogyne incognita* on grapes in relation to host biochemistry. *International Journal of Agricultural Research*, **2**: 776-785.

Schindler, A.F. (1961). A simple substitute for Baermann funnel. *Plant Disease Reporter*. **45:** 747 – 748.

Schmutterer, H. (1990). Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. *Annual Review of Entomology*, **35**: 271–297.

Seinhorst, J. W. (1966). Killing nematodes for taxonomic study with hot FAA 4:1, *Nematologica*, **12**:178.

Sen, D. (2017). Population fluctuation of soil inhabiting nematodes in relation to soil temperature and moisture at guava orchard in West Bengal, India. *Records of the Zoological Survey of India*, **117**(4):376-382.

Sen, P. K. and Verma, B. S. (1954). Studies on the dieback disease of guava *(Psidium guajava* L.) – A survey of the incidence of the disease in Jhargram area, West Bengal. *Proc. of the* 41*st Indian Sci. Cong.*, Sec. X. Agric. Sci. pp. 258.

Senthilkumar T. and Rajendran, G. (2004). Biocontrol agents for the management of disease complex involving root-knot nematode, *Meloidogyne incognita* and *Fusarium moniliforme* on grapevine *(Vitis vinifera), Indian Journal of Nematology*, **34**(1):49-51.

Sharma, A., Pathak, A., Sahgal, M., Meyer, J. M., Wray, V. and Johri, B. N. (2007). Molecular characterization of plant growth promoting rhizobacteria that enhance peroxidase and phenylalanine ammonialyase activities in chille (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum* Mill.). *Archives of Microbiology*, **188**: 483-494.

Sharma, N., Sharma, K. P., Gaur, R. K. and Gupta, V. K. (2011). Role of chitinase in plant defense. *Asian Journal of Biochemistry*, **6**: 29-37.

Sharma, S., Siddiqui, A. U. and Parihar, A. (1999). Pathogenicity of root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 on groundnut. *Indian Journal of Nematology*, **29**(2): 240.

Shreenivasa, K. R., Krishnappa, K., Reddy, B. M. R., Ravichandra, N. G., Karuna, K. and Kantharaju, V. (2005). Integrated management of nematode complex on banana. *Indian Journal Nematology*, **35**(1): 37-40.

Singh, R. K. and Singh, A. K. (2015). Studies on the population dynamics and integrated management of *Fusarium oxysporum* f.sp. *psidii* (Fop), a cause of guava (*Psidium guajava*) wilt. *Archives of Phytopathology and Plant Protection*, **48**: 785-796.

Singh, B. and Lal, S. B. (1953). Wilt of guava. *Agriculture and Animal Husbandry*, **3** : 78-79.

Singh, U. S., and Nath, R. P. (1996). Pathogenicity of root-knot nematode, *Meloidogyne incognita* on papaya. *Indian Journal of Nematology*, **26**(1): 115-116.

Sontirat, S. (1989). Plant disease: root-knot nematode. Extension and Training office Kasetsart University Bangkhen, Bangkok,Thailand.

Sonyal, S. (2015). Investigation on wilt complex in pomegranate and its management. *Ph.D. Thesis.* University of Agricultural Sciences, Bengaluru, India. 146 pp.

Soumya, D. M. (2015). Studies on root-knot and wilt complex in blackpepper (*Piper nigrum* L.) caused by *Meloidogyne incognita* (Kofoid and White) Chitwood and *Fusarium solani* (Mart.) Sacc. *M.Sc. Thesis*. University of Agricultural and Horticultural Sciences, Shivamogga, India. 88 pp.

Srivastava, J. N., Singh, A. K., Dwivedi, B. P., and Shukla, D. N. (2009). Management of guava (*Psidium guajava* L.) wilt (*Fusarium oxysporum* f. sp. *psidii*) through bioagents. *Environment and Ecology*, **27**(4A):1704-1706.

Srivastava, S. S. (2002). Efficacy of organic amendments in the management of root knot nematode (*Meloidogyne incognita*) infecting papaya. *Indian Journal of Nematology*, **32**(2): 183-184.

Srivastava, S., Singh, V. P., Kumar, R., Srivastava, M., Sinha, A. and Simon, S. (2011). *In vitro* evaluation of carbendazim 50% WP, antagonists and botanicals against *Fusarium oxysporum* f. sp. *psidii* associated with rhizosphere soil of guava. *Asian Journal of Plant Pathology*, **5**:46–53.

Suarez, H. Z., Rosales, L. C. and Rondon, A. (1999). Synergistic effect of the fungi *Macrophomina* and *Fusarium* with the root-knot nematodes, *Meloidogyne* spp. on decline of guava. *Nematology Mediterranea*, **27**: 79-82.

Suarez, Z.H., Rosales, L. C., Rondon, A. and Gonzalez, M. S. (1998). Root histopathology of *Psidium guajava* attacked by *Meloidogyne incognita* race 1 nematode and *Macrophomina phaseolina* and *Fusarium oxysporum* fungi. *Venezuelan Phytopathology*, **11**: 44–47.

Suhag, L. S. (1976). Observations in guava decline in Haryana and its control. *Pesticides*, **10:** 42-44.

Sukhakul, S. (2006). Pathogenicity of plant-parasitic nematodes on root-knot and slow decline diseases of guava. *Kamphaengsean Academics Journal*, **4**(2): 1-9.

Taylor, C. E. and Murant, A. F. (1966). Nematicidal activity of aqueous extracts from raspberry canes and roots. *Nematologica*, **12**: 488-494.

Tenuta, M., and Lazarovits, G. (2002). Ammonia and nitrous acid from nitrogenous amendments kill the microsclerotia of *Verticillium dahliae*. *Phytopathology*, **92**(3): 255–264.

Walia, R. K. and Poornima, K. (2017). Nematode management in horticultural crops for improving productivity. In: "Doubling Farmers Income through Horticulture" (Ed. K.L. Chadha et ll.), The Horticulture Society of India, New Dehli, 429-439.

Willers, P. and Grech, N.M. (1986). Pathogenicity of the spiral nematode, *Helicotylenchus dihystera* to guava. *Plant Disease*, **70** : 352.

Willers, P. and Welgemoed, C. P. 1993. Root-knot nematode damage to guavas in South Africa. Inligtings bulletin Instituut vir Tropiese en Subtropiese Gewasse. **251**: 21-23.

Yadav, B. S. and Verma, A. C. (1981). Effect of various inoculums levels of root- knot nematode, *Meloidogyne incognita* on brinjal, S*olanum melongena* L. (Abstact of papers presented at Nematology symposium held at Coimbatore, India, February 1981) *Indian Journal of Nematology,* **11**: 97.

Yang, B. and. Eisenback, J. D. (1983). *Meloidogyne enterolobii* (Meloidogynidae), a Root-knot Nematode Parasitizing Pacara Earpod Tree in China, *Journal of Nematology,* **15**(3): 381-391.

Zaitoun, F. M., Hamad, Y. K., Magda, M., Fahmi, Sabreen, M. and Ziyada. (2015). Role of certain bioagents against guava decline disease and in enhancement of the growth of guava trees. *Journal of Phytopathology and Pest Management*, **2**(3): 43-54.

ABSTRACT

Title of Thesis	:	**Studies on the incidence and management of guava decline involving root-knot nematode and fungi**
Name of the Degree Holder	:	**Mr. Madhu, M. R.**
Admission Number	:	2015A25D
Title of Degree	:	**Doctor of Philosophy**
Name and Address of Major Advisor	:	**Dr. K. K. Verma** (Major Advisor) Principal Scientist, Deptt. of Nematology CCS Haryana Agricultural University, Hisar, Haryana
Degree Awarding University	:	Chaudhary Charan Singh Haryana Agricultural University, Hisar
Year of Award of Degree	:	2019
Major Subject	:	Nematology
Total Number of Pages in Thesis	:	95 + ix
Number of Words in Abstract	:	349

Key words: Guava decline, *Meloidogyne incognita*, *Fusarium oxysporum* f.sp. *psidii*, Phytonematodes, neem cake, *Pseudomonas fluorescens*, *Trichoderma viride*, *Purpureocillium lilacinum*

The present investigation was conducted for the incidence and management of guava decline caused by root-knot nematode, *M. incognita* and fungus, *F. oxysporum* f.sp. *psidii*, in Haryana. The survey results of Hisar, Jind, Sirsa and Fatehabad districts of Haryana revealed the occurrence of nine phytonematode genera and three fungal genera from infected guava orchards. Among them, *M. incognita* and *F. oxysporum* f. sp. *psidii* were predominant pathogens involved in causing guava decline. The maximum frequency of occurrence of *M. incognita* was recorded in Fatehabad (72.2%), followed by Hisar (63.2%), Jind (56.3%) and Sirsa district (53.3%). Among four districts surveyed, the mean of guava decline incidence was maximum in Jind (51.6%) followed by Sirsa (49.4%), Hisar (40.4%) and Fatehabad district (36.6%). The experiment on pathogenicity of *M. incognita* revealed that the significant reduction of plant variables were observed at the inoculum level of 1000 j_2 and onwards and which was considered to be pathogenic level to guava plants. The pathogenicity of *F. oxysporum* f. sp. *psidii* on guava seedlings was proved and inoculum level of 6g mycelium/kg soil was considered to be pathogenic level to guava plants. The interaction of *M. incognita* and *F. oxysporum* f. sp. *psidii* showed that the statistically lowest plant growth parameters were recorded in nematode 10 and 20 days prior to fungus and the significantly lowest nematode reproduction factors were recorded in fungus 20 and 10 days prior to nematode inoculation. Significantly highest root rot was observed in plants inoculated with *M. incognita* 10 days prior to inoculation of *F. oxysporum* f.sp. *psidii* (43.67%) followed by nematode 20 days prior to fungus (39.34%). The incorporation of deoiled cakes *viz*., neem and mustard cake @ 30g/kg soil enhanced plant growth parameters and reduced the nematode reproduction factors irrespective of individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii*. Among different bio-agents tested, the treatments receiving combined formulation of *T. viride*, *P. fluorescens* and *P. lilacinum* @ 10ml/kg soil recorded maximum plant growth parameters and minimum nematode reproduction factors irrespective of whether individual or combined inoculation of *M. incognita* and *F. oxysporum* f.sp. *psidii.*

MAJOR ADVISOR **SIGNATURE OF STUDENT**

HEAD OF THE DEPARTMENT

CURRICULUM VITAE

(a) Name	:	MADHU, M. R.
(b) Date of birth	:	02-03-1991
(c) Place of birth	:	Vill.Sangahalli,
(d) Mother's name	:	Smt. Manjula
(e) Father's name	:	Shri. Rudrappa, M.
(f) Permanent address	:	Sangahalli, Channagiri (TQ), Davanagere (District), Karnataka, Pin code-577231
(g) Mobile	:	+91 9964137019
(h) E-mail	:	mrmadhu51@gmail.com

(i) Academic qualifications:

Degree	University/ Board	Year of passing	OGPA	Subjects
M.Sc. (Agri.)	University of Agricultural sciences, Dharwad-580005	2014	8.58	Plant Pathology
B.Sc.(Agri.)	University of Agricultural Sciences, Bangalore-560065	2012	7.77	Agriculture

(j) Co-Curricular Activities:

(k) Medals/Honours received

- Awarded with **ICAR-SRF** during **Ph.D**

List of Publications

Research Paper:

- Madhu, M. R., Verma, K. K. and Vinod Kumar. (2019). Distribution, prevalence and intensity of guava decline in western Haryana, *Journal of Entomology and Zoology Studies*, 7(4): 521-524.
- Madhu, M. R., Basavarajappa, M. P., Chidanand Lokapur and Anand, I. D. (2018). Efficacy of fungicides in inhibition of *Alternaria carthami* causing leaf spot of safflower *In vitro*, *International Journal of Chemical Studies*, 6(1): 1051-1053.
- Madhu, M. R., Basavarajappa, M. P., Anand, I.D. and Chidanand Lokapur. (2017). Morpho-Physiological Variability in *Alternaria carthami* Chowdhury Causing Safflower Leaf Spot. *International Journal of Current Microbiology and Applied Sciences*,6 (10):3244-3250

MADHU, M. R.

UNDERTAKING OF THE COPY RIGHT

I, **Madhu, M. R.** Admission No. **2015A25D,** undertake that I give the full copyrights to the CCS HAU HISAR, of my thesis entitled **"Studies on the incidence and management of guava decline involving root-knot nematode and fungi".** I also undertake that patent, if any, arising out of the research work conducted during the programme shall be filed by me only with due permission of the competent authority of CCS HAU, Hisar.

(SIGNATURE OF THE STUDENT)