INVESTIGATION ON ASSOCIATION OF *Ceratocystis fimbriata* Ell. and Halst. AND *Meloidogyne incognita* (Kofoid and White) Chitwood IN CAUSING WILT OF POMEGRANATE AND IT'S MANAGEMENT

MADHUSHRI S. KERAKALAMATTI

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF HORTICULTURE, BAGALKOT
UNIVERSITY OF HORTICULTURAL SCIENCES,
BAGALKOT, KARNATAKA, INDIA

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MADHUSHRI S. KERAKALAMATTI
ID No. UHS16PGM770

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF HORTICULTURE, BAGALKOT - 587 104
UNIVERSITY OF HORTICULTURAL SCIENCES,
BAGALKOT - 587 104

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DEPARTMENT OF PLANT PATHOLOGY

C E R T I F I C A T E

This is to certify that the thesis entitled **"INVESTIGATION ON ASSOCIATION OF *Ceratocystis fimbriata* Ell. and Halst. AND *Meloidogyne incognita* (Kofoid and White) Chitwood IN CAUSING WILT OF POMEGRANATE AND IT'S MANAGEMENT"** submitted in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (HORTICULTURE)** in **PLANT PATHOLOGY** to the University of Horticultural Sciences, Bagalkot, is a record of bonafide research work carried by **MADHUSHRI S. KERAKALAMATTI, UHS16PGM770** under my guidance and supervision and that no part of the thesis has been submitted for the award of any degree, diploma, associateship, fellowship or other similar titles.

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Members: **1.**_____
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2. _____
(KIRAN KUMAR K. C.)

3. _____
(RUDRESH D. L.)

4. _____
(RAGHAVENDRA G.)

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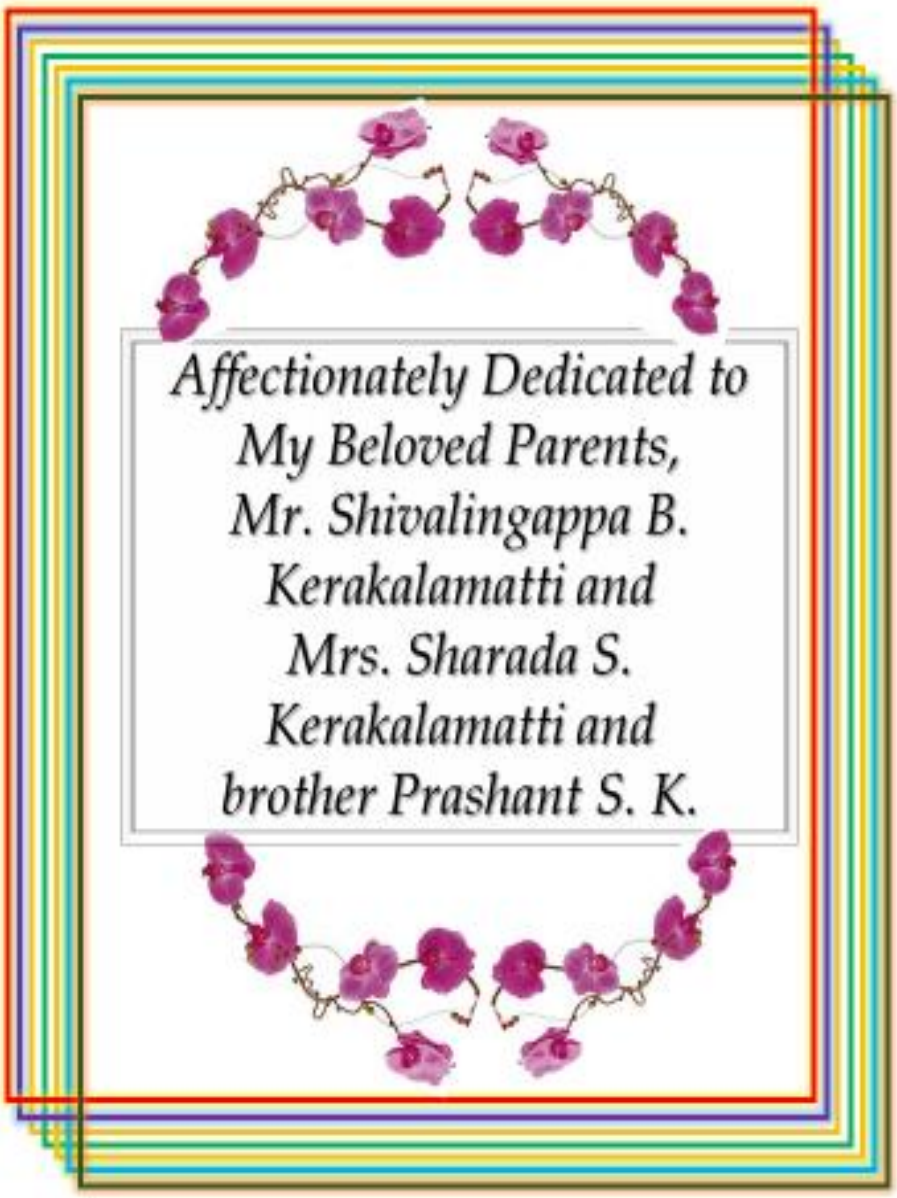
Affectionately Dedicated to
My Beloved Parents,
Mr. Shivalingappa B.
Kerakalamatti and
Mrs. Sharada S.
Kerakalamatti and
brother Prashant S. K.

CONTENTS

Contd……

LIST OF ABBREVIATIONS USED IN THESIS

Sl. No.	Abbreviations	
1	%	Per cent
2	mm	Millimetre
3	µm	Micro meter
4	^{0}C	Degree Celsius
5	ppm	Parts per million
6	cm	Centimetre
7	kg	Kilogram
8	ml	Millilitre
9	g	Gram
10	mg	Milligram
11	m^2	Meter square
12	µl	Micro litter
13	ha	Hectare
14	t	Tonnes
15	C.D	Critical difference
16	S.*Em*	Standard Error of Mean
17	*viz.*	As fallows
18	*et al.*	Et alli (and other)
19	MT	Metric Tonne

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1. INTRODUCTION

Pomegranate (*Punica granatum* L.) a popular fruit, also called as "Fruit of Paradise" is a deciduous shrub or a small tree belongs to the family Lythreceae, having 2n=16 number of chromosome and it is native to Iran. The pomegranate fruit is considered as symbol of fertility and prosperity. The basket of pomegranate was chosen as a symbol of plenty for the 18th International Horticultural Congress, held in 1970 (Singh, 1985). It is extensively cultivated in Spain, Morocco and other countries around the Mediterranean, Egypt, Iran, Afghanistan, Arabia and Baluchistan. It is one of the most adaptable subtropical minor fruit crops and its cultivation is increasing very rapidly.

Pomegranate cultivation is a highly lucrative and remunerative agriculture business in India. It is regarded as a "vital cash crop", grown in an area of 224 thousand ha with a production of 2650 thousand MT and it occupies sixth place in fruit export market of India (Anon, 2018). Maharashtra is the largest producer occupying 2/3rd of total area in the country followed by Karnataka, Andhra Pradesh, Gujarat and Rajasthan. Karnataka state has an area of 28.09 thousand ha with a production of 328.92 thousand MT (Anon, 2018) where this crop has spread across different districts *viz.*, Vijayapur, Bellary, Bagalkot, Koppal, Chitradurga, Belagavi, Davangere, Tumkur, Bangaluru and Kalaburgi.

Pomegranate is one of the important fruit crops of tropical and sub-tropical region. Pomegranate is extremely heat tolerant and perform best when temperature is above 29.4℃ for at least 120 days a year. The trees are also drought tolerant however, supplemental irrigation is necessary during tree establishment and is critical for commercial fruit production. Pomegranate performs best on deep loamy soils but will still grow quite well in sandy and clay soil. Trees grow best in a soil pH range of 5.5 to 7.2. Though pomegranate can tolerate short period of standing water, they prefer well drained soil.

Its adaptability to a wide range of climatic conditions, hardy nature, low water requirement, good response to high-tech horticultural practices, high yield, high returns, magical therapeutic values and increasing demand for table and processed products as well as high export potential has made pomegranate a popular fruit of

tropical and sub tropical regions in recent times. Attractive red external fruit colour, red coloured and fully developed arils, high TSS and sugar acid blend are important attributes in assessing quality of pomegranate fruits.

On the farmer's context, a highly remunerative crop helping for improving the economic status of growers and also other work force benefitting as a subsistence occupation particularly in regions such as Maharashtra and Karnataka. The alluring monetary return per unit area from this crop has resulted in steady increase in area, production and export of pomegranate. The most popular varieties suitable for processing and table use of pomegranate are Ganesh, Mridula, Arakta, Bhagwa, and Super Bhagwa. It is a high value crop and fruit has high demand in the international market being nutraceutically rich.

Pomegranate is a good source of carbohydrates and minerals such as calcium, iron and sulphur. It is rich in vitamin C and citric acid is the most predominant organic acid in pomegranate. Glucose (5.46%) and fructose (6.14%) are the main sugars with no sucrose in fruits. The fruits of pomegranate are known to possess pharmaceutical and therapeutic properties. Sweet varieties are mildly laxative, sour types are good for curing inflammation of stomach and heartache. In India, there is a common adage 'Ek Anar Sau Bimar' meaning one fruit cures hundred diseases. The flower buds are very useful in Ayurveda for managing bronchitis. The bark of the stem, root and rind of the fruit is used for slimming, control of dysentery, diarrhoea and killing tape worms. Modern research reports revealed that medically active compound isolated from different part of pomegranate can cure number of human diseases. Including coronary heart disease, cancer inflammation, hyperlipidaemia, diabetes, cardiac disorders, hypoxia, ischemia, aging, brain disorder and AIDS (Pearson, 1997).

Successful cultivation of pomegranate in recent years has met with different traumas of pest and diseases. Bacterial blight, wilt, anthracnose, shot hole bore, thrips, fruit borer are some of the diseases and pests. Wilt caused by *Ceratocystis fimbriata* Ell. & Halst has become a major threat. In recent years, the wilt incidence in traditional pomegranate growing areas of Bagalkot and Vijayapur districts of Karnataka has assumed an alarming situation. It was first noticed in two areas of the

Vijayapur district in 1990. This was once deemed as a minor disease, but now has become a serious threat for pomegranate production resulting in severe yield losses.

Around 1993, rapid spread of this disease was observed in the entire Vijayapur district. The cause was not identified until 1995. In 1996 the fungus *C. fimbriata* was isolated from discoloured stem, root, and branch tissues of wilted plants. The initial symptoms of the disease are yellowing of foliage of one or a few branches of a tree followed by yellowing and drooping of foliage of the entire tree. At the same time one or two stems of the tree showed wilting and it took few weeks to some months for the entire tree to completely wilt. Although yellowing of leaves normally proceeded acropetally, occasionally some plants revealed wilting symptoms all of a sudden by senescing the entire foliage at once. Wilt infected plants often revealed dried foliage and fruits attached to the branches for many months. Vertical stem cracking was also observed as one of the peculiar characteristic symptoms in some wilt infections. In many orchards diseased trees were observed dying due to wilt in patches, thereby indicating the spread of the disease from an infested to an adjacent healthy tree (Sharma *et al.,* 2010).

The disease was prevalent in parts of Maharashtra, Karnataka, Andhra Pradesh, Gujarat and Tamil Nadu states (Jadhav and Sharma, 2009). Despite many factors conducive for the high severity of disease, selection of seedlings for planting, soil borne nature of pathogen and also association with plant parasitic nematodes are of prime importance. The estimation of yield losses due to root knot nematode, *Meloidogyne incognita* in pomegranate has been reported to the extent of 17.3 per cent (Jain *et al*., 2010). Their association with wilt complex is documented by Sonyal (2010). This might be the reason for the current rampant spread of the disease in south Indian states. This has drawn the attention of the present study to know the incidence of wilt complex in north Karnataka. Hence, a survey in the worst hit area of north Karnataka is the need of the hour to assess the disease incidence, severity, association of pathogens, and also to know prevalence with respect to locality, cultivar *etc.* To develop management practices, thorough understanding of the causal organism with regard to nutritional and physiological characters are pre requisites. Further the role of *M. incognita* in predisposing wilt need to be ascertained as established in other crops. This will give an idea in designing management practices.

Use of fungicide is an important component of management for plant disease caused by fungi. Hence, different fungicides need to be evaluated both *in vitro* and *in vivo*. The oil cakes, nematicides and biological control agents are important components for the management of nematodes. Therefore, an effort is needed in this regard to see the efficacy of some fungicides, oil cakes, nematicides and biological agents against the wilt causing pathogens both in laboratory and field.

Keeping in view of these things, the present investigations on wilt of pomegranate was directed in the current study to elucidate some of the critical aspects of the disease and the pathogens with the following well defined objectives:

Objectives:

1. To conduct survey for the assessment of incidence of wilt complex of pomegranate caused by *Ceratocystis fimbriata* and *Meloidogyne incognita* in Bagalkot and Vijayapur districts.

2. To study the interaction of *Ceratocystis fimbriata* and *Meloidogyne incognita* in causing wilt of pomegranate.

3. To evaluate fungicides, nematicides, oil cakes and bioagents against *Ceratocystis fimbriata* and *M. incognita, in vitro* and *in vivo.*

2. REVIEW OF LITERATURE

Pomegranate is an important fruit crop affected by many pest and diseases. Among the different diseases, pomegranate wilt caused by *Ceratocystis fimbriata*, once deemed as a disease of minor importance, became a serious threat for pomegranate production in recent years. Root knot nematode is also known to predispose increase wilt disease. The disease assumed its severity in all the growing areas resulting severe yield losses both in terms of quality and quantity. The information available on this disease, pathogen and management strategies is very meager. Hence, the literature pertaining to the wilt of pomegranate along with information of disease and pathogen on related crops is reviewed here as under.

2.1 History and distribution of wilt complex of pomegranate

Somasekhara and Wali (1999) first noticed wilt of pomegranate in two areas of the Vijayapura district (16°49 N; 75°43E) of Karnataka, in 1990. Prominent pomegranate growing states showed the presence of wilt of pomegranate with varying severity. Maharashtra, recorded 49.2 per cent, Karnataka recorded 61.11 per cent while Andhra Pradesh recorded 8.69 per cent (Jadhav and Sharma, 2009).

Sharma *et al.* (2008) and Sharma (2009) revealed pomegranate wilt as one of the important limiting factors in pomegranate cultivation in Maharashtra, Karnataka and Andhra Pradesh and reported *C. fimbriata* as one of the major causes of wilt disease among other biotic and abiotic factors at National Research Center (NRC) Solapur during 2005-09.

Xu *et al.* (2011) found that a new devastating disease was observed on pomegranate (*Punica granatum* L.) in Panzhihua-Xichang region of Sichuan province, southwest China that caused losses estimated to be 30 per cent as surveyed by 10 orchards. Characteristic symptoms were yellowing and wilting of leaves. Initial symptoms occurred on shoots, but later, leaves of the whole tree turned yellow and wilted, causing extensive defoliation and dieback. The xylem of the trunk turned brown to black with a star burst like pattern.

Nasira *et al.* (2011) first reported the occurrence of *Meloidogyne incognita* on pomegranate in Pakisthan. Alam *et al.*, 2015 reported pomegranate wilt from Pakistan

where pomegranate (*Punica granatum* L.) was cultivated on 11,200 ha. During August 2015, survey in Punjab province recorded a new disease causing 21 per cent loss of trees in 50 orchards.

2.1.2 Symptoms of pomegranate wilt caused by *Ceratocystis fimbriata*

Somasekhara and Wali (1999) observed that the various symptoms of disease include all the leaves in the plant turning to yellow or light green in colour, yellowing of leaves in the single branch, sudden wilting of leaves, complete wilting of the plant and pin holes with brown discoloration in the stem. Huang *et al.* (2003) reported that older pomegranate bushes, showing initial yellowing symptoms and wilting of leaves on one to several branches, followed by sudden death of the bush within 3 to 4 weeks in Yunnan began dying. Roots of diseased plant appeared brown to black and irregularly shaped lesions were observed when the bark was removed.

Jadhav and Sharma (2009) reported that the *C. fimbriata* is soil borne and survive in soil through their thick walled conidia, aleurioconidia and chlamydospores, respectively. Based on the symptomatology studies, the disease appears as yellowing and then drooping of foliage of one or more branches of the plant. Plants may take few days to 2-3 months to reveal complete wilting. However, some plants showed sudden drooping of all leaves of the plant resulting in complete wilting within one or two days of symptom initiation. The vascular wilt caused by *C. fimbriata* is characterized by greyish discolouration of bundles and adjoining tissues.

Sharma *et al.* (2010) reported that initial wilt symptoms manifested as yellowing of foliage of one or few branches of a tree followed by yellowing and drooping of foliage of the entire tree. At times only one or two stems of the tree showed wilting and it took a few weeks to some months for the entire tree to completely wilt. Wilt infected plants often revealed dried foliage and fruits attached to the branches for many months. Vertical stem cracking was also observed as one of the peculiar characteristic symptoms in some wilt infections. In many orchards diseased trees were observed dying due to wilt in patches, thereby indicating the spread of the disease from an infected to adjacent healthy tree. However, in some orchards wilt infection were spotted unevenly at different locations. Splitting of root and stem bark and particularly lower branches, or cross and vertical sections of diseased plant parts

generally revealed dark greyish brown streaks in vascular and adjoining cortex tissues.

Khosla (2013) revealed that the initial symptoms observed on pomegranate cultiver-23 were yellowing of the leaves and dropping of one or two branches. Wilted leaves typically became dry and curled rather suddenly but remained attached to the tree, followed by sudden death of the bush within 3 to 4 weeks. The peeling of bark of the stems of infected bushes showed deep brown to black staining in the outer xylem of the diseased plants from down to upward in the stem and branches. The cross sections of the affected trunk revealed distinctive starburst like brown black discoloration in the stem portion.

Initial symptoms appeared only on shoots; later, leaves turned yellow, then wilted. In severe cases, defoliation and dieback was accompanied by black streaks in the xylem, followed by death of the tree. The disease would be more severe in older (>15 years) than in younger plantings (Alam *et al.,* 2015). Wilt symptoms initiate yellowing of leaves and drying of one or more branches. The plant appeared devitalized, leaves turned pale yellow starting from lower branches/limbs and progressed upwards. Partial wilting of the tree with drying and death of some branches were common symptoms while in severe cases the defoliation and complete wilting of plants within 2-3 months periods seen. Fruit drop occurred, the entire plant wilted from top to bottom (Chaudhari *et al.,* 2016).

Raja (2017) reported that in field condition, symptoms initiated as yellowing of leaves of one or more branches and the plant appeared devitalized, leaves turned pale yellow starting from lower branches/limbs and progressed upwards. The partial wilting of the tree with drying and death of some branches were common symptoms. In severe cases the defoliation and complete wilting of plants within 2-3 months periods was observed. Fruit drop was also occurred, the entire plant wilted from top to bottom and wilt infected plants often had dried foliage and fruits attached to the branches for many months. In some orchards diseased plants were died due to wilt in patches, thereby indicating the spread of the disease from an infected to an adjacent healthy orchard. Splitting of root or vertical sections of diseased plant parts showed dark grayish brown streaks or distinctive star burst like brown black discoloration in vascular and adjoin cortex tissues was observed.

Somu (2017) observed that wilt infected plants produced typical symptoms of yellowing and drooping of foliage of one or few branches of a plants. Later, one or two stems of the plants showed wilting and entire plant completely wilted within 2-3 months. The blue strains produced by the fungus can be observed on the wilted branch from bottom to tip. In severe cases rotting of root bark was also observed.

2.1.3 Symptoms caused by *Meloidogyne incognita*

Root knot nematodes are known to cause non epidemic diseases which leads to slow decline in yields which spreads gradually and steadily year by year. The damage caused by Root Knot Nematodes (RKN) can be visible from above ground and below ground with signals of root galling on most hosts as a major associated symptom (Kofoid, 1998).

Symptoms produced by nematode were yellowing and premature leaf shedding with severe stunted growth depending on nematode populations in the field and sharp decline in plant fruiting (Duncan, 1991). The infestation of RKNs results in temporal wilting of plants due to disturbance on the uptake of nutrients, water and plant metabolic activities and this has a serious impact on yield (Murukesan, 2008).

The below ground symptoms include galls or knots on the roots or tubers of numerous crops as a result of plant response to RKNs which occurs as result of feeding on the vascular tissues (Duncan, 1991). The vascular tissue of many plants was often targeted and plays a major role in pathogen and host plant interactions where an intimate relationship is maintained (Favery *et al.,* 2016).

The galls (giant cells) produced on plant roots causes physiological disturbance and further delay plant growth due to poor sink source relationship resulting in poor yields (Soltani, *et al.,* 2013; Barcala & Cabrera, 2015). The galls vary in size from pin head to large size due to different levels root knot infestations on host plant and at times they may coalesce to form large secondary galls. The size of galls also depends upon the host plants and nematode species involved. A clear and typical symptom of root knot nematode infestation produce ‘thick root’ appearance; the egg masses or females are concealed inside the root tissue (Mohiddin and Khan, 2014).

2.1.4 Morphology of *Ceratocystis fimbriata*

Morphological studies made by Somasekhara and Wali (1999) and Hung *et al.* (2003) indicated that *C. fimbriata* was belonging to Latin American group. Perithecia were black with a globose base (130 to 300 μm). Ascospores exuded from the apex of the perithecium neck in a long coil and were small, hyaline, and hat shaped (3.8 to 5.0 μm long X 2.3 to 4.0 μm wide). Conidiophores were septate and hyaline to dark greenish brown. Hyaline conidia, 8 to17 μm long X 6 to15 μm wide, were usually produced in chains of 10 or more. Thick walled endoconidia were globose to oval, olive brown, and 8 μm to 20 μm in diameter.

Two types of conidia were produced after 5 days: primary conidia (cylindrical, hyaline, 8 to 12 × 3 to 6 μm) and secondary conidia (hyaline, barrel shaped, 8 to 12 × 5 to 7 μm). Endoconidiophores with primary conidia were 10.5 to 35.5 × 2.6 to 5.2 μm. Perithecia produced on carrot discs were dark brown to black and the base was 153 to 281 μm in diameter. Ascomatal necks were 514 to 653 μm long, dark brown to black, lighter in color at apices, tapering from base (25 to 48 μm diameter) to apex (14 to 26 μm diameter). Ostiolar hyphae were 43 to 81 μm long. Ascospores were hyaline, hat shaped, 3 to 4 μm long, and accumulated in a sticky matrix at the tips of perithecial necks. Mycelium was initially hyaline but became dark greenish brown after 7 days. Dark brown, thick walled aleurioconidia (11 to 14 × 9 to 12 μm) appeared on culture plates after 2 months. Based on morphological characteristics, the fungus was identified as *Ceratocystis fimbriata* on sweet potato (Engelbrecht and Harrington, 2005). *C. fimbriata* on pomegranate produces spherical perithecia with long neck and releases ascospores through the neck canal. The pathogen also forms cylindrical hyaline endoconidia and spherical to ovoid thick walled brownish aleurioconidia (Jadhav and Sharma, 2009).

A one week old culture of *C. fimbriata* infecting pomegranate isolated on PDA medium revealed dark grayish green growth consisting of septate mycelium, endoconidia, aleurioconidia and long necked perithecia. Endoconidia were hyaline, cylindrical and formed endogenously in hyphae. Aleurioconidia were thick walled pyriform, truncate at the base, Perithecia were blackish in colour, globose to sub globose and produced ascospores. Ascospores were liberated by early ascus

deliquescence and were discharged passively through the ostioler hyphae towards the end of perithecial neck (Sharma *et al.,* 2010).

The wilt causing *C. fimbriata* grew well on potato dextrose agar and oat meal agar and produced whitish grey mycelium which changed to brown colour with age owing to production of micro, macro conidia and perithecium. Perithecia were black with a globose base (130 to 300 µm). Ascospores exuded from the apex of the perithecium neck in a long coil and were small, hyaline, and hat shaped (3.8 to 5.0 µm long x 2.3 to 4.0 µm wide). Conidiophores were septate and hyaline to dark greenish brown. Hyaline conidia, 8 to17 µm long x 6 to 15 µm wide, were usually produced in chains of 10 or more. Thick walled endoconidia were globose to oval, olive brown and 8 to 20 µm in diameter (Sonyal, 2010).

Chaudhari *et al.* (2016) identified *C. fimbriata* based on morphological characters such as mycelium which was white gray and later changed to brown colour with age owing to production of micro and macro conidia and perithecium. Microscopic examination of a fifteen days old culture revealed septate conidiophores and hyaline conidia (10 to 15 µm long) and perithecia were black with a globose base (100-300 µm). Ascospores exuded from the apex of the perithecium neck in a long coil and were small, hyaline and hat shaped (Soni and Kanwar, 2016).

Raja (2017) studied the growth characters of *C. fimbriata* on carrot bait followed by oat meal agar. As the growth progresses, production of endoconidia, aleurioconidia and perithecium was observed. The black colored perithecia with a globose base were observed with size of 181.1 x 131.2 µm, exuding small, hyaline and hat shaped ascospores from the apex of the perithecium neck which measure 5.13 x 4.27 µm. The endoconidia were hyaline, cylindrical and formed endogenously in hyphae with average size was 23.6 x 4.90 µm and aleurioconidia were thick walled ellipsoidal or pyriform, golden brown in colour with size of 18.5 x 10.10 µm borne singly or in chain.

Somu (2017) studied the morphology of *C. fimbriata* fungus infecting pomegranate. The mycelium was whitish grey colour in the beginning which later on

changed to brown colour. As the growth progressed, production of endoconidia, aleurioconidia and perithecium was observed. The black colored perithecia with a globose base were observed with size of 192.1 x 103.4 µm, exuding small, hyaline and hat shaped ascospores from the apex of the perithecium neck which measured 4.13 x 3.26 µm. The endoconidia were hyaline, cylindrical and formed endogenously in hyphae having average size of 25.1 x 4.12µm. Aleurioconidia were thick walled ellipsoildal or pyriform, golden brown in colour with size of 18.6 x 12.10 µm. They borne singly or in chain.

2.1.5 Morphology of *Meloidogyne incognita*

Eisenback and Hirschmann (1981) studied on head shape and stylet morphology of males of 90 populations of *M. arenaria*, *M. hapla*, *M. incognita*, and *M. javanica* from geographic regions of the world were compared by light microscopy (LM). Head morphology differed in size and shape of the head cap, annulation of the head region, and width of the head region relative to the first body annule. Differences in stylets occurred in size and shape of the cone, shaft, and knobs. Populations of *M. javanica* varied with respect to the presence of head annulations.

The male has long, thin, cylindrical shape of a worm but the lip region has a distinct head cap, which includes a labial disc surrounded by lateral and medial lips. The stylet usually less robust and shorter, 18-24 µm long for many species. Infective second stage juveniles, often free in the soil, are usually 0.3-0.5 mm long; they are less robust, the stylet is delicate with small basal knobs, under 20 µm long, and the head skeleton weak. The median oesophageal bulb is well developed and the oesophageal glands are extensive, overlapping the intestine for several body widths, mainly ventrally; the tail is conoid, often ending in a narrow rounded terminus, but tail length is variable, 1.5-7 anal body widths between species, it often ends in a clear hyaline region, the extent of which can help to distinguish species. (Sasser and Carter, 1985).

Suresh *et al.* (2017) characterized the perineal pattern of *M. incognita* infecting tomato and pomegranate in Tamil Nadu by the presence of high dorsal arch with squarish shape. The striae were smooth to wavy, closely spaced and varied from wavy to zigzag on dorsal and lateral side. Dorsal arch was high and rounded in case of

female nematode. The average total length of the second stage juveniles of *M. incognita* is 405 µm (346 - 463 µm).

2.1.6 Pathogenicity

Somashekhar *et al.* (2000) observed wilt symptoms in artificially inoculated pomegranate seedlings after 12 months of inoculation. Pathogenicity of *C. fimbriata* studied on one and half year old plants of *cv.* Ganesh in pots revealed wilt symptoms initiation in inoculated plants after 39 days of pathogen application and symptoms were continued to develop till another 5 months when all the treated plants succumbed to infection by the pathogen. Wilts infection was observed in both treatments having plants with wounded and unwounded roots, indicating the entry of the pathogen even without wounds. Similarly, Sharma *et al.* (2010) confirmed the pathogenicity of *C. fimbriata* on pomegranate tree.

Inoculation with an isolate of *C. fimbriata* were made by inserting mycelium with perithecia from 12 days old culture growing on PDA into root wounds made with a sterile scalpel on five pomegranate plants and then covering the wounds with parafilm. Sterile medium was placed in an equal number of wounded bushes, and 5 days later, all bushes exhibited symptoms. No symptoms were observed on control bushes. The first visible symptom was a small area of blackened tissue near the point of inoculation. Lesions expanded slowly, but they expanded more rapidly upward than downward. The fungus was reisolated on PDA from roots of all artificially inoculated bushes (Huang *et al.*, 2003).

The pathogen *C. fimbriata* was isolated from the bark peel of collar region of wilt infected pomegranate plant and established on PDA slants. The pathogenicity of the purified culture was tested on one year old potted pomegranate plants. Wilting symptoms were observed on inoculated plants (Khosla, 2013).

Chaudhari *et al.* (2016) carried out the pathogenicity test by soil inoculation method. After four months of inoculation with *Fusarium oxysporum,* Schl., *C. fimbriata* and nematode, the artificially inoculated pomegranate plants started showing initially typical wilting symptoms such as yellowing of leaves, wilting of plant and brownish discoloration of vascular tissues of roots. Subsequently complete wilting of plants was noticed. The yellowing of leaves started at 90 days of planting.

However, control plants did not show the wilting up to 120 days of planting. The Bhagwa variety was tested for pathogenicity and found susceptible to both *Fusarium oxysporum,* Schl. and *C. fimbriata* fungus pathogen.

Balaganur (2016) carried out the pathogenicity test on 3 months old pomegranate seedling by taking 1-2 cm of mycelia mass from fully grown colonies on PDA and was placed onto the stems of healthy pomegranate plants making collar wedges. The inoculation site was wrapped with cotton cloth (moistened with sterile distilled water) and plastic film. The inoculated plants were kept in greenhouse (average temperature of 27° C) for further observation. The organism was reisolated using PDA medium from artificially inoculated plants showing typical symptoms of disease confirming the Koch postulates of establishing the pathogen and pathogenicity.

Raja (2017) carried out the pathogenicity test by inoculating the pathogen on 2 months old pomegranate seedlings. The first symptoms appeared like yellowing of leaves in some twigs or branches, followed by drooping and drying of leaves as the external signs. The leaves turned pale yellow starting from lower branches and progressed upwards. Later, partial wilting of the plant with drying and death of some branches took place. The pathogen produced wilting symptoms 40 days after inoculation. Again, the fungus was re isolated from such wilted plants from pots and was found to resemble the original culture of *C. fimbriata* thus proving the pathogenicity.

Somu (2017) proved the pathogenicity *by* inoculating the pure culture of *C. fimbriata* to one month old pomegranate *cv.* Bhagwa raised in sterile soil along with a control plant. The pathogen produced wilting symptoms after 60 days of inoculation. The plants started showing initially typical wilting symptoms such as yellowing of leaves in some twigs or branches, followed by drooping and drying of leaves. Brownish discoloration of vascular tissues were observed when affected root split opened. Blue stains on stem were also noticed. The pathogen was reisolated from infected parts showing such symptoms and compared with original culture of *C. fimbriata,* thus confirmed the pathogenicity. The control plants which were not inoculated with the fungus did not show any symptoms of disease.

2.2 Survey for the assessment of incidence and severity of wilt complex of pomegranate caused by *Ceratocystis fimbriata* and *Meloidogyne incognita*

Plant parasitic nematodes are found in all agricultural regions of the world and any crop is likely to suffer from these parasites. They may also be additive to other combination of plant parasitic nematodes with fungal pathogens, which is sufficient to induce heavy crop losses (Zacheo, 1993).

Somasekhara *et al.* (2000) reported that, out of 54,866 plants surveyed in 128 locations 6,745 plants have found wilted and caused monitory loss about Rs. 67.45 lakhs during 1996-1999. Hung *et al.* (2003) conducted survey in pomegranate production areas of China and reported the increasing occurrence of the diseases. The pomegranate wilt was detected in 17 of 50 plantings surveyed. Disease was more severe in older plantings than in younger plantings. Disease incidence will be 1 per cent in 1 to 5 year old bushes, 3.6 per cent in 6 to 10year old bushes and 6 per cent in bushes more than 10 years old.

Sudheer *et al.* (2007) conducted random survey of root knot nematode on different cultivars (Ganesh, Mridula and Bhagwa) of pomegranate in Anatapurum district in Andhra Pradesh. The intensity of root knot nematode damage increased with increase in age of the plant. The maximum population of 370 juveniles/gm of soil + 150 to 350 egg masses/g of roots observed on 5 years old plants of pomegranate.

Benagi *et al.* (2009) conducted a roving survey to understand the disease situation in the region, during Mriga and Hasta bahar of 2008-09 in north Karnataka *viz.*, Bagalkot, Bijapur, Belgaum, Gadag and Koppal districts. Plantations of at least three years and above were selected for survey, where wilt was recorded in the range of 0.1 - 33.3 per cent. The higher per cent wilt incidence of 7.59 was observed in Bellary district.

Jadhav and Sharma (2009) conducted surveys in important pomegranate growing states and which revealed that wilt prevalence was 49.2 per cent in Maharashtra, 61.11 per cent in Karnataka and 8.69 per cent in Andhra Pradesh. In Maharashtra out of 92,185.34 ha area under pomegranate, 23,857.86 ha (25.88%) was

reported to be wilt affected. Wilt prevalence was high in districts of Satara, Pune, Nashik, Ahmednagar and Sholapur. Somasekhara *et al.* (2009) conducted a systematic survey on pomegranate wilt incidence in Karnataka (1996-2005) which showed 5.69 per cent incidence, accounting a monetary loss of approximately Rs. 3.43 crores. In Karnataka, maximum wilt prevalence was in Bagalkot followed by Koppal and Vijayapur districts. In Andhra Pradesh wilt was prevalent in only 8.69 per cent orchards of Ananthpur district.

Sharma *et al.* (2010) carried out survey on major pomegranate area in India during 2005-09 and revealed disease prevalence of wilt in states of Maharashtra (49.2%), Karnataka (61.11%) and Andhrapradesh (8.69%). In general, wilt was prevalent in 47.57% of orchards of which only 5.82% had serve wilt infection, 10.3% moderate and 37.7% mild wilt infection. Wilt was prevalent on all important cultivars of all ages ranging from 2 to 20 years.

Sonyal (2010) conducted survey and surveillance on incidence of wilt complex of pomegranate in northern Karnataka and revealed that the severity of disease was more in Bellary district followed by Koppal, Gadag, Vijayapur, Bagalkot and Raichur districts. During the period of survey new plant parasitic nematodes *viz., Meloidogyne incognita, Helicotylencu dhystera, Xiphinema* sp. *Dorylamid* and *Rotylenchulus reniformis* were found in Karnataka

Baig *et al.* (2013) worked on the pomegranate orchards under flood irrigation which were found to be severely affected by the disease. Of the ten districts surveyed, highest (14.47%) wilt incidence was recorded in Nasik district. Comparatively maximum average wilt incidence (13.53%) was recorded in the orchards with flood irrigation. Achari *et al.* (2014) conducted roving survey in pomegranate orchards of Bijapur district during 2012-13 to know the incidence of wilt diseases. The result revealed that, wilt incidence was noticed in range of 0.0 to 63.33 per cent. The higher incidence of wilt was noticed in neglected or less cared orchards.

Suresha *et al.* (2014) had undertaken a preliminary survey to find out the incidence and severity of wilt disease complex of pomegranate in Chitradurga, Davanagere, Tumkur and Chikmagalur districts of central Karnataka. The study

revealed that the wilt complex caused by *Ceratocystis fimbriata* and root knot nematode *Meloidogyne incognita* was observed in all the districts surveyed. But, overall the disease incidence was more in Chitradurga (32.5%) followed by Davanagere (29.5%) and Tumkur (27.00%) districts. However, the least incidence of 18.5% was observed in Chikkamagalur district.

Balaganur (2016) conducted roving survey on wilt incidence of pomegranate in different district of Karnataka. The highest wilt incidence of 23.22 per cent was recorded in Vijapura district and lowest incidence was recorded in Belagavi and Bellary districts with 3.22 and 2.75 per cent respectively. Chaudhari *et al.* (2016) conducted survey in fifteen villages of Rahuri tehsil in Ahmadnagar district and the results revealed that, maximum wilt disease incidence was recorded in Guha (26.22 %), which was followed by Pimpalgaon (25.53 %) and Kangar (20.04 %). The minimum wilt disease incidence was recorded in Ghorpadwadi (7.53 %).

Shruthi *et al.* (2016) carried out roving survey to assess severity of wilt of pomegranate in major pomegranate growing regions of north eastern Karnataka during *kharif* 2016-17. The survey revealed that the wilt caused by *C. fimbriata* was observed in all the districts surveyed and maximum disease incidence was recorded in Vijapura district (38.13%) followed by Koppal district (32.6%) and lowest incidence was recorded in Raichur district (7.8%). Highest disease incidence was observed in older orchards than new orchards.

Raja (2017) conducted fixed plot survey to know the wilt incidence of pomegranate in different district Karnataka. The survey revealed that Sira taluk of Tumakuru district recorded highest mean wilt (33.34%) and least incidence (11.54%) was recorded in Shorapur taluk of Yadgir district. In Karnataka state, the mean incidence of pomegranate wilt recorded was 24.13 per cent.

Somu *et al*, (2017) carried out survey in major pomegranate growing districts of Karnataka to know the incidence of wilt during 2015-16, which revealed that among the villages of different district surveyed the highest mean incidence (45.80%) of pomegranate wilt was noticed in Govindkoppa village followed by Kaladgi village (27.05%) in the Bagalkot taluk of Bagalkot district and the least disease incidence (1.00%) was noticed in the Bajjapanhatii village of Hosadurga talku, Chitradurga

district. Among the districts, the highest mean incidence of wilt (15.27%) was recorded in Bagalkot district followed by Vijayapura district (6.23%). The lowest incidence of pomegranate wilt (3.75%) was recorded in Chirtradurga district. Orchards of four years and above old planted under black soil showed higher incidence of wilt along with shot hole borer and root knot nematode infection.

2.3 Study of interaction of *Ceratocystis fimbriata* and *Meloidogyne incognita* in causing wilt of pomegranate

Atkinson (1892) for the first time reported the presence of *Meloidogyne incognita* along with *Fusarium oxysporum* in the rhizosphere of cotton plants which increased the severity of wilt caused by fungus. Since then nematode fungus complexes have been receiving increasing attention during the recent years and investigations covering this subject have been reviewed by several workers (Sasser, 1985; Khan and Reddy, 1993).

Byther and Morre (1974) established infection by atomizing a measured amount of conidial suspension of *C. paradaxa* into the soil and through mixing was done to achieve the inoculum density of $10^{1\ to}\ 10^{5}$ spores /g of soil. Observations indicated that predisposition of fungal diseases by plant parasitic nematodes required a minimum level of nematode infection. Gaber *et al*. (1979) observed that low population density of the fungal propagules (650 per gram of soil) and 50 larvae (*Meloidogyne incognita)* per 500 g of soil in combination with high population density of fungus (47×10^{3} to 77×10^{3} propagules / g of soil) could result in marked wilted symptoms in cotton.

Padilla *et al.* (1980) studied the interaction between *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *pisi* on pea and found that the concomitant inoculation of the nematode and the fungus at planting caused the death of plants after 45 days. Possible involvement of nematodes with fungal pathogens in causation of pod rot in ground nut has been suspected by Cheng *et al.* (1989). Studies were made by Ramanath and Dwivedi (1981) to determine the role of *Meloidogyne javanica* on the development of wilt caused by *Fusarium oxysporum* f. sp. *ciceri.* Plants grown in soil infested with *Fusarium* and *Meloidogyne* advanced the appearance of wilt by 15 days in chickpea cultivar T-3, than plants grown in soil infested by *Fusarium* alone. More

than 56 per cent plants wilted by combined infection of both nematode and fungus and only 32 per cent plants wilted in fungus alone treatments.

Singh *et al.* (1981) observed that simultaneous inoculation of *Meloidogyne incognita* and *Fusarium oxysporum* or inoculation of *Meloidogyne incognita* 10 days prior to fungus drastically reduced plant height and fresh shoot weight with high wilt incidence in French bean. Sharma and Cerauskas (1985) observed greater reduction in shoot and root weights in chickpea due to simultaneous inoculation of *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *ciceri* than inoculation of either of them. Goel and Gupta (1986) observed that inoculation of *Meloidogyne javanica* one week prior to *Fusarium oxysporum* inoculation to seedling of chickpea or simultaneous inoculation of both pathogens or inoculation of fungus one week prior to nematode resulted in reduced plant growth when compared to individual inoculations.

Mani and Sethi (1987) studied the effect of combined inoculation of *Meloidogyne incognita, Fusarium oxysporum* and *Fusarium solani* on growth of chickpea cv. JG-62, which was found to be additive in nature. However, when nematode was established one week prior to fungus the resultant effects was more than additive. Occurrence of *Meloidogyne incognita* in combination with both the fungi not only increased the severity of diseases but also shortened the incubations period by 6 – 11 days for disease expression when nematode preceded *Fusarium solani.* The nematode development and multiplication was adversely affected by *Fusarium solani* irrespective of time of inoculation. *Fusarium oxysporum* did not affect the nematode population significantly. Significant number of root knot nematode larvae along with Fusarium from wilted plants of soybean were isolated in microplot of Botany Department, Lucknow University. Neeta (1990)

Marimuthu, (1991) reported in betelvine that the nematode may predispose *Phytophthora palmivora,* which is able to infect through wounds caused by *Meloidogyne incognita.* Umamaheshwari (1991) reported that inoculation of nematode prior to fungus reduced the incubation period for the appearance of wilt symptom by a week in a late wilt variety of chickpea K-850. The fungus did not affect the development of nematode in the roots. Singh *et al.* (1994) observed that inoculation of nematode alone, simultaneously with fungus and 10 days prior to the

fungus drastically reduced the plant height and fresh shoot weight and increased wilt in french bean and recorded maximum wilt.

Krishna Rao and Krishnappa (1994) observed that inoculation of nematode (*Meloidogyne incognita)* along with fungus (*Fusarium oxysporum* f. sp. *ciceri*) at lower levels resulted in 19.88 per cent wilt incidence when compared to 6.66 per cent with fungus alone.

Krishna Rao and Krishnappa (1996) reported occurrence of *Meloidogyne* and *Fusarium* wilt disease complex in chickpea in most of the districts of Karnataka except in Mandya and Kolar. The population levels of nematode and fungus ranged from 11.7 to 132.5 juveniles per 250 cc soil and 5 g root and 4.8 to 34.8 × 103 cfu per g soil, respectively.

Patel *et al.* (2000) observed interaction of *Fusarium oxysporum* f. sp. *ciceri* with *Meloidogyne incognita* on chickpea cv. Dahood yellow revealed that the organisms either individually or in combinations reduced plant height and fresh root and shoot weights significantly, but the reduction was made by *Meloidogyne incognita* as compared to *Fusarium oxysporum* f. sp. *ciceri.* Among combined inoculations, simultaneous inoculation of both pathogens and maximum suppressing effect on growth of chickpea plants as compared to preceding or succeeding inoculation of fungus and nematodes. Root gall index and nematode multiplications on chickpea were maximum when nematodes were inoculated alone but it was reduced in the presence of fungus. The severity of disease increased when root knot nematode was present with the fungus. Maximum wilting of plant was observed when the fungus and nematode were inoculated simultaneously.

Interaction between *Meloidogyne incognita* and *Fusarium oxysporum* was studied on black gram cv. T-9. The combined inoculation, plant growth along with rhizobial nodulation was more adversely affected. Simultaneous inoculation of both the pathogens and nematode inoculation prior to fungus synergistically reduced the plant growth and nodulation. It was accompanied by an increase in the disease intensity. These effects were less significant when fungus preceded the nematode inoculation, the multiplication of nematodes and galling on roots increased with pre inoculation of fungus (Mahapatra and Swain, 2001).

Patil *et al.* (2003) reported that the prevalence and severity of different diseases and nematode problems in north eastern Karnataka, on medicinal crops such as Coleus, Long pepper, Ashwagandha *etc.* He revealed the presence of root knot, damping off, rhizome rot, seed rot, seedling blight and dieback diseases and estimated the loss to the tune of 5-20 per cent.

The survey carried out on *Coleus forskohlii* by Shresti (2005) to know the incidence of root knot disease in northern districts of Karnataka revealed the association of root knot nematode (*Meloidogyne* spp.) with fungi namely *Sclerotium rolfsii*, *Rhizoctonia bataticola*, and *Fusarium chlamydosporum.* Senthamarai *et al.* (2006a) carried a glasshouse experiment to study the interaction of *Meloidogyne incognita* and *Macrophomina phaseolina* on *Coleus forskohlii.* The nematode multiplication was adversely affected when fungus was inoculated prior to nematode. Simultaneous inoculation of nematode and fungus as well as nematode followed by fungus 15 days later, caused 100 per cent root knot disease and significant reduction in plant growth compared to the inoculation of fungus alone or fungus inoculation prior to nematode. Akhtar *et al.* (2007) studied individually as well as in combinations of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *pisi* causing significant reduction in all the growth parameters of *Pisum sativum* as compared to uninoculated plants. Simultaneous inoculation of both the pathogens and nematode inoculation prior to fungus significantly reduced the plant growth. The reproduction of nematode and galling on roots decreased with pre inoculation of fungus, while the infection of fungus increased in the presence of nematode.

Sonyal (2010) studied on interaction of fungal pathogen *Ceratocystis fimbriata* with plant parasitic nematodes. However, in the interaction study, the *C. fimbriata* + *M. incognita* showed less shoot and root length and shoot and root weight followed by fungal giant culture (*C. fimbriata*) compared to other treatments. Imran Khan, (2017) studied on interaction of combined inoculation of *C. fimbriata* and *M. incognita*. The least shoot and root length was recorded in the combined treatment inoculated with *C. fimbriata* and *M. incognita* followed by inoculation of giant culture of *C. fimbriata* which were on par with each other as compared to control.

2.4 *In vitro* evaluation of fungicides, bioagents, nematicides and oil cakes against *Ceratocystis fimbriata* and *Meloidogyne incognita*

2.4.1 *In vitro* evaluation of non systemic and systemic fungicides against *C. fimbriata*

Xiujian *et al.* (2000) checked the fungicides for the control of *Ceratocystis fimbriata* in sweet potatoes in Fujian, China using PDA plate cultures in the laboratory. In PDA plate culture, a high inhibitory effect was noted with thiophanate methyl, carbendazim and liguoling which protected >90% of stored tubers, whereas protection by chlorothalonil was 20.7%. Vijaya *et al.* (2007) tested five systemic and non systemic fungicides in two concentrations against *C. paradoxa* causing sett rot of sugar cane. Among the systemic fungicides carbendazim and propiconazole were most effective in complete inhibition of the pathogen at both the concentrations of 0.05 and 0.1%, while benomyl and hexaconazole inhibited only at 0.1 per cent concentration. Among the non systemic fungicides tested thiram was found best followed by captan at both the concentrations of 0.1 and 0.2%, whereas mancozeb and copper oxychloride were least effective.

Sharma *et al.* (2010) conducted *in vitro* studies on wilt of pomegranate and revealed efficacy of many fungicides like carbendazim (0.1%), propiconazole (0.1%), hexaconazole (0.1%), mancozeb (0.2%), captan (0.2%), boric aicd (0.1%) and bioagent *Trichoderma viride* preparation (0.2%) in providing complete inhibition of the pathogen.

Sonyal (2010) reported that among systemic fungicides, complete inhibition was shown by propiconazole, tricyclazole at all concentrations tested. The least mycelial growth inhibition was observed in carbendazim at 0.1 per cent (57.21%), 0.2 per cent (60.36%) and 0.3 per cent (68.32%). Among non systemic fungicides tested copper oxychloride was more effective than other fungicides at 0.1 per cent (95.92%), 0.2 per cent (97.03%) and 0.3 per cent (98.14%). The least mycelial growth inhibition was observed in chlorothalonil and captan at all concentration. Among combi product Carboxin 37.5 WP + thiram 37 (vitavax power) was completely effective in all concentrations. SAAF 75% WP (carbendazim + mancozeb) was effective only at 0.2 per cent (92.58%) and 0.3 per cent (93.14%).

Kishore and Bhardwaj (2011) evaluated six fungicides at their standard doses to know their antifungal properties against *C. fimbriata.* The result revealed 100 per cent inhibition of pathogen in case of carbendazim (0.05%), propiconazole (0.15%), benomyl (0.05%) and a combiproduct of captan + hexaconazole (0.1%). Individually captan (0.3%) was least effective against *C. fimbriata.* Poussio *et al.* (2012) evaluated three fungicides against *C. fimbriata* causing mango sudden death *viz.,* Topsin-M (thiophanate methyl), Alliate (fosetyle aluminum) and Nativo (trifloxystrobin and tebuconazole) at concentrations of 25, 50, 75, and 100 ppm under *in vitro* condition. The result revealed that there was no mycelial colony growth in Topsin-M at any dose, hence it was found effective fungicide, followed by Nativo and Alliate, as compared to control.

Apet *et al.* (2015) evaluated six systemic fungicide at concentration of 500 ppm, 1000ppm and four non systemic fungicides at concentration of 1000 ppm, 2000 ppm against *C. paradoxa* causing pineapple disease of sugarcane. Among the systemic fungicides *viz.*, carbendazim, propiconazole and hexaconazole with highest average mycelial growth inhibition of 94.44 per cent, 94.44 per cent and 91.66 per cent, respectively followed by non systemic fungicides thiram (79.64%) and captan (77.07%).

Balaganur (2016) tested the bioefficacy of different fungicides against *C. fimbriata* at 100, 300 and 500 ppm concentrations *in vitro*. The result showed that carbendazim and combi product tebuconazole 50% + trifloxystrobin 25% WG were found effective by recording inhibitions of 98. 92, 100, 100 per cent and 96.5, 97.61, 98.79 per cent at 100, 300 and 500 ppm concentrations tested respectively. Chaudhari *et al.* (2016) evaluated five systemic and 4 non systemic fungicides at concentrations of 0.05%, 0.1%, 0.15% and 0.2%, 0.25%, 0.3% respectively against *C. fimbriata* causing wilt of pomegranate. The result revealed that at all concentration hexaconazole and tricyclazole completely inhibited colony growth of pathogen. Among non systemic fungicides, copper oxy chloride and mancozeb were found most effective.

Khan *et al.* (2017) reported that among systemic fungicides tested, cent per cent inhibition of mycelial growth was seen in propiconazole followed by

hexaconazole 94.65 per cent, respectively followed by non systemic fungicides thiram (74.35 %) followed and copper oxy chloride (70.52 %). Raja (2017) reported that out of nine systemic fungicides tested, carbendazim, hexaconazole, thiophanate methyl, propiconazole and tebuconazole showed 100 per cent inhibition at all concentrations 0.05%, 0.10% and 0.15% tested. In case of combi fungicide molecules, hexaconazole + zineb, carbendazim + mancozeb, trifloxystrobin + tebuconazole and captan + hexaconazole were found highly effective at all the concentrations (0.10%, 0.20% and 0.30%).

Somu (2017) tested thirteen systemic fungicides against *C. fimbriata* by poisoned food technique. The mycelial growth of the fungus was inhibited 100 per cent by carbendazim, flusilazole, tebuconazole, tebuconazole + trifloxystrobin, fenamidone + mancozeb, difenoconazole, thiophanate methyl, propiconazole, propiconazole + difenoconazole, hexaconazole and tricyclazole at 0.1, 0.2 and 0.3 per cent concentrations. Among non systemic chemicals cymoxanil + mancozeb, mancozeb and captan inhibited the mycelia growth completely at 0.1, 0.2 and 0.3 per cent concentration.

2.4.2 *In vitro* evaluation of fungal and bacterial bio agents against *C. fimbriata*

Brain *et al.* (1946) reported the mechanism involved in inhibition of the *C. paradoxa* due to the release of antibiotic by *T. viride*. Hlume and Shields (1970) reported that reduction in mycelial growth of the *C. paradoxa* may be competition between *C. paradoxa* and *T. viride* for nutrition and other growth factors. Chet *et al.* (1981) reported that the mechanism involved in inhibition of the *C. paradoxa* was due to coiling effect around the hyphae. Mukherji *et al.* (1988) reported that reduction in mycelial growth of the *C. Paradoxa* may be due to penetration of the antagonistic hyphae into hyphae of pathogen at the place of contact. Sampang (1989) reported the first document of biological control of *C. paradoxa* on sugarcane sett rot.

Whipps (1992) reported that *T. harzianum* showed antagonistic behaviour towards *C. paradoxa*. The powerful antagonistic behaviour of the *T. harzianum* can be attributed to competition, parasitism and antibiosis or by synergistic combination of these modes of action. Guevarra (1992) conducted field trials in sugarcane. He found that when the setts were treated with *Gliocladium delinquescens* and

G. fimbriatum against *C. paradoxa* gave significant reduction in yield when sown in soils infected with the pathogen.

Karthikeyan (1996) reported that the mechanism involved in inhibition of the *C. paradoxa* is due to the release of antibiotic by *T. viride.* Joshi (1999) reported that *T. viride* inhibited 73.50 per cent of mycelia growth of *C. paradoxa*. Talukder *et al.* (2007) reported that *T. harzianum* was found effective antagonist to *C. paradoxa*, the causal organism of pineapple disease of sugarcane. Sharma (2009) reported that *T. viride* bioformulation resulted in 86.6% growth inhibition of *C. fimbriata* under *in vitro* condition. Soil application of *Trichoderma* sp. + *Paecilomyces* sp. at 25 g with 2 kg well decomposed farm yard manure around the trunk of pomegranate trees helps to prevent wilt infections (Raghuvanshi, 2007).

Rahman *et al.* (2009) evaluated different *Trichoderma* isolates against *C. paradoxa*, which causes the pineapple disease of sugarcane. The study showed that *Trichoderma* isolates have a good antagonistic effect on *C. paradoxa* mycelial growth and *T. harzianum* IMI-392432 was the most potential strain to control the pineapple disease pathogen. Sonyal (2010) revealed that *T. harzianum* and *T. viride* showed maximum inhibition of *C. fimbriata* (100%) within four days and completely inhibited the perithecium production as well as grows over the pathogen.

Mahalingam *et al.* (2011) evaluated the antagonistic potentiality of some soil fungi against *Ceratocystis paradoxa* causing pineapple disease in sugarcane by dual culture method. The experimental result showed that the maximum percentage inhibition of *C. paradoxa* was with *T. koeningii* (75.00) followed by *Gliocladium virens* (73.80), *T.viride* (73.80), *T.hirsuta* (72.30), *A. awamori* (70.00), *A. niger* (69.20), *T.harzianum* (56.90), *T.glaucum* (53.80), *Trichothecium* (53.80) and *P. citrinum* (23.10). Apet *et al.* (2015) evaluated five fungal and one bacterial antagonist against *C. paradoxa* causing pineapple disease of sugarcane. The results revealed that all the bioagents tested exhibited fungistatic or antifungal activity against *C. paradoxa* and significantly inhibited its growth over untreated control. Amongst the bioagents tested, *T. viride* was found most effective with highest inhibition (77.40%). followed by *T. harzianum* (70.74%), *T. hamatum* (69.44%), *T. koningii* (69.26%), and *P. fluorescens* (67.36%). The antagonists *T. longibrachiatum* was found less effective with 51.11 per cent mycelial inhibition.

Balaganur (2016) reported that among 17 strains of *P. putida* isolates, UHSPs 2 showed 96.67 per cent inhibition. Among 24 strains of *T. harzianum,* UHSTh 5, UHSTh 43 and UHSTh 48 showed complete inhibition, whereas *B. subtilis* isolates Bs-3b and Bs-27 recorded inhibition of 87.77% and 86.66% respectively. Khan *et al.* (2017) tested the efficacy of different bio agents against *C. fimbriata* causing wilt disease of pomegranate. Among them *C. fimbriata*, *T. harzianum* were found to be the most effective.

Raja (2017) reported that *T. harzianum* (Th-R) and *T. viride* (Diamond) recorded inhibition of 100 per cent mycelial growth of *C. fimbriata. P. fluorescens* (RP-46) inhibited to an extent of 42.65 per cent and there was no inhibition by *B. subtilis* (BS-1). Somu (2017) tested the efficacy of different bio agents against *C. fimbriata* causing wilt disease of pomegranate. Among the bio agents tested, *Trichoderma harzianum*-55 recorded the maximum per cent inhibition of mycelial growth (76.00%). It was found to be significantly superior to the rest of the bioagents tested. This was followed by *Trichoderma viride*-27 (70.33%), *Trichoderma viride* (PDBC) (67.00%), *Trichoderma viride* (64.00%), *Trichoderma harzianum*-1 (54.67%), *Trichoderma harzianum*-2 (51.00%) and *Pseudomonas fluorescens* (45.00%). Whereas, the minimum parasitic activity was noticed in case of *Bacillus subtilis* 1 and 2 which inhibited 40.67 and 41.67 per cent of *C. fimbriata* colony.

2.4.3 *In vitro* evaluation of nematicides and oil cakes against *C. fimbriata* and *M. incognita*

Among the several methods of managing the plant diseases, soil amendment are one of the effective methods Amendments in the form of plant debris, green manures, farmyard manures, compost, oil cakes and fertilizers are known to improve crop productivity by improving nutrient status and soil tilth. Addition of amendments to soils might increase microbial activities in soil to suppress diseases (Sivaprakasam, 1991).

Neem cake was more effective in reducing nematode population and improving tomato yield in pot experiments. Lower dose of neem showed a reduction in nematode numbers compared to untreated control (Jain and Gupta, 1997).

Vijayalakshmi (2000) reported that aqueous extracts of neem seed and neem cake as root dip treatments were effective against *Meloidogyne incognita* infection in tomato.

Mansoor (2006) reported neem leaf, seed powder, oil cake and two nematicides *viz.*, carbofuran and phorate alone and in combination reduced the root knot development caused by *Meloidogyne incognita* in tomato. Highest reductions in the nematode infections and corresponding improvement in plant growth was noted in pots treated with oil cake combined with carbofuran.

2.4.4 *In vivo* evaluation of non systemic and systemic fungicides against *C. fimbriata*

Sharma and Mehrotra (1984) observed the efficacy of carbendazim and benomyl for the control of *C. paradoxa*. Taylor and Ryam (1984) reported that budded sugarcane setts when treated with any of the fungicides such as agallol, carbendazim, tridadimefon or benomyl, growth of sett rot pathogen was effectively minimized. Raid *et al.* (1991) reported the increase in millable cane population, yield and sugar yield due to sett treatment with propiconazole. Salalrajan (1994) reported that sett treatment with carbendazim reduced the disease of sugarcane sett rot caused by *C. paradoxa*.

Kalaimani *et al.* (1996) reported that preplant application of carbendazim at 0.05 per cent gave 54 per cent less incidence of sugarcane sett rot. Natarajan (1997) reported that carbendazim at 0.5 g/l was effective against sett rot of sugarcane caused by *C. paradoxa* when applied after 25 days of planting.

The field trial on integrated disease management against sett rot of sugarcane *C. paradoxa* was conducted during 2003-04 and 2004-05 in sick soils, involving sett treatment with carbendazim @ 0.1 per cent and *T. harzianum* @ 10 g/l along with soil application of FYM @ 25 t/ha and vermicompost @ 2.5 t/ha which reduced the sett rot incidence significantly and improved both the quantitative and qualitative yield and yield attributes. Sett rot incidence decreased to a greater extent during second year. It is imperative from the study that usage of all control measures *viz.*, chemical, biological and cultural in conjunction, will give better control of the disease than following either of the methods separately (Yadahalli *et al.*, 2005).

Jadhav and Sharma (2009) reported that drenching of wilt infected pomegranate plants and also adjacent healthy plants with carbendazim (0.2%) propiconazole (0.2%) + chlorpyriphos (0.2%) is effective method of disease management. Somasekhara *et al.* (2009) found propiconazole, boric acid and phosphoric acid as effective chemicals against wilt pathogen. The infected plants were removed and soil was sterilized with formalin solution before new planting and the effective fungicides propiconazole (0.1%) along with boric acid (25 g/plant) were applied to the partially infected surrounding plants.

Sharma *et al.* (2010) reported that *Ceratocystis fimbriata* under field conditions soil drenching of wilt affected pomegranate plants by *Ceratocystis fimbriata* and adjacent healthy plants with carbendazim or propiconazole (0.2%) + chlorpyriphos (0.2%) has resulted in effective wilt management. Masood *et al.* (2014) conducted a field experiment on mango sudden death disease caused by *C. fimbriata.* In this study, two fungicides namely Topsin M and Aliette at the rate of 75 g L^{-1} and 150 g L^{-1} were applied through trunk injection method, three times at an interval of 10 days. The disease severity significantly decreased from 18. 7% to 7.10% (57 % reduction) in year 2007 while from 21.42 % to 14. 82% (35 % reduction) in year 2008.

2.4.4.1 *In vivo* evaluation of fungal and bacterial bio agents against *C. fimbriata*

Somasekhara (2002) reported that the soil application of bacterial culture, *Bacillus subtilis* was effective in field condition in reducing pomegranate wilt. Potential biological agents *viz., T. viride, T. polysporum, T. hamatum* and *T. aureoviride* used for seed treatment against *Certocystis paradoxa* causing black seed rot in oil palm sprouted seeds *in vitro*. *T. polysporum* significantly reduced the percentage infection to 13 and 10%, 14 days of post inoculation. Eziashi *et al.* (2006) reported that *T. viride* 18.3 and 11.7%, *T. hamatum* 22 and 15%, *T. aureoviride* 23.7 and 13%, benlate solution 21.7 and 18.3 % and control 80 to 30% for wounded and unwounded seeds, respectively.

Sonyal (2010) evaluated different chemicals and bio agents under field condition among which propiconazole was significantly superior than all other treatments followed by difenoconazole and *T. harzianum* + *P fluorescens*. Somu

(2017) reported that fungal bioagents were better than bacterial bioagents in inhibiting the growth of *C. fimbriata*. The maximum reduction in colony growth was observed in *Trichoderma harzianum*-55 which was very effective when compared to all other bioagents tested. Whereas, the minimum parasitic activity was noticed in case of *Bacillus subtilis* 1 and 2.

2.4.4.2 *In vivo* evaluation of nematicides and oil cakes against *C. fimbriata* and *M. incognita*

Amendments of soil with decomposable organic matter and oil cakes are recognized as the most efficient method of changing soil and rhizosphere environment, thereby adversely affecting the life cycle of pathogens and enabling the plant to resist the attack of pathogens through better vigour or altered physiology. It was also reported that chemicals like ammonia (Khan *et al.*, 1974) and fatty acids (Sitaramaiah and Singh, 1978) liberated during the decomposition of neem cake could be one of the factors involved in nematode control. Apart from this, the neem cake itself contains formaldehyde (0.25%), which is another factor responsible for nematode control.

Siddiqui and Khan (1986) tested phorate, phenamiphos and carbofuran each at 1.0,1.5 and 2.0 kg a.i./ha under field conditions against root knot nematode, *M. incognita* infesting pomegranate. The results revealed that the carbofuran at 2 kg a.i./ha was found to be significantly superior in reducing root knot index and increasing the yield of pomegranate upto 52 per cent. Sivakumar and Marimuthu (1986) reported in betel vine that a significant reduction of 44.40 per cent over control in nematode population with neem cake applied at 100 kg per ha. Narashimhalu and Bhaskaran (1987) found that application of neem cake at 5 t/ha reduced root rot incidence in sugarcane. Dasgupta and Gupta (1989) reported the effect of different soil amendments on wilt of pigeon pea. Darekar *et al.* (1989) reported that soil treatment with neem (*Azadirachta indica*) cake at 2.5 t/ha followed by kranj (*Pongamia pinnata*), mahua (*Bassia latifolia*) and castor (*Ricinus communis*) cakes have also been found effective in the management of root knot nematode.

Darekar *et. al.* (1989) conducted a field experiment on pomegranate (cv. Ganesh) where non edible oil cakes *viz.*, neem, karanj, mahua, castor and biogas sludge were applied at 2.5 tones /ha 20 days before "Bahar" (Blossom) treatment. The nematicide, carbofuran 3 G at 6 kg a. i. /ha was applied at the time of "Bahar" treatment. The observations on pre and post treatment nematode populations and yield per tree revealed that all the treatments were significantly superior over untreated control in reducing the nematode population and increasing the yield of pomegranate. Reddy and Khan (1991) reported groundnut and neem cakes along with carbofuran were used to manage *Meloidogyne incognita* on okras in India. Groundnut cake + carbofuran gave significantly higher okra fruit yield compared to control.

Nambiar and Ramanujam (1992) observed the survival of chlamydospores of *Thielaviopsis paradoxa* causing stem bleeding of coconut was greatly reduced by neem cake. The efficacy of decomposed products of groundnut, mustard and neem seed cake was investigated. The results indicated that organic amendments showed fluctuations in the mortality of *M. incognita*. Among, organic amendments mustard was the most nematoxic (Goswami *et al.*, 1993).

Rao *et al.* (1995) found that carbendazim and tridemorph with regulated fertilizer dose and five kilograms of neem cake checked the sett rot disease and increased yield. Devi and Das (1998) reported that neem cake was superior in reducing population of root knot nematode, gall index and increasing the yield attributing characters of carrot at dosage of 1.0 and 1.5 tonnes per ha followed by saw dust, poultry manure and water hyacinth at higher doses. Yadahalli (2008) reported that neem cake application recorded significant increase in germination (68.53%) and reduction in the incidence of sett rot (27.83%) of sugarcane caused by *C. paradoxa*.

Radwan *et al.* (2013) conducted the glass house experiment on efficacy of granular nematicides namely cadusafos, carbofuran, ethoprop, fosthiazate and oxamyl against *Meloidogyne incognita* associated with tomato. The results revealed that fosthiazate had the highest nematicidal effect with 97.52 % reduction in galls and 96.45 % juveniles in soil, while cadusafos was relatively least effective causing 77.51 and 86.63 % reduction in galling and J 2 population respectively.

2.5 Integrated disease management in nematode fungal disease complex

A field experiment was conducted to study the effect of organic amendments, nematicides, fungicides and their combination on the crop yield, wilt and root knot disease of betel vine. The results revealed that maximum yield (13.81 kg/ha) was obtained in the bed treated with carbofuran at 1.5 kg a.i. per ha + neem cake urea at 50 + 50 kg per ha + 0.5 per cent Bordeaux mixture. The wilt percentage and root knot index were minimum in the two treatments *viz.,* carbofuran and aldicarb at 1.5 kg a.i. per ha, both with the combination of neem cake and 0.5 per cent Bordeaux mixture. Carbofuran was effective in reducing the population and development of *Meloidogyne incognita* in tomato and improving plant growth at all concentrations tested. But, soil drench at 50, 100 and 500 ppm gave effective control (Haq and Saxena., 1986). Darekar *et al.* (1990) studied the efficacy of granular nematicides against *Meloidogyne incognita* in tomato nursery. Carbofuran and aldicarb at 0.6 g a.i. per m^2 had reduced nematode populations to a greater extent with highest yields of 27.40 and 25.67 per cent, respectively over control.

Sivakumar and Vidyasekaran (1990) reported that performance of *Paecilomyces lilacinus* @ 2g/kg, farmyard manure @ 4% (W/W) their combination for control of *Meloidogyne incognita* on *C. forskohlii*. A significant increase was found in all plant growth characters *viz.,* shoot weight, root weight, tuber yield. Pandey (1995) reported that application of neem seed cake at 5, 10, 20 g per kg, aldicarb at 0.0020 g a.i. per kg, carbofuran at 0.0015 g a.i. per kg, powdered leaves of *Adhatoda vasica* and *Murraya koenegii* at 50 and 100 g per kg of soil significantly reduced root knot nematode population in soil and roots of Japanese mint. Singh and Vinodkumar (1995) reported the efficacy of neem cake at two per cent w/w and carbofuran at two kg a.i. per ha in reducing the population of *Meloidogyne incognita* as well as increasing shoot length, shoot dry weight, root fresh weight, number of leaves of Japanese mint. De *et al.* (1996) reported coating of chickpea with biocontrol agents *viz., B. substilis*, *G. virens*, *T. harzianum* and *T. viride* and carboxin (Vitavax) significantly controlled *F. oxysporum* f. sp. *ciceri* wilt by 30 to 45.8 per cent. Integration of biocontrol agents and carboxin significantly increased seed yield by 25.4 and 42.6 per cent as did carboxin treatment alone.

Inoculation with *Trichoderma viride* + *Glomus mosseae* gave the best result in controlling the *Fusarium* wilt and resulted in maximum growth, yield and root forskolin concentration of Coleus. The next best treatment was *Pseudomonas fluorescens* + *Trichoderma viride* followed by *Glomus mossae* + *Pseudomonas fluorescens* and *Trichoderma viride* alone. (Boby and Bagyaraj, 2003). Chaitali *et al.* (2003) reported that efficacy of groundnut cake and neem cake at 5 per cent w/w and *T. viride* at 2 g mycelial mat of fungus per 500 g of soil for control of disease complex caused by *Rhizoctonia bataticola* and *Meloidogyne incognita* on okra.

Akhtar *et al.* (2005) reported the efficacy of bavistin + carbofuran followed by *A. indica* seed powder, *T. harzianum* + *P. fluorescens*, *M. koenigii* leaves, carbofuran, *T. harzianum*, *P. fluorescens*, bavistin, neem leaves against *Meloidogyne incognita* – *Fusarium oxysporum* disease complex on *Vigna mungo*. Highest increase in the fresh and dry weight of root.

A field experiment was conducted to study the management of collar rot disease of *Coleus forskohlii* using bioagents, organic amendments and chemicals in different combinations. Treatments with *T. viride* + nemato recorded lower per cent wilt incidence (12.76), followed by treatment with *Trichoderma harzianum* alone (18.87) and *Pseudomonas fluorescens* alone (19.98). Lowest population of root knot nematode recorded in treatment with *Trichoderma viride* + nemato (873.33) followed by treatment with carbofuran alone (1066.67) and nemato alone (1180.00) and lowest number of galls per 5 g root recorded in treatment with *Trichoderma viride* + nemato (10.13) followed by treatment with carbofuran alone (14.93) (Shresti, 2005).

Experiments were conducted to find out comparative performance of bioagents, botanicals and fungicides in management of root rot of *Coleus forskohlii* (Wild.) Briq. caused by *Fusarium chlamydosporum* and *Rhizoctonia* in pot culture. Among different bioagents, botanicals and fungicides tested in different combinations, the treatments with parthenium + propiconazole + *T. viride* and parthenium + captan + *T. viride* were found better with least per cent mortality (33.33%). The next best treatment was carbendazim alone, neem + propiconazole + *T. viride*, Pongamia + propiconazole + *T. viride* and neem + captan + *T. viride* recorded 41.66 per cent mortality (Sachidananda, 2005a). Nisha and Sheela (2006) reported that integration of soil solarization in the nursery for 15 days and application

of either *P. lilacinus*, *B. mucerans* or neem cake in the main field are the better treatments in improving biometric characters and reducing the nematode population in soil and root on Coleus (*Solenostemon rotundifolius*).

Tripati and Singh (2006) reported effect of compatible bio control agent along with mustard cake and furadon against *Meloidogyne incognita* in tomato. A significant increase in length and weight of root and shoot and considerable reduction in root galling as well as nematode population in soil and root was observed when plants were treated with *P. lilacinus* and *T. viride* in combination with mustard cake and furadon. Senthamarai *et al.* (2006b) tested the efficacy of bioagents against *M. incognita* in *Coleus forskohlii* under pot culture experiment. Results of investigation indicated that soil application of *Pseudomonas fluorescens* at the rate of 2.5 kg a.i. per ha showed increased plant growth and root knot nematode population both in soil and root.

Sunilkumar *et al.* (2006) reported that *T. harzianum* @ 5 g/kg of soil was highly effective against *M. incognita* when egg masses were inoculated 15 days prior to transplanting and fungus at transplanting time in absence or presence of neem cake. Paramasivan *et al.* (2007) studied antagonistic effect of fungal and bacterial antagonists against *Macrophomina phaseolina* on *Coleus forskohlii.* Results indicated that *T. viride* @ 2 g/kg of soil was effective as it suppressed the disease and enhanced the plant stand and tuber yield, followed by *T. harzianum* and *P. fluorescens.* Sonyal (2010) tested different fungicides, bioagents and botanicals against wilt complex of pomegranate in which propiconazole at 0.2 per cent, difenoconazole at 0.2 per cent concentration, *T. harzianum* + *P. fluorescence* and neem cake were effective in reducing the per cent disease incidence of wilt complex.

Shanmugam *et al.* (2015) evaluated sett rot management module against *C. paradoxa.* Among the different treatment combinations, bud chip treatment with *P. fluorescens* @ 10 g/l + *T. viride* @ 4 g/l + mixing of *P. fluorescens* @ 1kg/250 kg of coco peat and bud chip treatment with *P. fluorescens* @ 10 g/l followed by 0.1% thiophanate methyl treatment were found to be highly effective. Economic analysis of management modules showed that 1.87 and 1.78 benefit cost ratio for *P. fluorescens* + *T. viride* combination and 1.84 and 1.83 benefit cost ratio for *P. fluorescens* + thiophanate methyl combination.

Raja (2017) conducted a field experiment on wilt of pomegranate caused by *C. fimbriata* for two years. The result indicated that three drenching of propiconazole (0.2%), *T. viride* (diamond) (0.7 g/l) and *T. harzianum* (Th-R) (5 g/l) at an interval of 15 days showed maximum disease control with higher mean fruit yield and cost benefit ratio. Somu (2017) conducted a field experiment on wilt of pomegranate caused by *C. fimbriata* where propiconazole (0.2%), propiconazole + difenoconazole (0.2%), tricyclazole (0.2%) and tebuconazole (0.2%) drenching four times at an interval of 15 days showed the maximum disease control with higher fruit yields and net returns.

3. MATERIAL AND METHODS

The present investigation on wilt complex of pomegranate caused by *Ceratocystis fimbriata* was carried out during 2016-17 to 2017-18 at College of Horticulture, University of Horticultural Sciences, Bagalkot. During the research, laboratory studies were conducted at Department of Plant Pathology, College of Horticulture, UHS, Bagalkot and field experiments were conducted at farmer's field located at Tulasigeri of Bagalkot taluk. The details of material and methods used during the course of investigation are given here under.

3.1 General procedure

3.1.1 Cleaning of glassware

In all the experiments, Borosil and Corning glass wares were used. Petri plates of 90 mm diameter were used for isolation purpose and cultural studies. Conical flasks of 500 and 250 ml were used for preparation of various media. The glass wares were kept in a cleaning solution prepared by mixing 60 g potassium dichromate ($K_2Cr_2O_7$) and 60 ml of concentrated sulphuric acid (H_2SO_4) in one liter of water for 24 hours and afterwards they were washed in running water and cleaned with detergent powder. After rinsing twice in distilled water, they were air dried.

3.1.2 Sterilization

Glass wares and media were sterilized in autoclave at 15 lb pressure and temperature of 121.6 ^{0}C for 20 min.

3.2 Survey for the assessment of incidence and severity of wilt complex of pomegranate caused by *Ceratocystis fimbriata* and *Meloidogyne incognita* in Bagalkot and Vijayapur districts

To assess the extent of disease severity of wilt complex, intensive roving survey was conducted during 2016-17 in two important pomegranate growing district of northern Karnataka. *i.e.,* in Bagalkot and Vijayapur districts to know the incidence of *Ceratocystis fimbriata* and it's association with nematodes. The survey was conducted in major taluks, selecting 3 villages in each taluk and two fields in each village. Samples of soil and roots were collected from the rhizosphere of pomegranate

crop up to the root depth. The per cent disease incidence was calculated using the following formula.

$$\text{Per cent disease incidence} = \frac{\text{No. of plants showing wilting symptom}}{\text{Total number of plants}} \times 100$$

Each soil sample was filled in polythene bag and tied with a rubber band and labeled immediately. Information pertaining to the locality, crop history, *etc.* was also labeled along with the samples. Samples of soil and roots were analyzed on the day of collection or after keeping for a few days under refrigerated conditions. The nematode population from soil was estimated.

Nematode infected galled root system was scored by using a disease rating (0 to 5 scale) given by Taylor and Sesser (1978).

Grade	Number of galls per root
0	No galls per plant
1	1-2 galls per plant
2	3-10 galls per plant
3	11-30 galls per plant
4	31-100 galls per plant
5	More than 100 galls per plant

3.2.1 Collection of diseased samples and identification of the *C. fimbriata* and *M. incognita*

The roots and soil samples of pomegranate plants which are severely infected by *C. fimbriata* and *M. incognita* were collected during survey. The pathogen, *C. fimbriata* was isolated and then nucleus culture was maintained on potato dextrose agar slants and kept in a refrigerator at 4° C, for further use in all the laboratory and field studies.

3.2.2 Isolation of *C. fimbriata*

The fungus was isolated following standard tissue isolation technique. The black discolored stem bits along with some healthy portions were surface sterilized in 1:1000 mercuric chloride solution for 60 seconds and washed thoroughly thrice in sterile distilled water to remove the traces of mercuric chloride, if any. The infected tissues from diseased roots and stem were cut into small strips of about 1 cm and carrot baited for *Ceratocystis fimbriata* by placing these between two carrot slices in desiccator for 6-7 days. Ascospores mass that developed on carrot slices were transferred to PDA in petri plates. The Petri dishes were incubated at room temperature (25 ± 1^0C) and observed periodically for fungal growth. The pure colonies which developed from the bits were transferred to PDA slants and incubated at room temperature for 15 days. Pure culture of the fungus was obtained by single hyphal tip isolation method.

3.2.3 Hyphal tip isolation

This method was followed for maintaining of pure culture. Dilute spore suspension of the pathogen was prepared in sterilized distilled water containing eight to ten spores per ml from 15 days old culture. One ml of such suspension was spread uniformly on two per cent solidified water agar plates and observed for spores under the microscope. Single spore was marked with a marker on backside of the Petri plate and it was allowed to germinate. Such plates were periodically observed for spore germination under microscope. The hyphae growing from each cell of the single spore was traced and marked with marker. The tip of the hyphae was cut carefully and transferred to PDA plates and incubated at 25 ± 1^0C for 15 days. Later, mycelial bits of the fungus were transferred to the center of Petri plates containing PDA and incubated at 25 ± 1^0C for 15 days. Saltation or sectoring was observed in the culture to confirm the pure culture of the fungus.

3.2.4 Maintenance of the culture

The hyphal tip cultures of the fungus were sub cultured on potato dextrose agar slants and kept in laboratory at 25 ± 1^0C for 15 days. Such mother culture slants were preserved at 4^0C in refrigerator. Further, these cultures were sub cultured once in a month and used for future studies.

3.2.5 Processing of soil samples for extraction of *M. incognita*

The known quantity (200 cc) of soil from each sample was processed by combined Cobb's Sieving (using 100, 250 and 400 mesh sieves) and Baermann's funnel technique (Ayoub, 1977) and nematode population was estimated in representative samples as detailed below.

Procedure

1. Infected roots were cut off, made into small bits of 2.5 cm and split longitudinally
2. The cut roots were grinded by using warring blender
3. These were placed over tissue paper, spread on a wire gauge and kept in a Petri plate
4. Level of water was maintained in Petri plate and left undisturbed for 48 hours
5. Later, the suspension in the Petri plate was collected and observed for nematodes using stereo binocular microscope. Nematode population from this was finally estimated.

3.2.6 Preparation of perennial pattern and identification of root knot nematode species

1. The roots infested with root knot nematode was cut off
2. The females were dissected out from well developed galls of the root under stereo binocular microscope and transferred to Petri plate containing water. The posterior portion of the female was cut with a perennial pattern cutting knife (Taylor and Netscher, 1974) and the body contents were cleaned
3. Cleaned posterior portion of the female was further trimmed and transferred to a drop of glycerin on a clean microscopic slide
4. A cover slip was placed on it, sealed with nail polish and observed under stereo-binocular microscope. The species confirmation was done based on the perineal pattern as described by Chitwood (1949).

3.3 Study of interaction of *C. fimbriata* and *M. incognita* in causing wilt of pomegranate

Treatments involving individual inoculation of *C. fimbriata* and *M. incognita* as well as simultaneous inoculation of both these pathogens were done when the seedlings of four months old. *M. incognita* inoculum comprised of 1000 juveniles per kg of soil and *C. fimbriata* inoculum of about 200 g of giant culture per plant was used.

3.3.1 Preparation and inoculation *of C. fimbriata*

Fungal inoculum was prepared in the laboratory by inoculating 5 mm disc to the potato dextrose broth which was cut at periphery of the actively growing culture and incubated at 25 ± 1^{0}C for 15 days. The conidial suspension was prepared by adjusting conidial concentration to 10^{6} cfu/ml by adding sterile distilled water to the inoculum. The healthy susceptible cultivar Bhagwa seedlings were used in the study. Five hundred ml of the suspension was poured to the base of pomegranate plants by injuring the tertiary root with sterile blade in the pots.

3.3.2 Preparation of pure culture of *M. incognita*

The pure culture of the *M. incognita* population maintained on tomato roots at COH, Bagalkot. Infected plants were uprooted and roots were submerged in water to remove the adhered soil. Roots were cut into 2 cm pieces and left in a modified Baermann's funnel to get fresh second stage juveniles (J2) which was used as inoculum. Then one milliliter of *M. incognita* J2 suspension was pipetted into a counting dish and enumerated under a binocular microscope. Counting was repeated three times and the mean nematode count was recorded before inoculation.

The seedlings were inoculated with the suspension of *M. incognita* at the rate of 1000 second stage juveniles (J2) per pot three week after transplanting. Control pot was kept uninoculated. The inoculation was done around the root zone of the pomegranate seedling by making holes using pencil. The holes were covered after inoculation. Light watering was done as and when necessary.

3.3.3 Interaction study of *C. fimbriata* and *M. incognita*

For assessing the interaction of *C. fimbriata* and *M. incognita* on pomegranate, pot culture experiment was laid out in a Completely Randomized Design (CRD) with four replications under shade house condition. Inoculation was done by damaging tertiary roots of pomegranate with the help of sharp knife. The percentage of branches infected (secondary and tertiary) and per cent leaves infected were recorded at 15 days interval. The pots were monitored daily and recorded the following observations.

Experimental details

Crop : Pomegranate

Variety : Bhagwa

Design : CRD

Treatments : 6

Replications : 4

Treatment details

T1- Inoculation of *C. fimbriata* alone

T2- Inoculation of *M. incognita* alone

T3- Inoculation of *C. fimbriata* 15 days prior to inoculation of *M. incognita*

T4- Inoculation of *M. incognita* 15 days prior to inoculation of *C. fimbriata*

T5- Simultaneous inoculation of *C. fimbriata* and *M. incognita*

T6- Untreated control

3.3.4 Observations recorded

The following data were collected at 30,45 and 60 days after nematode inoculation (DAI).

Per cent wilt incidence

The per cent wilt infected plants due was calculated as follows.

$$\text{Per cent wilt incidence} = \frac{\text{No. of plants wilted}}{\text{Total No. of plants}} \times 100$$

Plant parameters

Root length (cm) : The root length was measured in centimetres from soil line to the tip of the root after the adhering soil was cut off using tap water.

Fresh shoot weight (g) : The pomegranate plant was cut at the crown level in each pot and the fresh shoot weight was measured using electronic balance soon after cutting.

Fresh root weight (g) : After cutting the top parts of the plants, all the pots were turned upside down with care, to dislodge the soil and the roots were made free from adhered soil. Finally, the plant roots were gently cut off with tap water to remove the adhering soil particles. The fresh root weight was measured using electronic balance.

Dry shoot weight (g) : The shoots were put in paper covers and brought to laboratory just after taking the fresh weight and kept in an oven at 60-65^{o}C for 24 hours and wilted dry shoot weight was measured using electronic balance.

Root knot index was estimated in grades by using disease rating scale (0 to 5) given by Taylor and Sesser (1978).

3.4 *In vitro* evaluation of fungicides, bioagents, nematicides and oil cakes against *Ceratocystis fimbriata* and nematodes

3.4.1 *In vitro* evaluation of non systemic fungicides against *C. fimbriata* through poison food technique

Six fungicides were tested *in vitro* against *C. fimbriata*. The fungicides were tested at 0.025, 0.05 and 0.1 per cent concentrations. Details of the fungicides are listed below.

Sl. No.	Common name	Chemical name	Trade name
1.	Captan 50% WP	N-trichoromethylthio- cyclohexane-1,2-ficarboxide (3aR,70s) -2-[(trichloromethyl) scelfonyl]-3ª,4,7,7atetrahdro-1H-isoinfole-1,3 (2H) -dione	Captan
2.	Chlorothalonil 75% WP	Tetrachloroisophthalonitrate	Kavach
3.	Copper oxy chloride 50%WP	Dicopper chloride trihyroxide	Blue copper
4.	Mancozeb 75%WP	Manganese ethylene bisdithiocarbomate plus zinc	Dithane M-45
5.	Mancozeb 63% + Carbendazim 12% WP	Methyl 1-1-2 benzimidazolecarbamates + zinc ion and manganese ethylene bis' dithiocarbamate.	SAAF
6.	Propineb 70%WP	Zinc propylenebisdithiocarbamate	Antracol

3.4.2 *In vitro* evaluation of systemic fungicides against *C. fimbriata*

Seven fungicides were tested *in vitro* against *C. fimbriata*. The fungicides were tested at 0.025, 0.05 and 0.1 per cent concentrations. Details of the fungicides are listed below

Sl. No.	Common name	Chemical name	Trade name
1.	Carboxin 37.5% WP + Thiram 37.5% WP	3- (3-5-dichlorophenyl) –N- (1-methyl ethyl) -2-4-dioxo-1-lemadazolidine carboximide + tetramethylthirumdisulphide	Vitavax power
2.	Difenoconazole 25%EC	trans, cis-3-chloro-4- [4-methyl-2-(1H-1, 2, 4-triazole-1-group methyl) -1, 3-dioxapentane-2 group] phenyl-4 chlorophenylether	Score

3.	Hexaconazole 5% EC	(RS) -2- (2,4, -diclorophenyl) -1- (1H-1,2,4-trizol-1-yl) -hexan-2-ol (C14H17Cl2N3O)	Contaf
4.	Propiconazole 25% EC	1-{2- (2,4-dichlorophenyl) -4-1 propyl-1-1, 3- dioxolan-2-yl} -1 4-1, 2,4-triazole	Tilt
5.	Tebuconazole 250 EC (25.9% w/w)	(*RS*) -1- (4,4-dimethyl-3- (1*H*,1,2,4-triazol-1-ylmethyl) pentan-3-ol	Folicur
6.	Thiophanate methyl 70% WP	Dimethyl [(1,2-phenylene) bis- (iminocarbonothioyl)] bis [carbamate] (56).	Topsin M
7.	Tricyclazole 75% WP	Tricyclazole mono-4-methylbenzenesulfonate	Baan

Poison food technique method was followed to test the efficacy of the above mentioned fungicides. The pathogen *C. fimbriata* was grown on PDA medium in Petri plates for fifteen days prior to setting the experiment. Fungicide suspension was prepared in PDA by adding required quantity of fungicide to obtain the desired concentration on the basis of active ingredient. Twenty ml of poisoned medium was poured in each of the sterilized Petri plates. Mycelial disc of 0.5 cm was taken from the periphery of ten days old culture, placed in the center and incubated at 25 ± 1^{0}C till growth of the fungus touched the periphery in control plate. Suitable checks were also maintained without addition of any fungicide. Three replications were maintained for each treatment. The diameter of the colony was measured in two directions and average was worked out. The per cent inhibition of growth was worked out. The per cent inhibition of growth was calculated by using the formula given by Vincent (1947).

$$I = 100 (C-T) / C$$

Where,

I = Per cent inhibition of mycelial growth

C = Growth of mycelia in control

T = Growth of mycelia in treatment

3.4.3 *In vitro* evaluation of bio agents through dual culture technique

Different isolates of bio agents were tested against *C. fimbriata.* The antagonists used in the study were collected from different sources as following

Sl. No.	Bio agents	Source
1	*Bacillus subtilis*	UHS, Bagalkot
2	*Paecilomyces lilacinus*	UHS, Bagalkot
3	*Pseudomonas fluorescens* (Commercial formulation available as 'Krushidhara')	UAS, Dharwad
4	*Pseudomonas putida*	UHS, Bagalkot
5	*Trichoderma harzianum* (Commercial formulation available as 'Krushidhara')	UAS, Dharwad
6	*Trichoderma* isolate-1	UHS, Bagalkot
7	*Trichoderma* isolate-2	UHS, Bagalkot
8	*Trichoderma* isolate-3	UHS, Bagalkot
9	*Trichoderma* isolate-4	UHS, Bagalkot
10	*Trichoderma* isolate-5	UHS, Bagalkot
11	*Trichoderma virens*	UHS, Bagalkot

About 20 ml of PDA was poured into sterile Petri plates and allowed to solidify. From previously grown young cultures of 0.5 disc of test fungus and respective bioagents were transferred aseptically to Petri plates simultaneously by leaving sufficient space in between two discs. In case of bacterial bio agent, mycelial discs of the fungus were kept at opposite ends and bacteria streaked at the center. Three replications were maintained for each treatment. The Petri plates were incubated at 25 ± 1^{0}C till the growth of colony touches the periphery in the control plate. Colony diameter of both the test fungus and bio agents were measured and per cent inhibition was calculated. Data were analyzed statistically.

3.4.4 *In vitro* evaluation of oil cakes against *M. incognita*

Sl. No.	Oil cakes	
	Common name	Botanical name
1.	Castor	*Ricinus communis*
2.	Jatropha	*Jatropha curcas*
3.	Mustard	*Brasssica juncea*
4.	Neem	*Azadirachta indica*
5.	Pongamia	*Pongamia pinnata*

Details of the oil cakes are given below.

Above mentioned oil cakes (2 g in powdered form) were added to 90 mm diameter sterilized petri plate containing 20 ml of distilled water. For each petri plate five female egg masses of *Meloidogyne incognita* were inoculated. The number of juveniles emerged were counted with needle at 24h, 48h and 72h intervals using stereomicroscope.

3.4.5 *In vitro* evaluation of nematicides against *M. incognita*

The nematicides tested are given below.

Sl. No.	Name of the chemical	Chemical name	Trade name	Dosage g/ Kg of soil
1.	Aldicarb 15G	2-Methyl-2- (methylthio) propanal o- (N-methylcarbamoyl) oxime	Temik	0.5
2.	Carbosulfan 25E	2, 2-Dimethyl-2, 3-dihydro-1-benzofuran-7-yl [(dibutylamino) sulfanyl] methylcarbamate	Marshal	0.75
3.	Cartap hydrochloride 50% SP	S, S'-[-2 (dimethylamino) trimethylene]-bis (thiocarbamate) hydrochloride	Cartap	0.5
4.	Phorate 10% CG	0, 0-diethyl-S (ethyl thiomethyl) dithiophophate	Thimet	0.45

Above mentioned nematicides at required concentrations were added to 90 mm diameter sterilized Petri plate containing 20 ml of distilled water. For each petri plate five female egg masses of *Meloidogyne incognita* were inoculated. The number of juveniles emerged were counted with needle at 24h, 48h and 72h intervals using stereomicroscope.

3.4.6 Efficacy of nematicides and oil cakes against *M. incognita* under pot culture

Above mentioned nematicides and oil cakes at required concentrations were added before and one week after inoculation of (1000 J_2 stage) *M. incognita* juveniles to pomegranate plant. Observations like disease and plant growth parameters were checked after 60 days of inoculation.

3.4.7 Evaluation of fungicides, bio agents, oil cakes and nematicides prior and after inoculation of *C. fimbriata* and *M. incognita* under pot culture

A pot trail was undertaken to find out the suitable fungicide/ nematicide/ organic amendment for the management of wilt. Both pre and post treatment of fungicide/ nematicide/ Oil cakes were tested against *Ceratocystis fimbriata* and *Meloidogyne incognita*. For post treatment the pots were simultaneously inoculated with *Ceratocystis fimbriata* and *Meloidogyne incognita* 15 days prior to the application of treatments. Plants with 10-20 per cent wilt infection were selected for the treatment. The experiment was laid out with 17 treatments and in each treatment single plant was tagged along with three replications. The efficacy of treatments (systemic, non-systemic fungicides, bio agent and organic amendment) was tested with one untreated control. The fungicide solutions were prepared and drenched by dissolving known quantity of fungicide in two liters of water. The observations were taken at 15, 30, 45 and 60 days intervals. List of fungicide, bio agents, nematicide and oil cakes are given below.

Sl. No.	Treatment	Concentration
1	Difenoconazole 25% EC	1.5 ml/l
2	Tricyclazole 75% WP	1.5 g/l
3	Propiconazole 25%EC	1.5 ml/l
4	Thiophanate Methyl 70% WP	1.5 g/l
5	Hexaconazole 5% EC	1.5 ml/l
6	Carboxin 37.5% WP + Thiram 37.5% WP	1.5 g/l
7	Tebuconazole 250 EC	1.5 ml/l
8	Mancozeb 63% WP + Carbendazim 12% WP	1.5 g/l
9	Mancozeb 75% WP	1.5 ml/pot
10	*Trichoderma harzianum*	100 g/plant
11	*Trichoderma virens*	100 g/plant
12	Phorate 10% CG	18 g/plant
13	Carbosulfan 25E	15 ml/pot
14	Neem cake	100 g/plant
15	Pongamia cake	50 g/pot
16	*P. fluorescence*	50 g/pot
17	Control	Untreated

3.5 Evaluation of fungicide, bio agents, oil cakes and nematicides against *C. fimbriata* and *M. incognita* under field conditions

A field trail was undertaken during 2017-18 at farmer's field in Tulasigeri under irrigated condition to find out the suitable fungicide for the management of wilt. Plants with 10-20 per cent infection of *C. fimbriata* and *M. incognita* were selected for the treatment. The experiment was laid out with 15 treatments. The efficacy of fungicides (systemic, non-systemic fungicides, bio agent and organic amendment and combinations) was tested with one untreated control. The fungicide solutions were prepared by dissolving known quantity of fungicide in water and ten liters of solution per tree was drenched at 15 days interval. Totally 5 drenches were made.

Treatment details

Location : Tulasigeri village

Variety : Bhagwa

Spacing : 4.5 m x 3 m

Sl. No.	Treatment	Concentration
1	Difenoconazole 25% EC	2 ml/l
2	Tricyclazole 75% WP	2 g/l
3	Propiconazole 25%EC	2 ml/l
4	Thiophanate Methyl 70% WP	2g/l
5	Hexaconazole 5% EC	2 ml/l
6	Carboxin 37.5% WP + Thiram 37.5% WP	2 g/l
7	Tebuconazole 250 EC	2 ml/l
8	Mancozeb 63% WP + Carbendazim 12% WP	2 g/l
9	*Trichoderma harzianum*	100 g/plant
10	Phorate	18 g/plant
11	Neem oil cake	100 g/plant
12	Propiconazole 25% EC + Phorate 10% CG	2 ml/l + 18 g/plant
13	Propiconazole 25% EC + Neem cake + *Trichoderma harzianum*	2 ml/l + 100 g/plant + 100 g/plant
14	Propiconazole 25% EC + Phorate + *Trichoderma harzianum*	2 ml/l + 18 g/plant + 100 g/plant
15	Control	Untreated

Observations were recorded with respect to percentage of branches infected *viz.*, primary, secondary and tertiary branches after drenching at 15, 30,45,60 and 90 days.

3.5.1 Economic analysis of the experiment

Total cost, total returns, benefit cost ratio, net returns and incremental cost benefit ratio were calculated for different fungicides under field condition against *C. fimbriata* and *M. incognita*.

3.6 Statistical analysis

The data obtained in the present investigations for various parameters were subjected to ANOVA for a completely randomized design for *in vitro* studies and randomized block design for *in vivo* studies after applying suitable transformations. The statistical analysis was done by using Web based Agricultural Statistics Package (WASP) 2.0 version and HAU-OPSTAT.

4. EXPERIMENTAL RESULTS

Pomegranate being one of the commercial horticulture crops is highly threatened by the wilt complex caused by *Ceratocystis fimbriata* and *Meloidogyne incognita*. The present investigation on wilt complex of pomegranate caused by *Ceratocystis fimbriata* and often association with *Meloidogyne incognita* was carried out during 2016-17 and 2017-18. The roving survey was conducted on severity of wilt complex of pomegranate in Bagalkot and Vijayapur districts of Karnataka. During the investigation, laboratory and pot experiments were conducted at Department of Plant Pathology, College of Horticulture, UHS, Bagalkot while disease management under field condition was conducted in a farmer's field located at Tulasigeri village of Bagalkot taluk. The results are presented in this chapter.

4.1 Survey for the assessment of incidence and severity of wilt complex of pomegranate caused by *C. fimbriata* and *M. incognita*

To assess the extent of severity of wilt complex, intensive roving survey was conducted during 2016-17 in Bagalkot and Vijayapur districts of northern Karnataka. The survey was conducted to know the incidence of *Ceratocystis fimbriata* and association of root knot nematode in pomegranate. The survey was conducted in all taluks of the two districts, selecting three villages in each taluka and two fields in each village, as explained in "Material and Methods." The data are presented in Table 1, 2 and 3.

The results revealed that among six taluks of Bagalkot district surveyed (Table 3), maximum total per cent disease incidence was recorded in Badami (19.91%) followed by Bagalkot (9.25%), Hunagund (9.03%), Jamakhandi (7.22%), Bilagi (6.99%) and Mudhol (4.08%). The average per cent yellowing, partial wilting and complete wilt symptoms observed in Badami taluk were 15.45%, 1.94% and 2.71%; in Bagalkot taluk 0.95%, 2.86% and 5.44%; in Hunagund taluk 1.29%,1.55% and 6.19%; in Jamakhandi taluk 1.80%, 1.86% and 3.56%; in Bilagi taluk1.58%, 1.43% and 3.99% and in Mudhol taluk 0.99%, 1.13% and 1.96% respectively. Among the villages of Bagalkot districts (Table 1) maximum disease incidence was noticed in

Table 1. Status of wilt of pomegranate in Bagalkot district as recorded in survey during 2017-2018

Sl. No	Taluk	Village		Age of the crop (years)	Soil type	Variety	Wilt incidence (%)					Root knot (Grade)	Shot hole borer (Grade)
							Yellowing	Partial wilt	Complete wilt	Total wilt	Average wilt		
1.	Badami	Anwal	Field-1	2	Black	Sindhoor	2.14	1.71	2.85	6.71	**5.46**	2-3	2-3
			Field-2	2	Black	Sindhoor	1.00	1.20	2.00	4.20		2-3	0-2
		Neerboodihal	Field-1	5	Black	Sindhoor	62.50	4.37	6.25	73.12	**71.12**	0	3-4
			Field-2	5	Black	Sindhoor	56.20	6.25	6.62	69.12		0	2-3
		Shellikeri	Field-1	4	Red	Kesar	1.11	1.00	1.60	3.70	**1.88**	0-2	2-3
			Field-2	3	Red	Kesar	0.13	0.13	1.33	0.06		0	0
		Yandigeri	Field-1	2	Black	Sindhoor	0.14	0.28	0.14	0.57	**1.19**	0	0
			Field-2	2	Black	Sindhoor	0.36	0.54	0.90	1.81		0	1-2
2	Hunagund	Kamatagi	Field-1	2	Red	Kesar	0.18	0.25	0.12	0.55	**0.88**	0-3	0
			Field-2	2	Red	Kesar	0.44	0.22	0.55	1.21		0-3	0-3
		Kelloor	Field-1	5	Sandy	Kesar	2.45	4.09	3.27	9.81	**15.43**	0-4	0
			Field-2	4	Red	Sindhoor	2.22	2.22	16.6	21.04		0	2-4
		Shiroor	Field-1	5	Red	Sindhoor	2.22	2.22	16.6	21.04	**10.77**	0	0-3
			Field-2	5	Red	Sindhoor	0.20	0.30	0.00	0.5		0	0
3	Bilagi	Bilagi	Field-1	2	Sandy loam	Kesar	3.33	0.83	16.6	20.76	**14.66**	0	0
			Field-2	8	Red	Ruby	1.42	4.28	2.85	8.55		0	0
		Mannikeri	Field-1	2	Red	Kesar	0.13	0.13	0.00	0.26	**1.67**	0	0-2
			Field-2	3	Red	Kesar	1.66	1.25	0.16	3.07		3-4	0
		Sunaga	Field-1	3	Red	Kesar	1.66	1.25	0.16	3.07	**4.66**	0-4	0
			Field-2	2	Black	Kesar	1.25	0.83	4.16	6.24		1	0-2

Contd…

Sl. No	Taluk	Village		Age of the crop (years)	Soil type	Variety	Wilt incidence (%)					Root knot (Grade)	Shot hole borer (Grade)
							Yellowing	Partial wilt	Complete wilt	Total wilt	Average wilt		
4	Jamakhandi	Gani	Field-1	7	Sandy loam	Kesar	2.63	1.05	10.50	14.18	**1.82**	3-5	0
			Field-2	3	Red	Kesar	1.00	2.00	6.00	9.00		0-3	0
		Konnoor	Field-1	2	Black	Kesar	2.38	1.58	0.63	4.59	**3.40**	0-2	0-3
			Field-2	2	Black	Kesar	4.41	5.88	2.05	12.34		2-4	0-2
		Mareguddi	Field-1	3	Red	Kesar	0.18	0.37	1.88	2.43	**1.59**	0	0-3
			Field-2	3	Black	Kesar	0.18	0.28	0.28	0.74		0	0-3
5	Mudhol	Chikkur	Field-1	3	Red	Kesar	0.83	2.59	1.85	5.27	**5.29**	2-4	0
			Field-2	4	Red	Kesar	1.25	0.50	3.55	5.30		1-4	0-3
		Malapoor	Field-1	4	Red	Kesar	1.25	0.50	1.00	2.75	**2.55**	3-4	0-3
			Field-2	2	Red	Kesar	1.17	0.70	0.47	2.34		0	0
		Shirol	Field-1	2	Black	Kesar	0.33	0.83	1.11	2.27	**4.41**	0-3	0
			Field-2	2	Red	Kesar	1.11	1.66	3.77	6.54		0-3	0-3
6	Bagalkot	Govindakoppa	Field-1	6	Black	Bhagwa	2.22	7.77	11.11	21.10	**11.39**	3-5	0
			Field-2	4	Black	Bhagwa	0.21	0.42	1.05	1.68		0-3	0
		Kaladgi	Field-1	4	Black	Bhagwa	0.88	0.55	5.88	7.31	**10.94**	0-4	0
			Field-2	6	Black	Bhagwa	1.71	4.28	8.57	14.56		0	0
		Tulasigere	Field-1	3	Red	Ruby	0.25	3.75	3.75	7.75	**5.42**	0	0-3
			Field-2	5	Red	Bhagwa	0.45	0.36	2.27	3.08		0	0

Table 2. Status of wilt of pomegranate in Vijayapur district as recorded in survey during 2017-2018

Sl. No	Taluk	Village		Age of the crop (years)	Soil type	Variety	Wilt incidence (%)					Root knot (Grade)	Shot hole borer (Grade)
							Yellowing	Partial wilt	Complete wilt	Total wilt	Average wilt		
1	Indi	Atharga	Field-1	5	Black	Bhagwa	3.42	2.73	6.84	12.99	**6.97**	0	0
			Field-2	10	Black	Bhagwa Kesar	0.30	0.15	0.50	0.95		0-4	3
		Benakanalli	Field-1	3	Black	Bhagwa Kesar	0.20	0.57	0.20	1.14	**1.79**	0	0-3
			Field-2	4	Black	Bhagwa	0.00	1.11	1.33	2.44		0	0-5
		Tamba	Field-1	4	Black	Kesar	1.11	1.38	1.11	3.6	**3.70**	0	0
			Field-2	6	Red	Kesar	1.60	1.6	0.60	3.8		0-4	0-3
2	Vijayapur	Aliyabad	Field-1	5	Black	Kesar	1.33	1.66	2.00	4.99	**4.69**	3-4	0
			Field-2	4	Black	Kesar	0.80	1.60	2.00	4.40		2-3	0
		Halli	Field-1	5	Black	Bhagwa	0.20	0.75	1.50	2.45	**2.30**	3-4	0
			Field-2	5	Black	Kesar	0.30	0.69	1.15	2.14		2-4	0
		Jumanal	Field-1	3	Black	Kesar	0.88	0.55	5.88	7.31	**10.94**	2-5	0
			Field-2	4	Black	Kesar	1.71	4.28	8.57	14.56		0-4	0
3	Muddebihal	Basarkod	Field-1	3	Black	Kesar	0.00	0.00	0.10	0.10	**0.17**	0	0
			Field-2	3	Black	Kesar	0.07	0.05	0.12	0.24		0	0
		Hadalageri	Field-1	3	Red, Black	Kesar	0.99	2.13	7.10	10.22	**6.35**	0-4	0-3
			Field-2	3	Red	Kesar	0.80	1.6	0.08	2.48		0-4	0-2
		Rakkasgi	Field-1	6	Red	Kesar	1.60	1.6	0.60	3.80	**4.40**	0-4	0-3
			Field-2	3	Black	Bhagwa	1.33	1.66	2.00	4.99		0-4	0

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Sl. No	Taluk	Village		Age of the crop (years)	Soil type	Variety	Wilt incidence (%)					Root knot (Grade)	Shot hole borer (Grade)
							Yellowing	Partial wilt	Complete wilt	Total wilt	Average wilt		
4	Basavana bagewadi	Hunasyal	Field-1	4	Black	Kesar	0.99	2.13	7.10	10.22	**10.02**	0-4	0-3
			Field-2	2	Black	Kesar	0.55	0.36	8.90	9.81		0	0
		Managuli	Field-1	3	Black	Kesar	0.80	0.66	0.26	1.72	**10.04**	0	0-2
			Field-2	3	Black	Kesar	0.80	0.36	17.2	18.36		0-1	0
		Sankanal	Field-1	2	Black	Kesar	0.25	3.75	3.75	7.75	**5.47**	0-3	0
			Field-2	2	Black	Kesar	0.55	0.36	2.27	3.18		0	0
5	Sindagi	Bommanajol	Field-1	5	Black	Bhagwa	1.42	3.42	2.85	7.69	**12.60**	0	0-2
			Field-2	3	Red	Bhagwa	3.75	6.25	7.5	17.5		0-2	0-1
		Kannolli	Field-1	3	Black	Kesar	0.5	0.16	0.33	0.99	**1.07**	0	0-3
			Field-2	3	Black	Kesar	0.19	0.57	0.38	1.14		0	0-3
		Tanda	Field-1	4	Black	Kesar	0.90	1.81	10.9	13.61	**26.81**	0	0-4
			Field-2	5	Black	Kesar	14	10	16	40		0-5	0

Table 3. Status of wilt of pomegranate in Bagalkot and Vijayapur districts as recorded in survey during 2017-2018

Sl. No.	District	Taluk	Yellowing (%)	Partial wilt (%)	Complete wilt (%)	Total wilt (%)	Root knot (Grade)	Shot hole borer (Grade)
1	Bagalkot	Badami	15.45	1.94	2.71	19.91	0-3	0-4
2		Bagalkot	0.95	2.86	5.44	9.25	0-5	0-3
3		Bilagi	1.58	1.43	3.99	6.99	0-4	0-2
4		Hunagund	1.29	1.55	6.19	9.03	0-4	0-4
5		Jamakhandi	1.80	1.86	3.56	7.22	0-5	0-3
6		Mudhol	0.99	1.13	1.96	4.08	0-4	0-3
Mean			**3.68**	**1.80**	**3.98**	**8.53**		
1.	Vijayapur	Basavana bagewadi	0.66	1.27	6.58	8.51	0-4	0-3
2.		Indi	1.11	1.26	1.76	4.15	0-4	0-5
3.		Muddebihal	0.80	1.17	1.67	3.64	0-4	0-3
4.		Vijayapur	0.87	1.59	3.52	5.98	4-5	0
5.		Sindagi	3.46	3.70	6.33	13.49	0-5	0-4
Mean			**1.38**	**1.80**	**3.97**	**7.15**		

Neerbudihal village of Badami taluk (71.12%) followed by Kellor village of Hunagund taluk (15.43%). The least disease incidence was observed in Kamatagi village of Hunagund taluk followed by Yendigeri village (1.19%) of Badami taluk.

With respect to Vijayapur district, the results revealed that among five taluks surveyed (Table 3) maximum total per cent disease incidence was recorded in Sindagi (13.49%) followed by Basavana bagewadi (8.51%), Vijayapur (5.89%), Indi (4.15%), and Muddebihal (3.64%). The average per cent yellowing, partial wilting and complete wilt symptoms observed in Sindagi taluk were 3.46%, 3.70% and 6.33%; in Basavana bagewadi 0.66%, 1.27% and 6.58%; in Vijayapur taluk 0.87%, 1.59% and 3.52%; in Indi taluk 1.11%,1.26% and 1.76%; and in Muddebihal taluk 0.80%, 1.17% and 1.67% respectively. Among the villages of Vijayapur districts (Table 2), maximum disease incidence was noticed in Tanda village of Vijayapur district (26.8%) and lowest incidence was observed in Basarkod village of Vijayapur taluk (0.17%) followed by Kannolli of Sindagi taluk (1.07%).

Incidence of wilt complex of pomegranate was noticed in all the places surveyed**.** Among two districts, slightly higher average Per cent Disease Index (PDI) of wilt was noticed in Bagalkot district (8.53%) compared to Vijayapura district (7.15%). Among 6 taluks of Bagalkot maximum average wilt disease incidence was noticed in Badami taluk (19.91%) followed by Bagalkot taluk (9.25%). Among 5 taluks of Vijayapura districts surveyed, average wilt disease incidence was noticed maximum in Sindagi taluk (13.49%) followed by Basavana bagevadi taluk (8.51%). The least disease incidence was observed in Muddebihal taluk (3.64%) of Vijayapura district followed by Mudhol taluk (4.08%) of Bagalkot district. Among the pomegranate orchards maximum disease incidence was noticed in Neerbudihal village of Bagalkot district (71.12%) followed by Tanda village of Vijayapur district (26.8%). Lowest incidence was observed in Basarkod village of Vijayapur district (0.17%) followed by Kamatagi village of Bagalkot district (0.88%).

Higher wilt disease incidence was noticed in orchards more than 4-5 years old (11.15%) and while it was less in orchards below 1-2 years (3.48%). Root knot nematode and shot hole bore association was noticed in orchards more than 4-5 years.

The maximum incidence of wilt complex disease was observed in sandy loam (17.47%) followed by black soil (10.08%), sandy soil (9.81%) and red soil (5.85%). It was evident from the survey that among the commonly growing pomegranate cultivars (*viz.*, Bhagwa, Kesar, Ruby and Sindhur) Bhagwa was found to be highly susceptible to *C. fimbriata*, root knot nematode and shot hole borer.

4.1.1 Symptoms of the disease caused by *C. fimbriata* and *M. incognita*

Wilt complex of pomegranate results in complete wilting of plant and is characterized by the initial symptoms as yellowing and wilting of leaves on one to several branches. At times only one or two stems of the tree showed wilting and it took a few weeks to some months for the entire tree to completely wilt. Although yellowing of leaves normally produced acropetally, occasionally some plants revealed wilt symptoms all of a sudden by senescing the entire plant's foliage at once (Plate 1). Wilt infected plants often revealed dried foliage and fruits being attached to the branches for many months (Plate 2c). The xylem of the trunk turned brown to black with a star burst like pattern with blue strains on stem was noticed (Plate 2a & b). In many orchards diseased trees were observed dying in patches (Plate 1e), there by indicating the spread of the disease from an infected to an adjacent healthy tree. However, in some orchards wilt infections were spotted unevenly at different locations. Below ground, symptoms like the dark black to greyish colour mycelial mat was observed on the root portion (Plate 3a) with characteristic fruity odour and root knot nematode associated roots showed large galls or knots throughout the root system of infected plants (Plate 3b). The galls were white in colour turned to light brown and hardy when they became old. The intensity of root knot nematode damage increased with increase in age of the plant. In general, more than four to five year old plants were severely affected by root knot nematode. Egg masses were observed inside as well as outside of the galls. More number of females were found in a single compound gall. Severe infection resulted in dying of whole tree causing severe yield losses leading to death of affected plants in a few weeks.

a) Initial yellowing of tertiary branches

b) Yellowing of secondary branches

c. Partial wilting of plant

d. Complete wilting of plant

e. Over view of the field showing patchy appearance of wilt

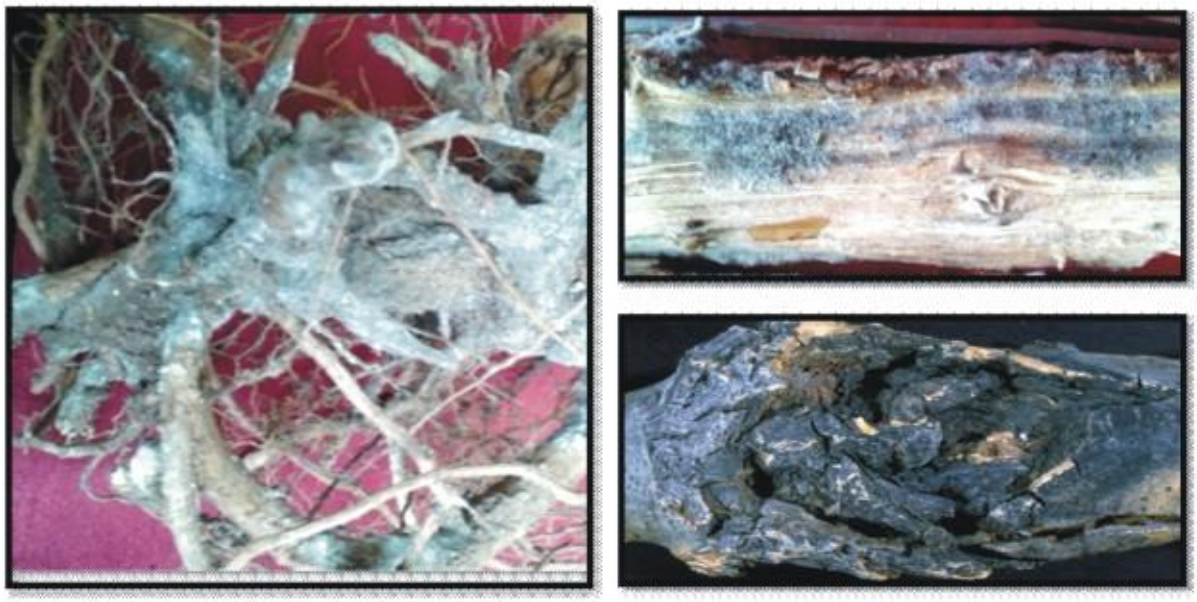
Plate 1: Different stages of wilt symptoms in pomegranate

a) Blue stains on twigs

b. Brown and black discoloration inside the stem

c. Dried fruits in wilt infected plants remain attached to twigs

Plate 2: Different symptoms of wilt in pomegranate



a) Mycelial growth of *C. fimbriata* on infected plant parts

b. Root knot infestation at different locations with varying severity

Plate 3: Below ground symptoms observed in wilt complex of pomegranate

4.1.2 Isolation of pathogens associated with disease complex in pomegranate

4.1.2.1 Isolation of *C. fimbriata* from wilt affected samples

Standard tissue isolation was followed to isolate *Ceratocystis fimbriata* culture from diseased sample as described in "Material and Methods". The pure culture was maintained on potato dextrose agar at 25 ± 1^{0}C. Sub culturing was done at every fortnight interval. The fungus isolated was confirmed as *C. fimbriata* based on their cultural and morphological characters (Plate 4).

The wilt fungus *C. fimbriata* grew well on potato dextrose agar and oat meal agar. The mycelium was whitish grey in the beginning which later changed into brown. As the growth progressed, production of endoconidia, aleurioconidia and perithecium was observed. Perithecia were black with a globose base. Ascospores exuded from the apex of the perithecium neck in a long coil which are small, hat shaped and hyaline. Conidiophores were septate and hyaline to dark greenish brown. Thick walled endoconidia were globose to oval, olive brown in colour. Aleurioconidia were thick walled, ellipsoidal or pyriform, golden brown in colour, borne singly or in chain.

4.1.2.2 Extraction of *M. incognita* from wilt affected samples

Soil samples collected from the field were brought to the laboratory. The known quantity (200 cc) of soil from each sample was processed by combined Cobb's Sieving and Baermann's funnel technique and nematode population was estimated by the method described in "Material and Methods". The nematode population was maintained on Pusa Ruby variety of tomato for further experiments.

The male root knot nematode has long, thin cylindrical shape of body with shorter and less robust stylet. The female was globose to saccate shape usually embedded in root tissues which are often swollen or galled. Body was soft, pearl white in colour. The paired gonads have extensive convoluted ovaries that fill the swollen body cavity. The posterior body having gelatinous matrix, which is excreted via the rectum to form an egg sac in which many eggs are deposited. The cuticle of the terminal region forms a characteristic perineal pattern.

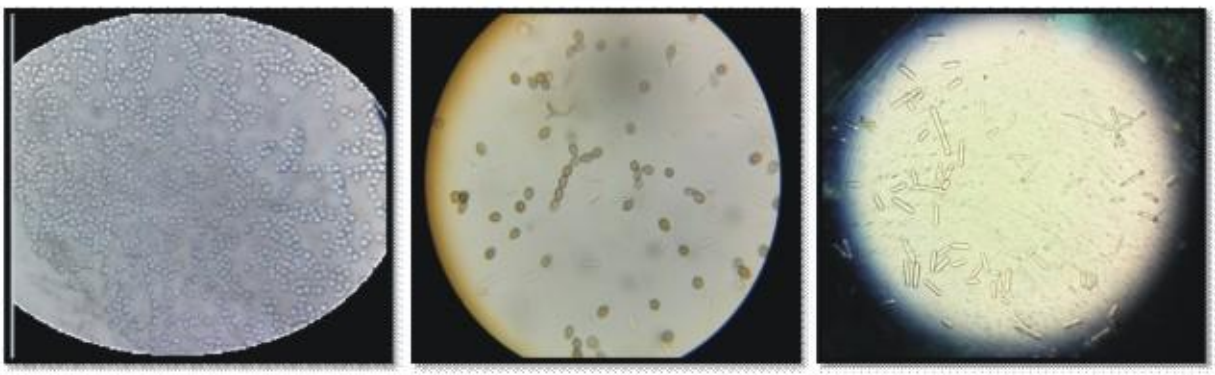
a) Isolation of *Ceratocystis fimbriata* from infected tissue using carrot bait

b) Isolation of *Ceratocystis fimbriata* from infected soil

c. long necked perithecia (40X)

c. long necked perithecia (40X)

d. Ascospores releasing from perithecia (40X)



e. Hat shape ascospores (40X)

f. Ellipsoidal aleurioconidia (40X)

g. Cylindrical endoconidia (X400)

Plate 4: Isolation and morphology of *Ceratocystis fimbriata*

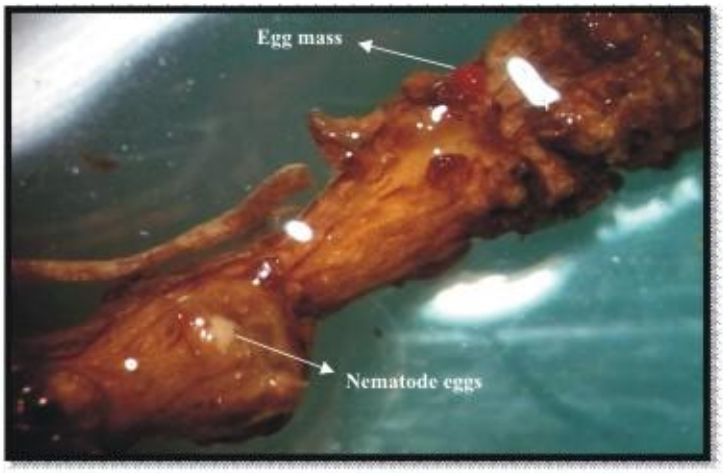
For morphological studies, perineal pattern is the most important morphological characters used for reliable species identification. The prevailing root knot nematode was identified as *M. incognita.* The root knot nematode species on pomegranate were characterized by the presence of high, squarish dorsal arch that often continued a distinct whorl in the tail terminal area. The striae were smooth to wavy. Sometimes, zig zagged and distinct lateral lines (Plate 5d).

4.1.3 Pathogenicity test for *C. fimbriata*

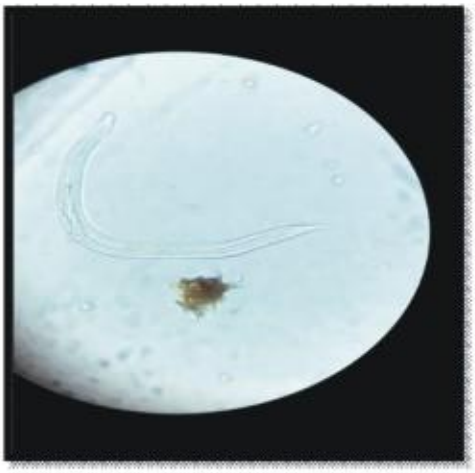
The pathogenicity of the isolated fungus was proved by inoculating the pure culture of *C. fimbriata* to six month old pomegranate *cv* Bhagwa raised in sterile soil along with a control plant without adding inoculum to sterile soil. The pathogen produced wilting symptoms after 45 days of inoculation. The plants started showing initially typical wilting symptoms such as yellowing of leaves in some twigs or branches, followed by drooping and drying of leaves. Brownish discoloration of vascular tissues was observed when affected root was split opened. Blue stains on stem were also noticed. The pathogen was reisolated from infected parts showing such symptoms and compared with original culture of *C. fimbriata,* thus confirmed the pathogenicity (Plate 6). The control plants which were not inoculated with the fungus did not show any symptoms of disease.

4.1.4 Pathogenicity test for *M. incognita*

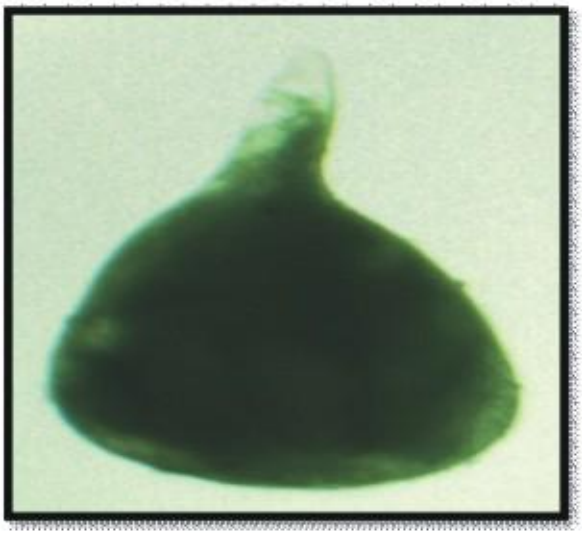
The pathogenicity of the isolated nematode was proved by inoculating the pure culture of *M. incognita* to six month old pomegranate *cv* Bhagwa raised in sterile soil along and 1000 J_2 stage root knot nematodes. The pathogen produced yellowing of foliage and stunting symptoms after 30 days of inoculation. Below ground symptom like root galls were observed (Plate 7). The pathogen was reisolated from infected roots and compared with original infestation of *Meloidogyne incognita,* thus confirmed the pathogenicity. The control plants which were not inoculated with the nematode did not show any symptoms of disease.



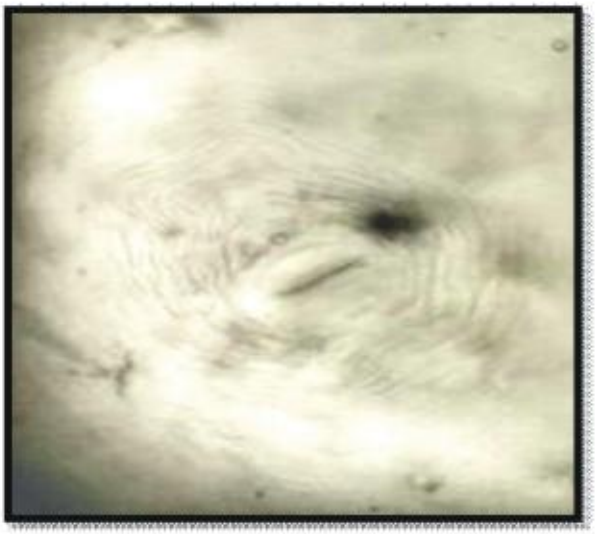
a. Root knot nematode eggs and egg mass on pomegranate root

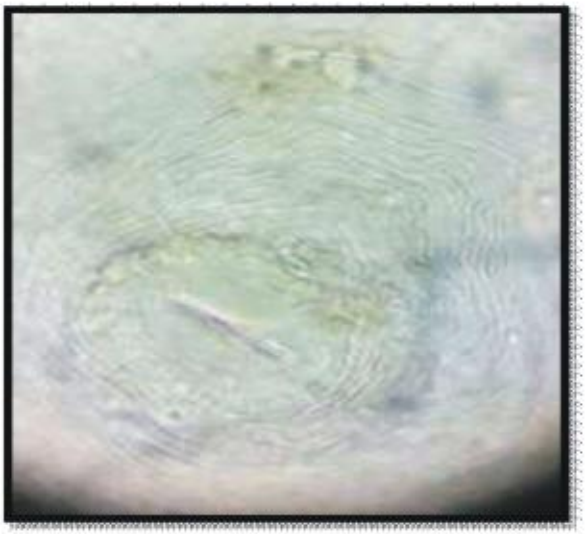


b. Male root knot nematode- *Meloidogyne incognita*



c. Female root knot nematode- *Meloidogyne incognita*





d. Perineal pattern of root knot nematode- *Meloidogyne incognita*

Plate 5: Morphology of *Meloidogyne incognita*

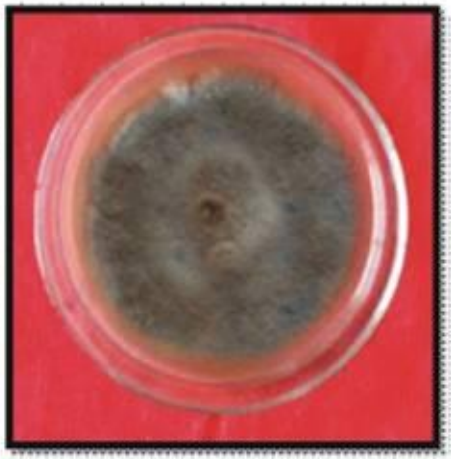
a) Untreated control

b) Yellowing of leaves and branches

c) Complete yellowing and defoliation

d) Complete wilting

Blue stain symptoms from infected twigs



Reisolation of pathogen form infected twigs

Plate 6: Pathogenicity test of *Ceratocystis fimbriata*

a) Root knot infected pomegranate plant

b) Multiplication and maintains of root knot nematodes in tomato plants

c) Tomato roots infected by *M. incognita*

d) Extraction of nematodes from Baermann funnel technique

e) Healthy plant

f) Root knot infected plant showing yellowing symptoms

g) Pomegranate roots infected by *M. incognita*

Plate 7: Pathogenicity test of *Meloidogyne incognita*

4.2 Interaction studies

4.2.1 Interaction of *Ceratocystis fimbriata* and *Meloidogyne incognita* in causing wilt of pomegranate

Disease complexes involving nematode and fungi have gained momentum in the recent years. An experiment was carried out during 2017 with six treatments under pot condition to know the interaction between *M. incognita* and wilt inducing fungi *C. fimbriata* on pomegranate. The treatments were laid out in CRD with 3 replications. Treatments were imposed by damaging tertiary roots. Data on percentage of branches infected (primary, secondary and tertiary) were recorded at 15 days interval and presented in Table 4 and Plate 8a, 8b and 8c.

Data obtained 15 days after inoculation revealed that there was significant difference among the treatments. Simultaneous inoculation of *C. fimbriata* and *M. incognita* (T5) at 15 days after inoculation showed 50.6% tertiary branches, 36.6% secondary branches and 45.6% leaves infection which is on par with inoculation of *C. fimbriata* 15 days prior to inoculation of *M. incognita* (T3) which recorded 29.3% tertiary branches, 24.4%, secondary branches and 6.2% leaves infection. These two treatments found significantly superior over all the treatments. In other treatments *viz.*, T1, T2, and T4 and T6 there were no such symptoms expressed.

Observations taken at 30 days after inoculation revealed that T5 showed 97.9% of tertiary branches infected and 100% of secondary branches infected and 79.3% leaves yellowing and dropping which was on par with T3 which recorded 66.1% of tertiary branches infected, 68.9% of secondary branches infected and 60.3% leaves with yellowing and dropping. These two treatments found significantly superior over all the treatments, followed by T1 (Inoculation of *C. fimbriata* alone) which recorded 29.3% tertiary branches, 36.7% secondary branches, 6.7% leaves infection.

Data obtained at 45 days revealed the significant increase in per cent branches infected and per cent leaves yellowing and dropped was observed in all the treatments except T4 and T6 (control). The wilt incidence at this stage was 100.0 per cent in secondary branches in both T5 and T3. In tertiary branches 100.0 and 87.0 per cent incidence was seen in T5 and T3 respectively. In T1 disease incidence was 49.4% in

Table 4. Development of wilt of pomegranate due to interaction of *Ceratocystis fimbriata* and *Meloidogyne incognita*

Treatment	Per cent branches infected														
	15 days after inoculation			30 days after inoculation			45 days after inoculation			60 days after inoculation			90 days after inoculation		
	II**	III***	Per cent leaves infected	II	III	Per cent leaves infected	II	III	Per cent leaves infected	II	III	Per cent leaves infected	II	III	Per cent leaves infected
T1	0.0 (0.0) *	0.0 (0.0)	0.0 (0.0)	36.7 (36.9)	29.3 (32.6)	6.7 (5.5)	50.0 (45.0)	49.4 (44.7)	15.5 (8.6)	50.0 (45.0)	67.7 (55.5)	53.6 (47.0)	65.0 (53.8)	77.7 (62.1)	82.7 (65.4)
T2	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	44.4 (41.7)	23.8 (29.2)	7.1 (5.8)	44.4 (41.7)	31.2 (33.7)	16.8 (9.0)	91.6 (80.0)	69.4 (56.6)	86.6 (68.5)	100.0 (90.0)	90.7 (75.8)	91.2 (72.7)
T3	24.4 (29.4)	29.3 (32.6)	6.2 (5.3)	68.9 (56.3)	66.1 (54.4)	60.3 (50.9)	100.0 (90.0)	87.0 (72.6)	75.2 (60.1)	100.0 (90.0)	100.0 (90.0)	94.4 (76.3)	100.0 (90.0)	100.0 (90.0)	98.3 (82.5)
T4	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	50.0 (45.0)	28.5 (32.2)	39.5 (38.9)	58.3 (50.0)	48.2 (43.9)	67.9 (55.5)
T5	36.6 (31.9)	50.6 (45.4)	45.6 (42.3)	100.0 (90.0)	97.9 (85.2)	79.3 (62.9)	100.0 (90.0)	100.0 (90.0)	99.3 (85.2)	100.0 (90.0)	100.0 (90.0)	99.3 (85.2)	100.0 (90.0)	100.0 (90.0)	99.3 (85.2)
T6	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
S. *Em*±	6.6	2.8	3.0	3.0	2.5	4.7	1.9	4.2	3.29	4.30	7.4	3.0	2.4	4.2	4.1
CD@1 %	20.7	8.7	9.3	9.4	7.9	14.5	5.9	13.0	10.2	13.4	23.0	9.3	7.4	13.0	12.7

*values in the parenthesis are arc sine transformed values

T1- Inoculation of *C. fimbriata* alone

T2- Inoculation of nematode alone

T3- Inoculation of *C. fimbriata* 15 days prior to inoculation of nematode

T4- Inoculation of nematode 15 days prior to inoculation of *C. fimbriata*

T5- Simultaneous inoculation of *C. fimbriata* and nematode

T6- Control

**Secondary branches

***Tertiary branches

a) Symptoms after 30 days of inoculation

b) Symptoms after 45 days of inoculation

c. Symptoms after 90 days of inoculation

R_1T_1- Inoculation of *C. fimbriata* alone
R_1T_2- Inoculation of nematode alone
R_1T_3- Inoculation of *C. fimbriata* 15 days prior to inoculation of nematode
R_1T_4- Inoculation of nematode 15 days prior to inoculation of *C. fimbriata*
R_1T_5- Simultaneous inoculation of *C. fimbriata* and nematode
R_1T_6- Control

Plate 8a: Successive development of wilt due to interaction of *Ceratocystis fimbriata* and *M. incognita* in pomegranate

Plate 8b: Effect of interaction of *C. fimbriata* and *M. incognita* in causing wilt of pomegranate on root and shoot parameters

R_4T_1- Inoculation of *C. fimbriata* alone
R_4T_3- Inoculation of *C. fimbriata* 15 days prior to inoculation of nematode
R_4T_5- Simultaneous inoculation of *C. fimbriata* and nematode
R_4T_2- Inoculation of nematode alone
R_4T_4- Inoculation of nematode 15 days prior to inoculation of *C. fimbriata*
R_4T_6- Control

tertiary branches and 50.00% in secondary branches. In T2, 31.2% wilt incidence in tertiary branches and 44.4% incidence in secondary branches was noticed. There was no symptom expression in T4 and T6 at 45 days of inoculation.

At 90 days after inoculation there was significant difference between the treatments. The treatment T5, T3 and T2 recorded 100% infection of secondary branches which were followed by T1 (65.0%) and T4 (58.3%). With respect to tertiary branches T5 and T2 showed 100% incidence followed by T2 (90.7%), T1 (77.7%) and T4 (48.2%). With respect to leaves T5 showed 99.3% yellowing and dropping symptoms fallowed by T3 (98.3%), T2 (91.2%), T1 (82.7%) and T4 (67.9%).

The affected shoots showing blue stain symptoms and roots showing brownish discolouration were observed in T1, T3, T4 and in T5 treatments. Root galls produced by root knot nematode was observed in T2, T3, T4 and T5. There was no blue stain, brownish discolouration and root galls in T6 (uninoculated control).

Data on root and shoot parameters due to interaction is presented in Table 5. Table 5 revealed that there is significant difference in treatments with respect to in fresh shoot weight, dry shoot weight, fresh root weight, dry root weight, shoot length and root length. All these parameters were found reduced in comparison to uninoculated control.

Among the treatments the number of root knots were counted in each plant in which T2 and T5 contain more than 50 galls per plant (Grade 4) and in T4; 25 to 30 galls per plant (Grade 3) were observed. There were no root galls in untreated T1 (Inoculation of *C. fimbriata* alone) and T6 (Control).

Data showed that the plants with simultaneous inoculation of *C. fimbriata* and *M. incognita* (T5) adversely affected plant growth parameters like fresh and dry weight of shoot, fresh and dry weight of root, shoot and root length per plant (Plate 8b).

The plants with simultaneous inoculation of *C. fimbriata* and *M. incognita* (T5) recorded least fresh shoot weight (28.13g), followed by T3 (34.73g) which is on par with T4 (35.86 g) and T2 (38.43). The highest fresh shoot weight (59.80 g) was

Table 5. Effect of interaction of *Ceratocystis fimbriata* and *Meloidogyne incognita* on growth parameters of pomegranate

Treatments	Root gall index (grade)	Fresh weight of shoots (g)	Dry weight of shoots (g)	Fresh weight of roots (g)	Dry weight of roots (g)	Root length (cm)	Shoot length (cm)
T1	0	51.70	24.46	27.96	16.93	9.46	91.33
T2	4	38.43	24.60	36.93	23.86	8.13	83.00
T3	4	34.73	20.53	23.80	14.43	7.90	87.33
T4	3	35.86	20.63	24.36	16.00	8.6	86.66
T5	4	28.13	17.30	23.33	12.50	7.16	65.00
T6	0	59.80	35.20	41.90	14.40	12.00	97.00
S. *Em*±		2.53	2.73	1.42	1.02	0.24	4.15
CD@1%		7.90	8.50	4.42	3.18	0.76	12.93

T1- Inoculation of *C. fimbriata* alone
T2- Inoculation of nematode alone
T3- Inoculation of *C. fimbriata* 15 days prior to inoculation of nematode
T4- Inoculation of nematode 15 days prior to inoculation of *C. fimbriata*
T5- Simultaneous inoculation of *C. fimbriata* and nematode
T6- Control

observed in T6 fallowed by T1 (51.70 g). There was maximum reduction in dry shoot weight found in T5 (17.30 g) followed by T3 (24.60 g), T4 (20.63 g), T1 (24.46 g), and T2 (24.60 g) compared to T6 (35.20 g).

The least fresh and dry weight of root was observed as 23.33 g and 12.50 g in T5, followed by T3 (23.80 g and 14.43 g). The maximum fresh weight and dry weight of root was observed in T6 (41.90 g and 14.40 g) which is on par with T2 (36.93g and 23.86 g).

The data on root length and shoot length revealed that there is significant difference in treatments. Among six treatments the least root length and shoot length was observed in T5 (12.50 cm and 7.16 cm) fallowed by T3 (7.90 cm and 87.33 cm) which is on par with T4 (8.6 cm and 86.66 cm) and T2 (8.13cm and 83.00 cm). The highest root length and root length was observed in control plant (12.00 cm and 97.00 cm).

4.3 Evaluation of fungicides, bioagents, nematicides and oil cakes against *C. fimbriata* and *M. incognita in vitro*

4.3.1 *In vitro* evaluation of non systemic fungicides against *C. fimbriata*

Six non systemic fungicides were tested at three different concentrations in the laboratory against the *C. fimbriata*. The result pertaining to the study are presented in the Table 6, Fig. 1b and Plate 9.

There was a significant difference between the non systemic fungicides with respect to per cent inhibition of mycelial growth. Mancozeb 63% + Carbendazim 12% was found most effective and significantly superior over all other treatments which inhibited 100 per cent growth of the fungus at all concentrations tested. The next best treatment was mancozeb and captan which inhibited 96.33 and 95.45 per cent growth of the fungus respectively. However, there was least inhibition in chlorothalonil (31.18%) and copper oxy chloride (52.27%) at all concentrations.

Among the concentrations, 0.1 per cent (84.35) was found significantly superior over 0.05 (74.54) and 0.025 (56.13) per cent concentration in inhibition of growth of the fungus. Interaction studies showed that mancozeb 63% + carbendazim

Table 6. *In vitro* evaluation of non systemic fungicides against the mycelial growth of *Ceratocystis fimbriata* through poison food technique

Sl. No.	Fungicide	Per cent inhibition of mycelial growth (mm)			Mean
		Concentration			
		0.025%	0.05%	0.10%	
1	Captan	94.73 (76.77) *	95.75 (78.18)	98.52 (83.07)	96.33 (79.34)
2	Chlorothalonil	21.59 (27.64)	28.78 (32.45)	43.18 (40.92)	31.18 (33.67)
3	Copper oxy chloride	18.18 (25.16)	60.22 (50.95)	78.40 (62.40)	52.27 (46.17)
4	Mancozeb	86.36 (68.44)	100.00 (90.05)	100.00 (90.05)	95.45 (82.85)
5	Mancozeb 63%+ Carbendazim 12%	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
6	Propineb	15.91 (23.49)	62.50 (52.49)	85.98 (71.84)	54.79 (49.28)
Mean		56.13 (51.92)	74.54 (65.70)	84.35 (73.06)	71.67 (62.47)
Source				S. *Em*±	CD@1%
Fungicides (F)				1.65	4.75
Concentration (C)				1.17	3.36
Fungicide x Concentration				2.85	8.22

*****Values in parenthesis are arc sine transformed values

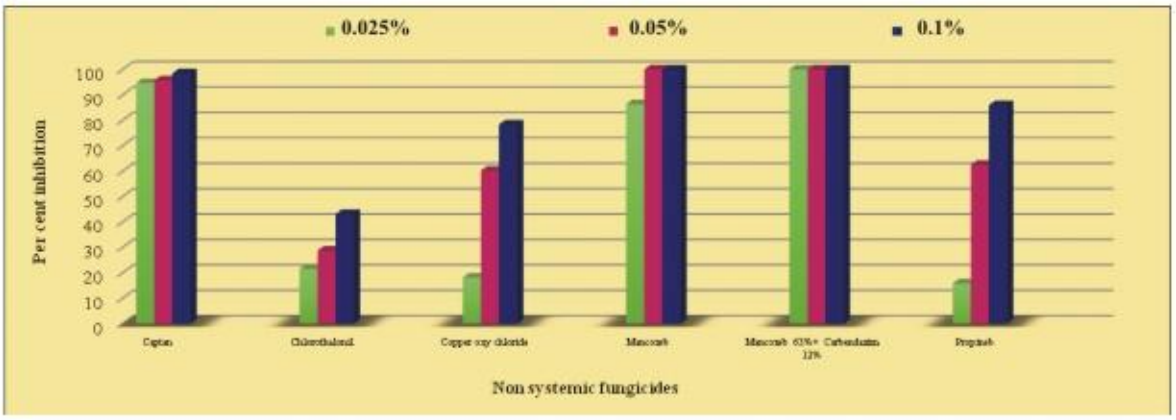


Fig. 1: Per cent inhibition of the mycelial growth of *Ceratocystis fimbriata* by non systemic fungicides

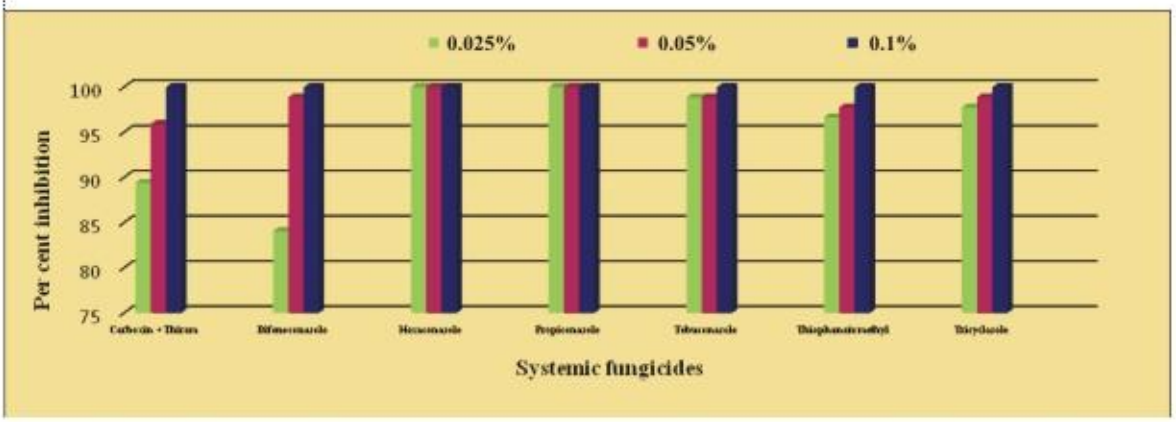


Fig. 2: Per cent inhibition of the mycelial growth of *Ceratocystis fimbriata* by systemic fungicides

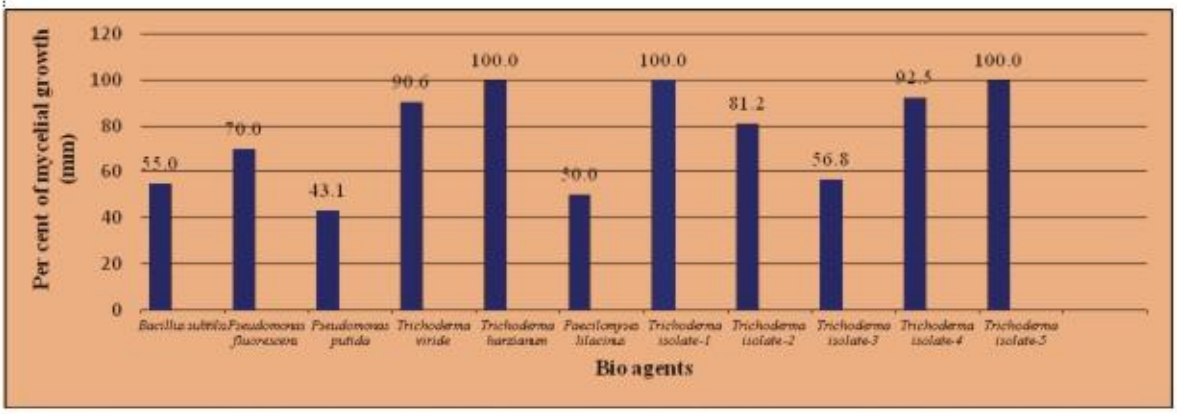


Fig. 3: Per cent inhibition of the mycelial growth of *Ceratocystis fimbriata* by different bioagents

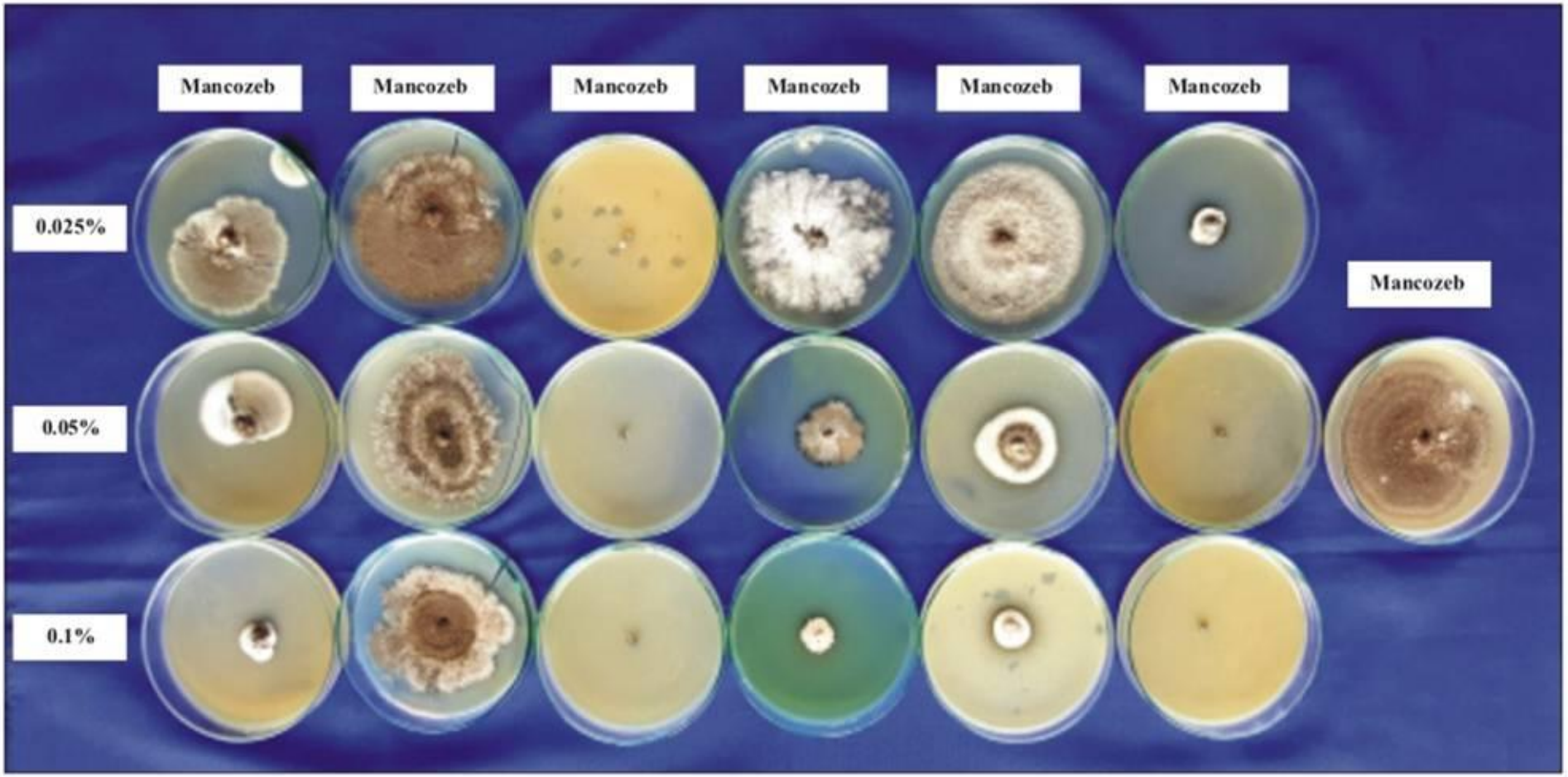


Plate 9: Inhibition of mycelial growth of *Ceratocystis fimbriata* by non systemic fungicides

12% inhibited the mycelial growth completely (100 per cent) at 0.025% and above concentration. Mancozeb inhibited the mycelia growth completely at 0.05 and 0.1 per cent whereas at 0.025 per cent there was 86.36 per cent inhibition. Captan inhibited the mycelial growth of fungus by 94.73, 95.75 and 98.52 per cent at 0.025, 0.05 and 0.1 per cent concentrations. Least per cent inhibition was found in chlorothalonil (21.59, 28.78, 43.18), copper oxy chloride (18.18, 60.22, 72.40) and propineb (15.91, 62.50, 85.98) at 0.025, 0.05 and 0.1% concentrations respectively.

4.3.2 *In vitro* evaluation of systemic fungicides against *C. fimbriata*

Seven systemic fungicides were tested against *C. fimbriata* by poisoned food technique at three concentrations in the laboratory for their efficacy against *C. fimbriata* as described in "Material and Methods". The results are presented in the Table 7, Fig. 2 and Plate 10.

The mycelial growth of the fungus was inhibited 100 per cent by hexaconazole and propiconazole at 0.025%, 0.05% and 0.1% concentrations. While all other systemic fungicides inhibited the mycelial growth completely at 0.1 per cent concentration. Least inhibition was found in difenoconazole (66.52 per cent) at 0.025 concentration.

4.3.3 *In vitro* evaluation of bioagents against *C. fimbriata* through dual culture technique

The competitive ability of antagonists against *C. fimbriata* was studied by dual culture method as described in "Material and Methods". The results obtained are presented in Table 8.

There was a significant difference between the bioagents tested with respect to per cent inhibition of mycelial growth of *C. fimbriata.* Among the bio agents tested, *Trichoderma harzianum, Trichoderma* isolate 1 *and Trichoderma* isolate 5 recorded the maximum per cent inhibition of mycelial growth (100%). It was found significantly superior to the rest of the bioagents tested. This was followed by *Trichoderma* isolate 4, *Trichoderma virens, Trichoderma* isolate 2 and *Pseudomonas fluorescens*. Whereas, the minimum parasitic activity was noticed in case of

Table 7. *In vitro* evaluation of systemic fungicides against the mycelial growth of *Ceratocystis fimbriata* through poison food technique

Sl. No.	Fungicide	Per cent inhibition of mycelial growth (mm)			Mean
		Concentration			
		0.025%	0.05%	0.10%	
1.	Carboxin +Thiram	89.40 (71.04) *	95.92 (78.47)	100.00 (90.05)	95.10 (79.85)
2.	Difenoconazole	84.07 (66.52)	98.88 (83.97)	100.00 (90.05)	94.31 (80.18)
3.	Hexaconazole	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
4.	Propiconazole	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
5.	Tebuconazole	98.88 (83.97)	98.88 (83.97)	100.00 (90.05)	99.25 (86.00)
6.	Thiophanate methyl	96.66 (79.51)	97.77 (81.45)	100.00 (90.05)	98.14 (83.67)
7.	Tricyclazole	97.77 (81.45)	98.88 (83.97)	100.00 (90.05)	98.88 (85.16)
Mean		95.26 (80.37)	98.62 (84.56)	100.00 (90.05)	
Source				S. *Em*±	CD @ 1%
Fungicides (F)				0.15	0.43
Concentration (C)				0.10	0.28
Fungicide x Concentration				0.26	0.74

*****Values in parenthesis are arc sine transformed values

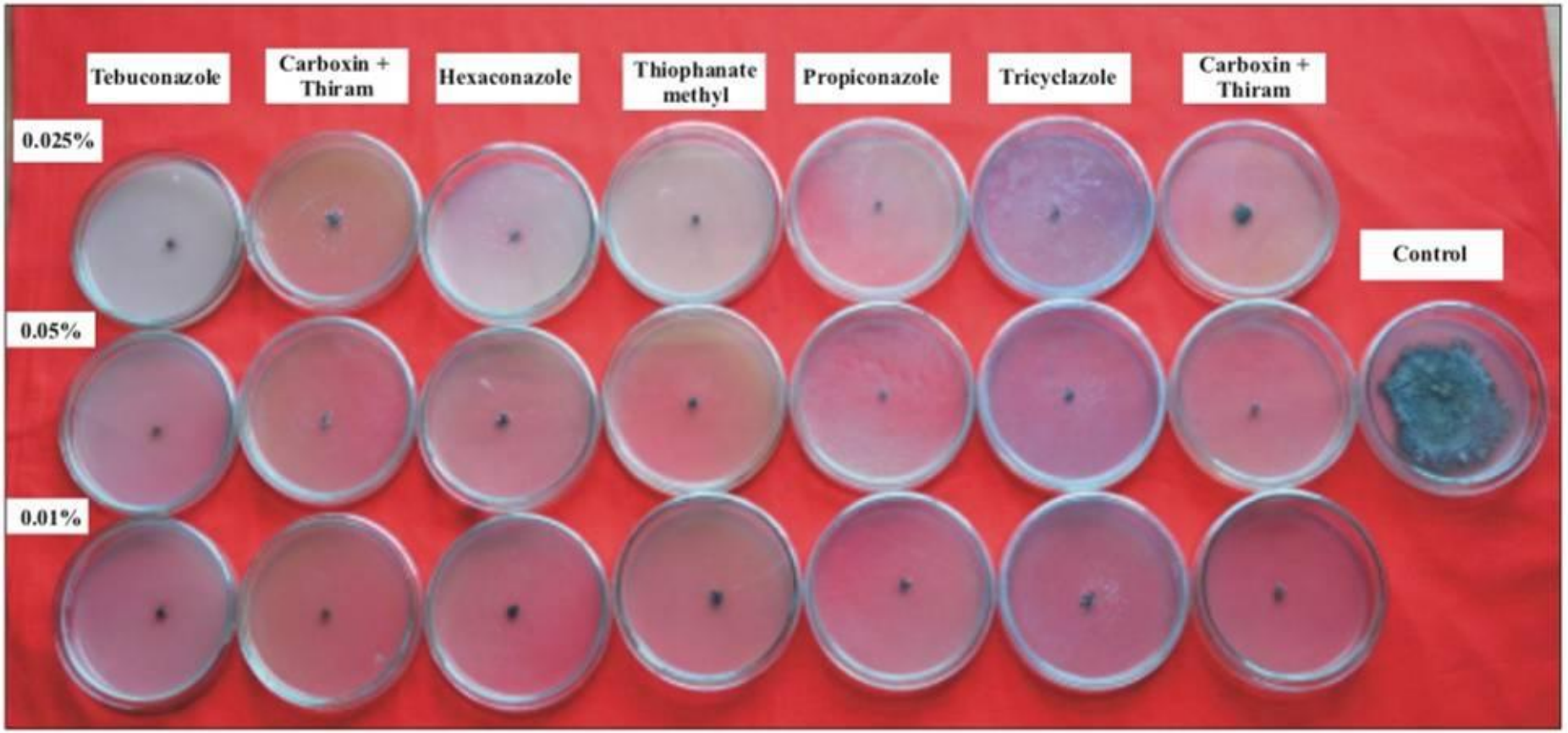


Plate 10: Inhibition of mycelial growth of *Ceratocystis fimbriata* by systemic fungicides

Table 8. *In vitro* evaluation of bioagents against *Ceratocystis fimbriata* through dual culture technique

Sl. No.	Bioagent	Percent inhibition of mycelial growth (mm)
1.	*Bacillus subtilis*	55.00 (47.85) *
2.	*Pseudomonas fluorescens*	70.00 (56.76)
3.	*Pseudomonas putida*	43.12 (41.00)
4.	*Trichoderma virens*	90.62 (72.30)
5.	*Trichoderma harzianum*	100.00 (90.00)
6.	*Paecilomyces lilacinus*	50.00 (44.98)
7.	*Trichoderma isolate* -1	100.00 (90.00)
8.	*Trichoderma isolate* -2	81.25 (64.31)
9.	*Trichoderma isolate*-3	56.87 (48.94)
10.	*Trichoderma isolate*-4	92.50 (77.08)
11.	*Trichoderma isolate*-5	100.00 (90.00)
S. *Em*±		2.50
CD @ 1%		7.34

*****Values in parenthesis are arc sine transformed values

Plate 11: Inhibition of mycelial growth of *Ceratocystis fimbriata* by different bio agents

Pseudomonas putida (43.12%), *Paecilomyces lilacinus* (44.98%), *Trichoderma* isolate 3 (48.94%) and *Bacillus subtilis* (55.00%).

4.3.4 *In vitro* evaluation of nematicides against *M. incognita*

Four nematicides were evaluated against number of juveniles emerged at 24, 48 and 72 hours interval against *M. incognita* in laboratory condition for their efficacy against the pathogen as described in "Material and Methods". The results are presented in the Table 9, Fig. 4

Among the nematicides phorate (0.01 g/ml) was found effective as it recorded significantly least numbers over all other nematicides with respect to number of juveniles emerged (1.56) followed by carbosulfan (11.22), carbofuran (11.33). Least inhibiting capacity was found in cartap hydrochloride (17.22) over the control.

The number of juveniles emerged increased steadily as number of hours increased. However, the increase was very less in phorate @ 0.01%/ml (1.33, 1.67 and 1.63) for juveniles emerged. This was followed by carbosulfan @ 0.2 µl/ml (4.33, 14.33 and 15.00). The control recorded (10.67, 15.67 and 25.67) for juveniles emerged.

4.3.5 *In vitro* evaluation of oil cakes against *M. incognita*

Five oil cakes were tested against number of juveniles emerged at 24, 48 and 72 hours interval against *M. incognita* in laboratory condition for their efficacy against the pathogen as described in "Material and Methods". The results are presented in the Table 10, Fig. 5

Among the oil cakes neem cake was found significantly superior over all other nematicides as it recorded least number of juveniles emerged (2.45) followed by pongamia cake (7.33), mustard cake (12.33), and jatropha cake (18.45) while the control recorded 26.00.

The number of juveniles emerged increased steadily as number of hours increased. However, the increase was very less in neem cake @ 0.01%/ml (0.00, 1.67 and 5.97) followed by pongamia cake @ 0.2 µl/ml (6.00, 7.67 and 8.33) for juveniles emerged. The control recorded (17.00, 27.00 and 34.00) for juveniles emerged.

Table 9. *In vitro* evaluation of nematicides against *Meloidogyne incognita*

Sl. No.	Nematicide	Number of juveniles emerged			
		@24 hours	**@48 hours**	**@72 hours**	**Mean**
1	Carbofuran (0.02 g/ml)	4.00 (2.23)	7.33 (2.89)	22.67 (4.86)	11.33 (3.33)
2	Carbosulfan (0.2 µl/ml)	4.33 (2.31)	14.33 (3.91)	15.00 (3.99)	11.22 (3.40)
3	Cartap hydrochloride (0.1 mg/ml)	10.33 (3.37)	16.67 (4.20)	24.67 (5.07)	17.22 (4.21)
4	Phorate (0.01 g/ml)	1.33 (1.52)	1.67 (1.63)	1.67 (1.63)	1.56 (1.59)
5	Control	10.67 (3.42)	15.67 (4.08)	25.67 (5.16)	17.33 (4.22)
Mean		6.13 (2.57)	11.13 (3.34)	17.93 (4.14)	
Source		S. *Em*±		CD@1%	
Nematicide		0.07		0.20	
Hour		0.05		0.15	
Nematicide X Hour		0.12		0.34	

* Values in parenthesis are √X+1 transformed values

a) Effect of one week prior application of nematicides on development of symptoms on pomegranate seedlings caused by root knot nematodes

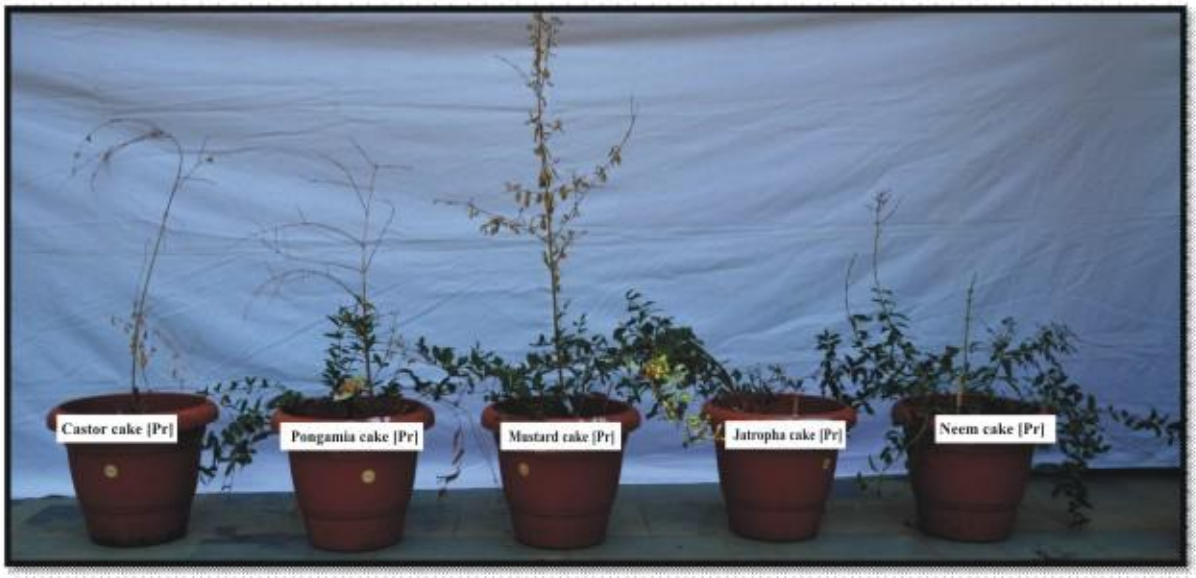
b) Effect of one week later application of nematicides on development of symptoms on pomegranate seedlings caused by root knot nematodes

Plate 12. Effect of nematicides on disease and plant growth parameter of pomegranate

Table 10. ***In vitro*** **evaluation of organic oil cakes against** ***Meloidogyne incognita***

Sl. No.	Nematicide	Number of juveniles emerged			
		@24 hours	@48 hours	@72 hours	Mean
1	Castor cake (0.1 g/ml)	10.33 (3.37)	14.00 (3.87)	23.00 (4.89)	15.78 (4.04)
2	Jatropha cake (0.1 g/ml))	5.67 (2.55)	20.67 (4.59)	29.00 (5.47)	18.45 (4.20)
3	Mustard cake (0.1 g/ml)	7.00 (2.83)	12.33 (3.64)	17.67 (4.32)	12.33 (3.60)
4	Neem cake (0.1 g/ml)	0.00 (1.00)	1.67 (1.48)	5.67 (2.58	2.45 (1.24)
5	Pongamia cake (0.1 g/ml)	6.00 (2.64)	7.67 (2.94)	8.33 (3.05)	7.33 (2.88)
6	Control	17.00 (4.24)	27.00 (5.29)	34.00 (5.92)	26.00 (5.15)
Mean		7.67 (2.77)	13.89 (3.64)	19.61 (4.37)	
Source		S. *Em*±		CD@1%	
Organic amendment		0.12		0.36	
Time		0.09		0.25	
Organic amendment X Hour		0.22		0.62	

*Values in parenthesis are √X+1 transformed values



a) Effect of one week prior application of oil cakes on development of symptoms on pomegranate seedlings caused by root knot nematodes

b) Effect of one week later application of oil cakes on development of symptoms on pomegranate seedlings caused by root knot nematodes

Plate 13. Effect of oil cakes on disease and plant growth parameter of pomegranate

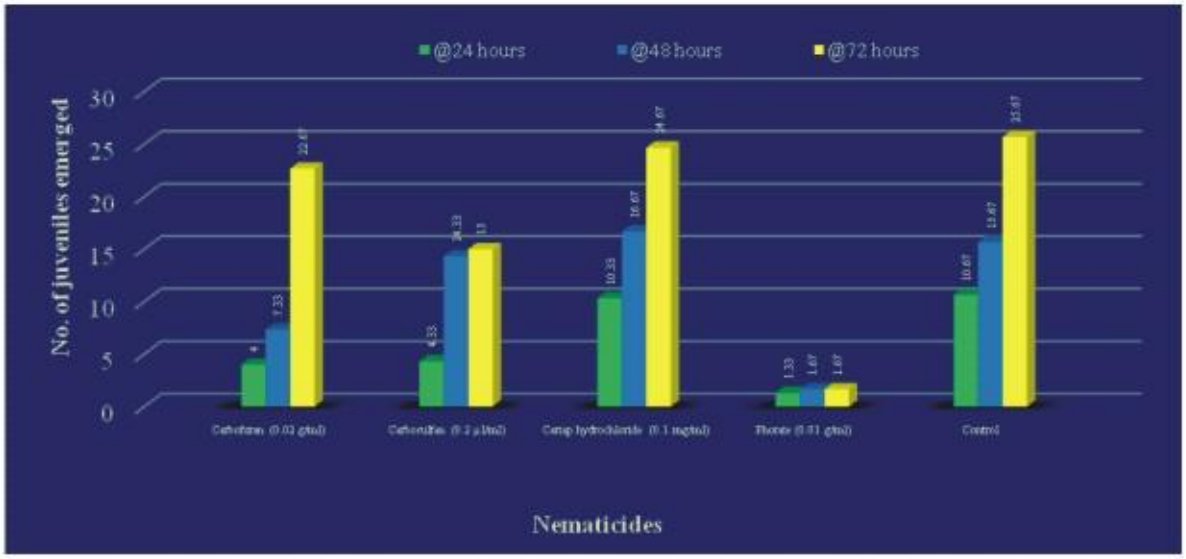


Fig. 4: Effect of nematicides on number of juveniles emerged

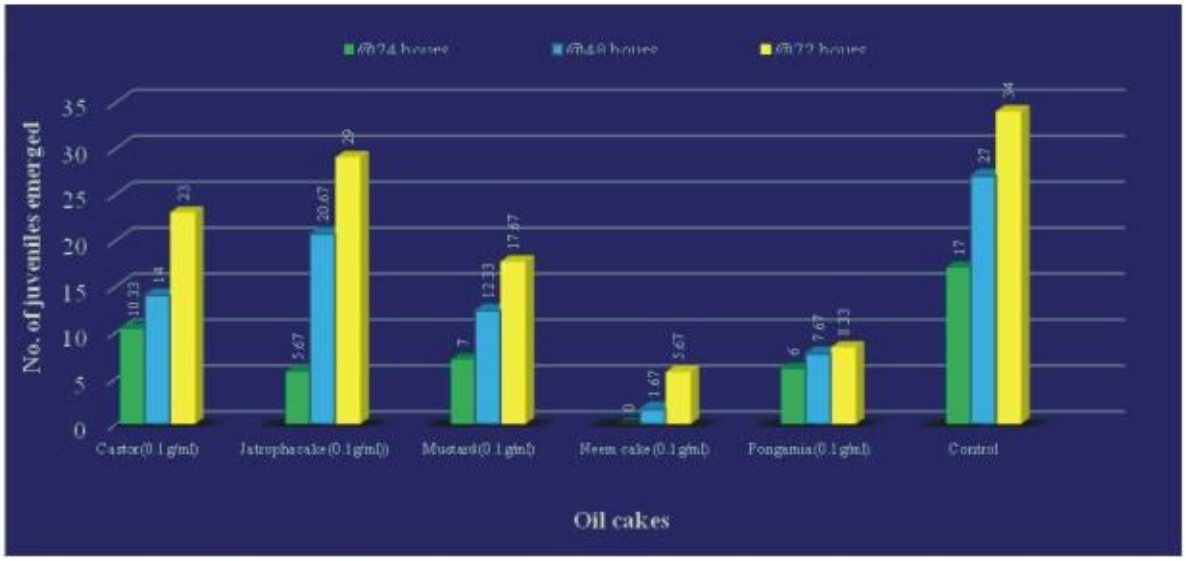


Fig. 5: Effect of oil cakes on number of juveniles emerge

4.3.6 Efficacy of nematicides and oil cakes against *M. incognita* under pot culture

Four nematicides and five Oil cakes were tested against *M. incognita* before and after inoculation of pathogen in pots to check the effect on plant growth parameters like fresh shoot weight, dry shoot weight, fresh root weight, dry root weight, shoot length, root length and disease parameters like yellowing of leaves and number of galls (grade) in all the treatments in comparison to uninoculated control. Data presented in Table 11, Plate 14a and 14b.

Among the treatments, neem cake and phorate applied plants prior and after inoculation of *M. incognita* showed higher fresh shoot weight, dry shoot weight, fresh root weight, dry root weight, shoot length, root length and less number of galls.

Application of phorate @ 9 g/pot and neem cake @ 50 g/pot before the inoculation of *M. incognita* did not recorded any galls. Treatment of phorate one week prior to inoculation of *M. incognita* plants recorded maximum number of fresh shoot weight of (103.0 g), dry shoot weight (71.6 g), fresh root weight (30.0 g), dry root weight (19.1 g), shoot length (95.0 cm) and root length (9.5 cm) found compared to other treatment. Treatment of neem cake one week after inoculation of *M. incognita* plants with maximum number of fresh shoot weight of (98.0 g), dry shoot weight (61.4 g), fresh root weight (32.0 g), dry root weight (23.5 g), shoot length (99.0 cm) and root length (9.5 cm). In both the treatments plants were healthy.

Treatment of neem cake one week prior to inoculation of *M. incognita* plants had high fresh shoot weight of (98.6 g), dry shoot weight (68.7 g), fresh root weight (29.0 g), dry root weight (18.5 g), shoot length (92.0 cm), root length (9.5 cm) and no galls found compared to control. And treatment of neem cake one week after inoculation of *M. incognita* plants also had high fresh shoot weight of (102.0 g), dry shoot weight (68.0 g), fresh root weight (28.0 g), dry root weight (21.1 g), shoot length (96.0 cm), root length (9.8 cm) and there are no galls formed compared to control. In both the treatments plants were healthy. There is high shoot and root weight was observed in castor cake treated plants. But the efficacy of castor cake was low compares to neem cake and phorate in case of nematode management.

Table 11. Evaluation of nematicides and oil cakes against *Meloidogyne incognita* under pot condition

Sl. No.	*Treatment		Prior application of nematicides and oil cakes								Later application of nematicides and oil cakes							
			Disease parameters		Plant growth parameters						Disease parameters		Plant growth parameters					
			Root knot index	Yellowing and wilting symptoms	Fresh weight of shoots (g)	Dry weight of shoots (g)	Fresh weight of roots (g)	Dry weight of roots (g)	Shoot length (cm)	Root length (cm)	Root knot index	Yellowing and wilting symptoms	Fresh weight of shoots (g)	Dry weight of shoots (g)	Fresh weight of roots (g)	Dry weight of roots (g)	Shoot length (cm)	Root length (cm)
1	Nematicides	Carbofuran (10 g/pot)	3	_	80.0	58.7	25.0	16.6	96.0	7.0	5	-	73.0	44.0	22.0	23.2	78.0	7.8
2		Carbosulfan (15 g/pot)	1	+	34.0	24.4	27.0	15.4	66.0	7.2	1	-	62.0	38.30	28.0	19.8	76.0	9.0
3		Cartap hydrochloride (10 g/pot)	2	+	28.5	15.2	10.0	10.6	73.0	8.3	1	+	21.0	17.1	24.0	10.0	87.0	8.5
4		Phorate (9 g/pot)	0	_	103.0	71.6	30.0	19.1	95.0	9.5	0	-	98.0	61.4	32.0	23.5	99.0	9.5
5	Organic oil cakes	Castor cake (50 g/pot)	2	+	77.0	26.3	14.0	6.0	122.0	8.0	1	-	104.0	45.0	42.0	38.7	140.0	7.9
6		Jatropha cake (50 g/pot)	4	_	50.0	29.1	23.0	13.1	97.0	7.9	3	+	32.0	19.1	12.0	19.8	75.0	8.3
7		Mustard cake (50 g/pot)	2	+	70.0	48.5	19.0	10.5	120.0	7.	1	+	93.0	61.3	25.0	19.2	90.0	8.0
8		Neem cake (50 g/pot)	0	_	98.6	68.7	29.0	18.5	92.0	9.5	0	-	102.0	68.0	28.0	21.1	96.0	9.8
9		Pongamia cake (50 g/pot)	2	_	70.0	37.6	14.0	17.1	60.0	8.7	2	+	52.0	27.7	16.0	11.3	92.0	8.7
10		Untreated control	4	+	26.5	13.3	12.5	7.16	65.0	5.2	4	+	26.5	13.3	12.5	7.1	65.0	5.2

*Each pot contains 20kg sterilized soil.

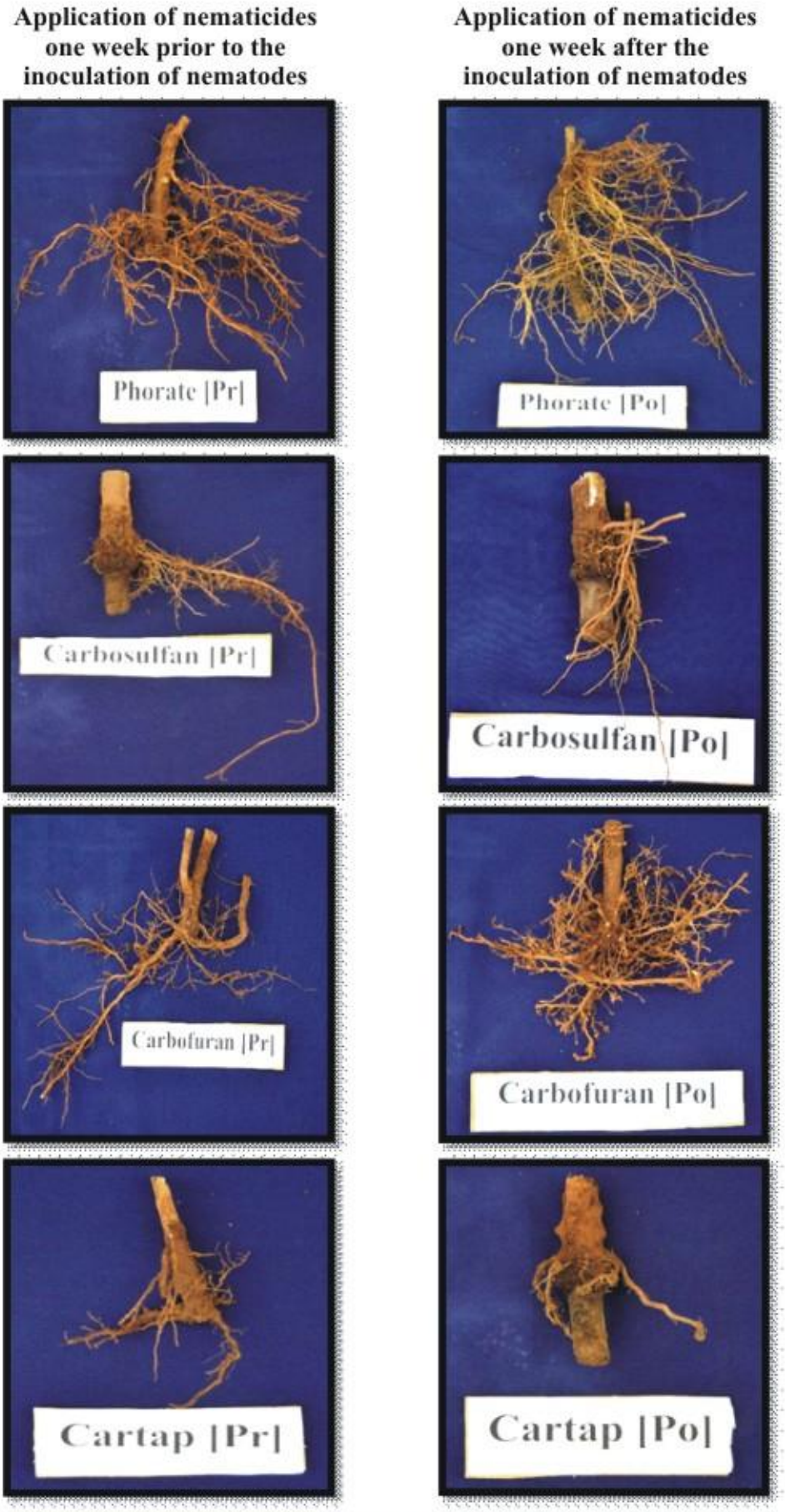


Plate 14: Effect of nematicides on gall index and root growth parameter of pomegranate

4.3.7 Evaluation of fungicides, bioagents, oil cakes and nematicides against wilt by simultaneous inoculation of *C. fimbriata* and *M. incognita*

4.3.7.1 Prior application of fungicides, bioagents, oil cakes and nematicides

Sixteen Fungicides, bioagents, oil cakes and nematicides were applied prior to the simultaneous inoculation of *Ceratocystis fimbriata* and *Meloidogyne incognita* under pot culture and evaluated against wilt. The results are presented in Table 12. There were no wilting symptoms up to 30 days. After 45th day gradual yellowing and wilting symptoms were noticed in difenoconazole, thiophanate methyl, mancozeb, pongamia cake and *P. fluorescence* treated plants. There was a steady increase in wilt symptoms at 60 in both secondary and tertiary branches in these treatments.

At 60 days after drenching maximum wilt incidence was seen in mancozeb treated plants which recorded 61.1% in secondary branches and 51.5% wilt incidence in tertiary branches and was on par with thiophanate methyl (30.6 and 51.3% in secondary branches and tertiary branches respectively). *T. virens* (30.6 and 50.9%), *P. fluorescence* (30.6 and 48.6%), pongamia cake (38.9 and 32.8), difenoconazole (26.1 and 32.0%), carbosulfan (6.7 and 11.1%), Phorate (4.8% and 6.7%) and Neem cake (4.2% and 4.8%) were next in order. Except these, all other treatments *viz.*, tricyclazole (0.1%), propiconazole (0.1%), hexaconazole (0.1%), carboxin + thiram (0.1%), tebuconazole (0.1%) and mancozeb + carbendazim (0.1%) were effective against wilt complex of pomegranate as they recorded zero per cent incidence.

4.3.7.2 Later application of fungicides, bioagents, oil cakes and nematicides

Sixteen Fungicides, bioagents, oil cakes and nematicides were applied after the simultaneous inoculation of *Ceratocystis fimbriata* and *Meloidogyne incognita* under pot culture and evaluated against wilt. The results are presented in Table 13. The results indicate that before drenching all plants showing 10-20 per cent wilting and yellowing but none of the plants completely wilted in all treatments.

Data obtained 15, 30, 45 and 60 days after drenching revealed that there was significant difference among the treatments. At 60 days after drenching propiconazole @ 0.1% recorded 22.2% of secondary branches infected and 17.0% of tertiary branches infected which was on par with tebuconazole @ 0.1% which recorded

Table 12. Effect of prior application of fungicides, bioagents, nematicides, and oil cakes (as precautionary) against simultaneous inoculation of *Ceratocystis fimbriata* and *Meloidogyne incognita*

Sl. No.	Treatment	No. of branches showing yellowing/wilting after drenching									
		Before drenching		15 days after drenching		30 days after drenching		45 days after drenching		60 days after drenching	
		II*	III**	II	III	II	III	II	III	II	III
1.	Difenoconazole (0.1%)	0.0 (0.0) +	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	26.1 (30.6)	25.9 (30.2)	26.1 (30.6)	32.0 (34.2)
2.	Tricyclazole (0.1%)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
3.	Propiconazole (0.1%)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
4.	Thiophanate methyl (0.1%)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	27.8 (26.7)	27.5 (30.7)	30.6 (33.5)	51.3 (45.6)
5.	Hexaconazole (0.1%)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
6.	Carboxin + Thiram (0.1%)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
7.	Tebuconazole (0.1%)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
8.	Mancozeb + Carbendazim (0.1%)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
9.	Mancozeb (0.1%)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	27.8 (26.7)	27.5 (30.7)	61.1 (51.5)	51.5 (45.9)
10.	*T. harzianum* (50g/pot)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	30.6 (33.5)	40.2 (39.2)	30.6 (33.5)	40.2 (39.2)
11.	*T. virens* (50g/pot)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	30.6 (33.5)	50.9 (45.5)
12.	Phorate (9g/pot)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	4.8 (7.4)	6.7 (8.9)
13.	Carbosulfan (15ml/pot)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	6.7 (8.9)	11.1 (11.7)
14.	Neem cake (50g/pot)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	4.2 (6.9)	4.8 (7.4)
15.	Pongamia cake (50g/pot)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	38.9 (38.5)	32.8 (34.7)	38.9 (38.5)	32.8 (34.7)
16.	*P. fluorescence* (50g/pot)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	30.6 (33.5)	43.8 (41.3)	30.6 (33.5)	48.6 (44.2)
17.	Control	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
S. *Em*±		-	-	-	-	-	-	4.8	3.3	3.6	2.6
CD@1%		NS	NS	NS	NS	NS	NS	14.1	9.5	10.6	7.5

+ Values in the parenthesis are arc sign transformed values.

* Secondary branches **Tertiary branches

Table 13. Effect of later application of fungicides, bioagents, nematicides, and oil cakes (as curative) against simultaneous inoculation of *Ceratocystis fimbriata* and *Meloidogyne incognita*

Sl No.	Treatment	No. of branches showing yellowing/wilting after drenching									
		Before drenching		15 days after drenching		30 days after drenching		45 days after drenching		60 days after drenching	
		II*	III**	II	III	II	III	II	III	II	III
1.	Difenoconazole (0.1%)	13.9 (21.8)+	15.9 (23.5)	13.3 (21.3)	15.9 (23.5)	22.2 (23.5)	16.4 (23.8)	38.9 (38.5)	40.0 (39.1)	61.1 (51.5)	66.7 (55.3)
2.	Tricyclazole (0.1%)	12.5 (20.7)	16.2 (23.6)	16.7 (24.1)	17.8 (24.9)	16.7 (24.1)	17.8 (24.9)	50.0 (45.0)	32.4 (34.6)	50.0 (45.0)	32.4 (34.6)
3.	Propiconazole (0.1%)	13.9 (21.8)	17.0 (24.3)	13.9 (21.8)	17.0 (24.3)	22.2 (23.5)	17.0 (24.3)	22.2 (23.5)	17.0 (24.3)	22.2 (23.5)	17.0 (24.3)
4.	Thiophanate methyl (0.1%)	13.9 (21.8)	17.8 (24.9)	38.9 (38.5)	27.0 (30.9)	38.9 (38.5)	27.0 (30.9)	29.6 (32.9)	47.6 (43.6)	38.9 (38.5)	53.2 (46.9)
5.	Hexaconazole (0.1%)	4.8 (7.4)	6.7 (8.9)	11.1 (11.7)	8.3 (10.0)	11.1 (11.7)	21.7 (27.7)	44.4 (41.7)	52.5 (46.4)	55.6 (48.2)	87.5 (77.4)
6.	Carboxin + Thiram (0.1%)	4.2 (6.9)	4.8 (7.4)	16.7 (15.0)	7.4 (9.4)	50.0 (45.0)	16.9 (24.2)	50.0 (45.0)	39.7 (39.0)	61.1 (56.7)	66.1 (54.5)
7.	Tebuconazole (0.1%)	15.6 (23.1)	16.7 (24.1)	44.4 (41.7)	38.9 (38.5)	44.4 (41.7)	38.9 (38.5)	44.4 (41.7)	38.9 (38.5)	44.4 (41.7)	38.9 (38.5)
8.	Mancozeb + Carbendazim (0.1%)	13.9 (21.8)	17.8 (24.9)	13.9 (21.8)	17.8 (24.9)	33.3 (35.2)	30.0 (33.1)	66.7 (60.0)	35.6 (36.6)	66.7 (60.0)	46.7 (43.1)
9.	Mancozeb (0.1%)	11.1 (11.7)	16.7 (19.8)	11.1 (11.7)	16.7 (19.8)	50.0 (45.0)	28.9 (32.4)	66.7 (60.0)	58.9 (50.1)	100.0 (90.0)	88.9 (78.2)
10.	*T. harzianum* (50g/pot)	12.5 (20.7)	14.5 (22.3)	33.3 (35.2)	23.3 (28.8)	33.3 (35.2)	23.3 (28.8)	33.3 (35.2)	38.3 (38.2)	44.4 (41.7)	58.3 (49.8)
11.	*T. virens* (50g/pot)	14.5 (22.3)	15.0 (22.6)	36.1 (36.7)	15.0 (22.6)	36.1 (36.7)	15.0 (22.6)	55.6 (48.2)	38.3 (38.2)	91.7 (80.0)	49.2 (44.5)
12.	Phorate (9g/pot)	12.5 (20.7)	17.8 (24.9)	12.5 (20.7)	23.3 (28.8)	33.3 (35.2)	23.3 (28.8)	66.7 (60.0)	46.7 (43.1)	83.3 (75.0)	70.0 (56.9)
13.	Carbosulfan (15ml/pot)	13.9 (21.8)	16.2 (23.6)	13.9 (21.8)	30.0 (33.1)	13.9 (21.8)	30.0 (33.1)	55.6 (53.5)	70.0 (56.9)	77.8 (66.5)	93.3 (81.1)
14.	Neem cake (50g/pot)	12.5 (20.7)	16.4 (23.8)	12.5 (20.7)	29.2 (32.2)	12.5 (20.7)	29.2 (32.2)	50.0 (45.0)	51.0 (45.5)	100.0 (90.0)	100.0 (90.0)
15.	Pongamia cake (50g/pot)	11.1 (11.7)	12.5 (20.7)	11.1 (11.7)	20.0 (26.6)	60.0 (50.7)	20.0 (26.6)	60.0 (50.7)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
16.	*P. fluorescence* (50g/pot)	16.4 (23.8)	16.7 (24.1)	61.1 (56.7)	24.2 (28.8)	61.1 (56.7)	24.2 (28.8)	72.2 (63.2)	62.5 (57.6)	100.0 (90.0)	100.0 (90.0)
17.	Control	16.7 (24.1)	22.2 (28.0)	50.0 (45.0)	66.7 (54.7)	50.0 (45.0)	66.7 (54.7)	100.0 (90.0)	83.3 (65.9)	100.0 (90.0)	100.0 (90.0)
S. *Em*±		2.6	3.0	7.5	4.9	6.6	2.7	9.0	4.6	7.5	5.5
CD@1%		7.4	8.6	21.7	14.1	19.0	7.7	26.0	13.2	21.8	15.8

+ Values in the parenthesis are arc sign transformed values.

* Secondary branches **Tertiary branches

44.4% of secondary branches infected and 38.9% of tertiary branches infected and these two treatments found significantly superior over all the treatments. Followed by this, tricyclazole @ 0.1 % recorded 50.0% of secondary branches infected and 32.4% of tertiary branches infected. Mancozeb + carbendazim @ 0.1% recorded 66.7% of secondary branches and 46.1 percentage of tertiary branches infected. The cent per cent infection of secondary and tertiary branches infected was recorded in neem cake @ 50 g/pot, pongamia cake @ 50 g/pot and *P. fluorescence* @ 50 g/pot and in control 100.0 percentage of 100.0 percentage of secondary branches and tertiary branches infected.

4.3.8 Efficacy of different chemicals (fungicides, bioagents, nematicides and oil cakes) against combined infection of *C. fimbriata* and *M. incognita* under field conditions

An experiment was carried out in a farmer's field at Tulsigeri village of Bagalkot taluk during 2017-18 using fourteen fungicides, bioagents, nematicides and different combinations of fungicides, bioagents and nematicides for the management of wilt complex of pomegranate under field condition. Treatments were imposed by drenching at 15 days interval. Data on percentage of branches infected (primary, secondary and tertiary) were recorded as presented in Table 14 and Plate 15.

Data obtained at 45 days after drenching revealed that tricyclazole @ 0.2%, propiconazole @ 0.2%, propiconazole @ 0.2% + phorate @ 18 g/plant and propiconazole @ 0.2% + neem cake @ 100 g/plant + *T. harzianum* @ 100 g/plant found very effective compared to all other treatments followed by tebuconazole @ 0.2% which recorded 50.0% of primary branches infected, 40.0% of secondary branches infected and 31.0% of tertiary branches infected. The maximum percentage of primary (50.0%) secondary (27.2%) and tertiary branches (44.8%) infected was recorded in neem cake @ 0.2%.

Data obtained at sixty days after drenching revealed that propiconazole @ 0.2% + phorate @ 18 g/ plant recorded 66.6% of primary branches infected, 27.2% of secondary branches infected and 42.1% of tertiary branches infected which was on par with propiconazole @ 0.2% recorded 50.0% of primary branches infected, 60.0%

Table 14. Efficacy of different fungicides, bio agents, nematicides, oil cakes and their combinations against wilt complex of pomegranate caused by *Ceratocystis fimbriata* and *Meloidogyne incognita* under field condition during 2017-18

Treatment	Per cent of branches showing yellowing/wilting																				
	Before drenching			15 days after drenching			30 days after drenching			45 days after drenching			60 days after drenching			90 days after drenching			% disease increase after 90 days		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
T1*	50.0	50.0	37.5	50.0	50.0	37.5	50.0	50.0	37.5	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	62.5	**0.0**	**0.0**	**25.0**
T2	66.6	57.1	28.5	66.6	57.1	28.5	66.6	57.1	28.5	66.6	57.1	28.5	66.6	57.1	35.7	66.6	71.4	42.8	**0.0**	**14.3**	**14.3**
T3	50.0	60.0	57.1	50.0	60.0	57.1	50.0	60.0	57.1	50.0	60.0	61.9	50.0	60.0	61.9	50.0	60.0	66.6	**0.0**	**0.0**	**9.5**
T4	50.0	33.3	42.8	50.0	33.3	42.8	50.0	33.3	42.8	50.0	41.6	50.0	50.0	50.0	57.1	50.0	58.3	67.8	**0.0**	**25.0**	**25.0**
T5	50.0	41.6	28.5	50.0	33.3	42.8	50.0	41.6	42.8	50.0	41.6	50.0	50.0	50.0	57.1	50.0	58.3	67.8	**0.0**	**16.7**	**39.3**
T6	50.0	33.3	45.4	50.0	33.3	45.4	50.0	33.3	45.4	50.0	33.3	45.4	50.0	33.3	65.1	50.0	33.3	65.1	**0.0**	**0.0**	**16.1**
T7	50.0	40.0	27.5	50.0	40.0	27.5	50.0	40.0	31.0	50.0	40.0	31.0	50.0	40.0	34.4	50.0	40.0	34.4	**0.0**	**0.0**	**6.9**
T8	50.0	50.0	37.5	50.0	50.0	37.5	50.0	50.0	37.5	50.0	66.6	50.0	50.0	66.6	62.5	50.0	66.6	75.0	**0.0**	**16.6**	**37.5**
T9	50.0	15.3	28.5	50.0	15.3	28.5	50.0	15.3	32.1	50.0	30.7	53.5	50.0	38.4	64.2	75.0	61.5	75.0	**25.0**	**46.2**	**46.5**
T10	66.6	18.7	27.7	66.6	18.7	27.7	66.6	18.7	33.3	66.6	31.2	55.5	66.6	43.7	61.1	66.6	56.2	77.7	**0.0**	**37.5**	**50.0**
T11	25.0	18.1	27.5	25.0	18.1	27.5	25.0	18.1	31.0	50.0	27.2	44.8	50.0	36.3	58.6	75.0	54.5	86.2	**50.0**	**36.4**	**58.7**
T12	66.6	27.2	39.4	66.6	27.2	39.4	66.6	27.2	39.4	66.6	27.2	39.4	66.6	27.2	42.1	66.6	27.2	44.7	**0.0**	**0.0**	**5.3**
T13	50.0	37.5	52.0	50.0	37.5	52.0	50.0	37.5	52.0	50.0	37.5	52.0	50.0	37.5	60.0	50.0	50.0	60.0	**0.0**	**12.5**	**8.0**
T14	18.7	33.3	43.6	18.7	33.3	59.0	18.7	33.3	59.0	18.7	33.3	59.0	18.7	33.3	59.0	18.7	40.0	59.0	**0.0**	**6.7**	**15.4**
T15	50.0	44.4	51.7	50.0	44.4	51.7	50.0	44.4	62.0	75.0	55.5	75.8	100.0	100.0	100.0	100.0	100.0	100.0	**50.0**	**55.6**	**48.3**

*

T_1-Difenoconazole @ 0.2%	**T_6**-Carboxin + Thiram @ 0.2%	**T_{11}**- Neem cake @100 g/plant
T_2-Tricyclazole @ 0.2%	**T_7**- Tebuconazole @ 0.2%	**T_{12}**- Propiconazole @ 0.2 %+ Phorate @ 18 g/plant
T_3-Propiconazole @ 0.2%,	**T_8**- Mancozeb +Carbendazim @ 0.2%	**T_{13}**- Propiconazole @0.2 % +Neem cake @ 100 g/plant +*T. harzianum* @100 g/plant
T_4-Thiophanate Methyl @ 0.2%	**T_9**- *Trichoderma harzianum* @100g/plant	**T_{14}**- Propiconazole @ 0.2%+ Phorate @ 18 g/plant +*T. harzianum* @ 100 g/plant
T_5- Hexaconazole @ 0.2%	**T_{10}**- Phorate @18 g/ plant	**T_{15}**- Control.

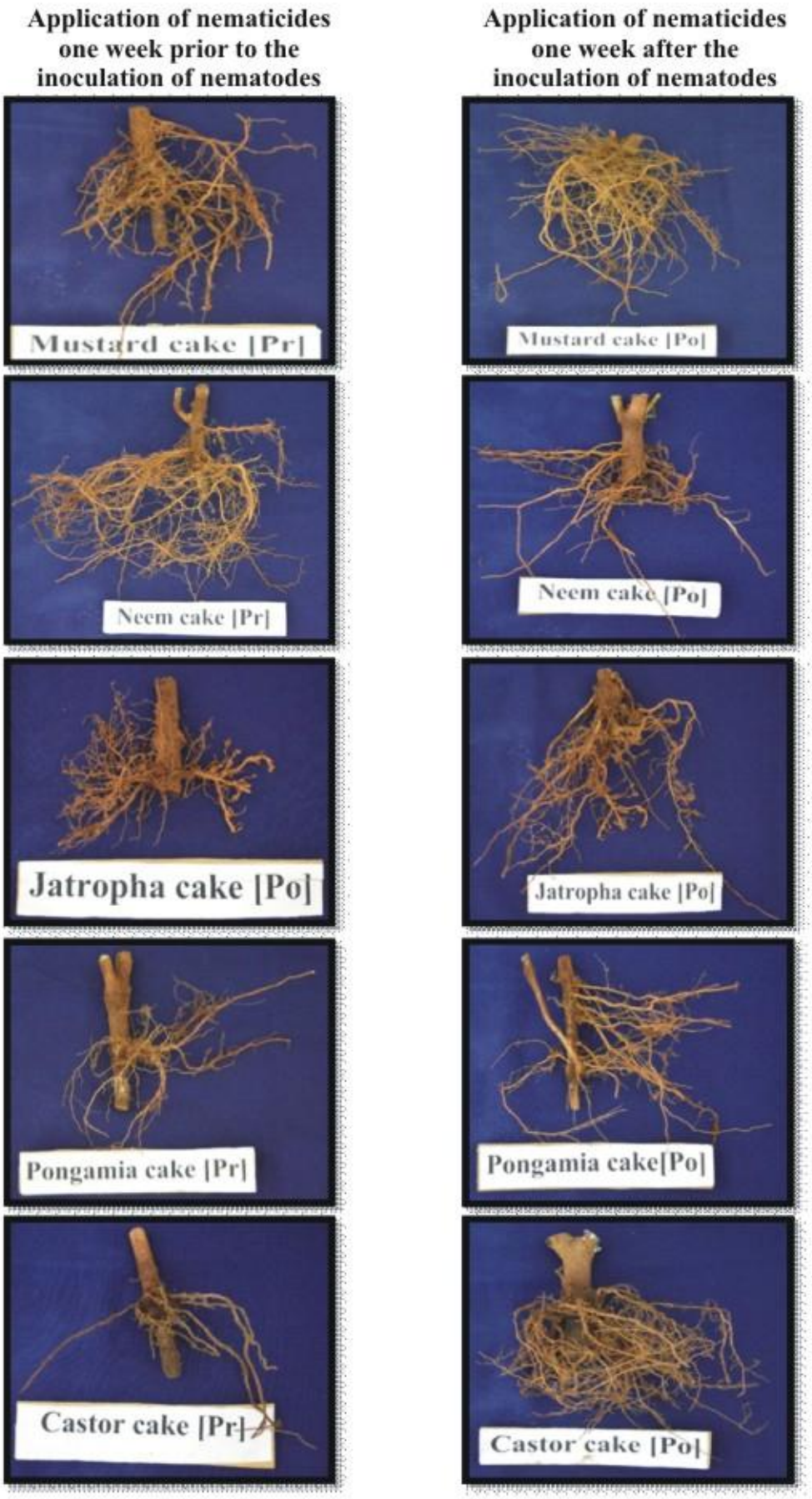


Plate 15: Effect of oil cakes on gall index and root growth parameter of pomegranate

of secondary branches infected and 61.9% of tertiary branches infected and these two treatments found effective over all the treatments. This was followed by tebuconazole @ 0.2% recorded 50.0 primary branches infected, 40.0% of secondary branches infected and tertiary 34.4% of tertiary branches infected and tricyclazole @ 0.2% recorded 66.6% of primary branches infected, 57.1% of secondary branches infected and 35.7% of tertiary branches infected. The maximum percentage of primary (50.0%) secondary (36.3%) and tertiary (58.6%) branches infected was recorded in neem cake @ 100 g/plant. In control cent per cent of wilt incidence was observed in primary, secondary and tertiary branches.

Data obtained at 90 days after drenching revealed that propiconazole @ 0.2% + phorate @ 18 g/ plant recorded 66.6% of primary branches, 27.2% of secondary branches and 44.7% of tertiary branches infected followed by propiconazole @ 0.2% + neem cake @ 100 g/plant + *T. harzianum* @ 100 g/plant recorded 50.0% of primary branches, 50.0% of secondary branches infected and 60.0% of tertiary branches infected. These two treatments found effective over all the treatments. This was followed by tebuconazole @ 0.2% which recorded 50.0% primary branches, 40.0% of secondary branches and tertiary 34.4% of tertiary branches infected and propiconazole @ 0.2% recorded 50.0% of primary branches infected, 60.0% of secondary branches infected and 66.6% of tertiary branches infected. The maximum percentage of primary (75.0%) secondary (54.5%) and tertiary (86.2%) branches infected was recorded in neem cake @ 100 g/plant. In control complete wilt incidence was observed in primary, secondary and tertiary branches.

4.3.8.1 Yield and economic analysis of field experiment on management of wilt complex

The economics of different combination fungicides tested under field condition against *C. fimbriata* and *M. incognita* are calculated and revealed here under Table 15. The highest yield of 14.58 t/ha was recorded in propiconazole + phorate with net returns of Rs. 7,40,423 which was followed by propiconazole + neem cake + *T. harzianum* (12.99 t/ha) with net returns of Rs. 610701, propiconazole (12.15 t/ha) with net returns of Rs. 597455. Control recoded highest percentage of disease and no fruits were recorded in this treatment all the plants died before harvest.

Before treatment | Ninety days after treatment

T_1: Difenoconazole @ 0.2%

T_2: Tricyclazole @ 0.2%

T_3: Propiconazole @ 0.2%

Plate 16. Efficacy of different chemicals against wilt complex of pomegranate caused by *C. fimbriata* and *M. incognita* under field condition during 2017-18

Plate 16 contd.....

Before treatment **Ninety days after treatment**

T_4: Thiophanate methyl @0.2%

T_5: Hexaconazole @0.2%

T_6: Carboxin + Thiram @0.2%

Plate 16 contd.....

Before treatment **Ninety days after treatment**

T_7: Tebuconazole @ 0.2%

T_8: Mancozeb + Carbendazim @ 0.2%

T_9: *Trichoderma harzianum* @ 100 g/plant

Plate 16 contd.....

Before treatment **Ninety days after treatment**

T_{10}: Phorate @ 18g/plant

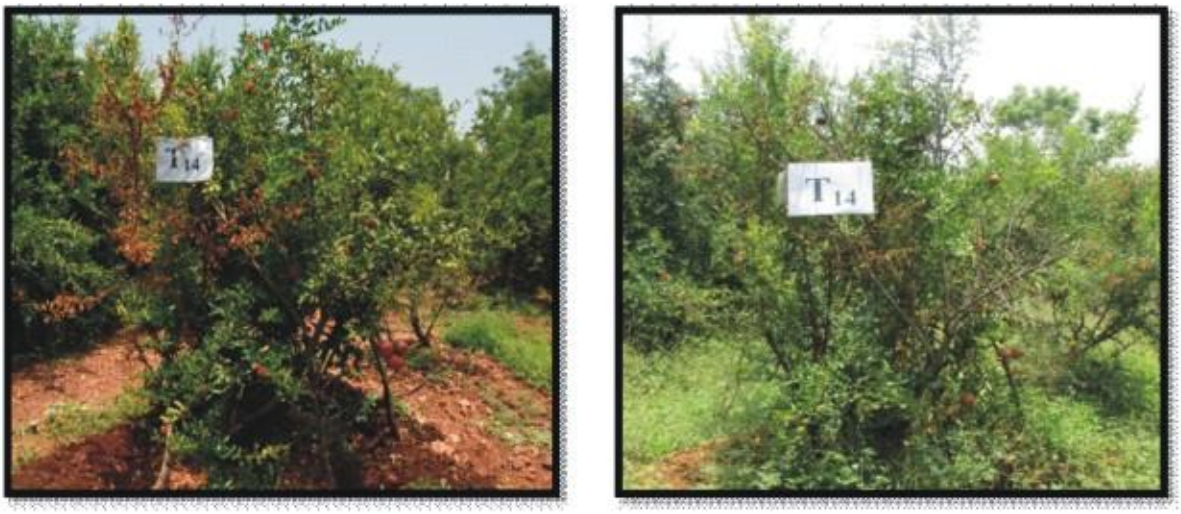
T_{11}: Neem cake @ 100 g/plant

T_{12}: Propiconazole @ 0.2% + Phorate @ 18 g/ plant

Plate 16 contd.....

Before treatment **Ninety days after treatment**

T_{13}: Propiconazole @0.2% + Neem cake 100 g + *T. harzianum* 100 g/ plant



T_{14}: Propiconazole @0.2% + Phorate 18 g +*T. harzianum* 100g/plant

T_{15}: Untreated control

Table 15. Economic analysis of fungicides, bio agents, nematicides, oil cakes and their combinations used against *C. fimbriata* and *M. incognita*

Sl. No	Treatment	Yield/ treatment (kg)	Yield/ha (t)	Cost of cultivation/ha (Rs)	Cost of treatments/ha (Rs)	Total cost/ha (Rs)	Total Returns/ha (Rs)	B:C	Net returns/ha (Rs)	ICBR
1	Difenoconazole @ 0.2%	9.70	7.18	3,00,609	1,92,400	4,93,009	5,74,240	1.16	81,231	1.91
2	Tricyclazole @ 0.2%	10.82	8.01	3,00,609	1,18,400	4,19,009	6,40,544	1.53	2,21,535	2.13
3	Propiconazole @ 0.2%	16.42	12.15	3,00,609	74,000	3,74,609	9,72,064	2.59	5,97,455	3.23
4	Thiophanate Methyl @ 0.2%	5.50	4.07	3,00,609	66,600	3,67,209	3,25,600	0.89	-41,609	1.08
5	Hexaconazole @ 0.2%	8.25	6.11	3,00,609	26,640	3,27,249	4,88,400	1.49	1,61,151	1.62
6	Carboxin + Thiram @ 0.2%	4.28	3.17	3,00,609	81,400	3,82,009	2,53,376	0.66	-1,28,633	0.84
7	Tebuconazole @ 0.2%	15.55	11.51	3,00,609	1,25,800	4,26,409	9,20,560	2.16	4,94,151	3.06
8	Mancozeb +Carbendazim @ 0.2%	9.33	6.90	3,00,609	37,000	3,37,609	5,52,336	1.64	2,14,727	1.84
9	*Trichoderma harzianum* @ 100 g /plant	3.24	2.40	3,00,609	7,400	3,08,009	1,91,808	0.62	-1,16,201	0.64
10	Phorate @18 g/ plant	2.00	1.48	3,00,609	6,808	3,07,417	1,18,400	0.39	-1,89,017	0.39
11	Neem cake @100 g/plant	2.33	1.72	3,00,609	1,850	3,02,459	1,37,936	0.46	-1,64,523	0.46
12	Propiconazole @ 0.2 %+ Phorate @ 18 g/plant	19.70	14.58	3,00,609	1,25,208	4,25,817	11,66,240	2.74	7,40,423	3.88
13	Propiconazole @0.2 % +Neem cake @ 100 g/plant +*T. harzianum* @100 g/plant	17.55	12.99	3,00,609	1,27,650	4,28,259	10,38,960	2.43	6,10,701	3.46
14	Propiconazole @ 0.2%+ Phorate @ 18 g/plant +*T. harzianum* @ 100 g/plant	3.60	2.66	3,00,609	1,32,608	4,33,217	2,13,120	0.49	-220097	0.71
15	Control.	0.00	0.00	3,00,609	0	300609	0	0.00	-300609	0.00

5. DISCUSSION

Pomegranate (*Punica granatum* L.) is a high value horticultural crop, suffers mainly due to wilt especially in northern Karnataka, which caused by the interaction of *Ceratocystis fimbriata* Ell. and Halst and *Meloidogyne incognita*. This has drawn the attention of the present study wherein, a survey in the north Karnataka to assess the disease prevalence with respect to locality, cultivar *etc*; interaction of *C. fimbriata* and *M. incognita* in the causation of the disease and management of the disease were studied. The results of the investigation are briefly discussed here under.

5.1 Survey for the assessment of incidence and severity of wilt complex of pomegranate caused by *C. fimbriata* and *M. incognita*

An intensive roving survey was carried out in Bagalkot and Vijayapur districts of north Karnataka, during 2016-17 and 2017-18 to know the incidence of wilt caused by *C. fimbriata* and *M. incognita* in pomegranate. The survey was conducted in major taluks, selecting 3 villages in each taluk and two fields in each village.

The findings of the present study revealed that the wilt complex caused by *C. fimbriata* and *M. incognita* was observed in all the taluks of Bagalkot and Vijayapur districts surveyed. Among two districts average per cent disease index (PDI) of wilt disease was noticed maximum in Bagalkot district (8.53%) than Vijayapur district (7.15%).Among six taluks of Bagalkot district surveyed maximum total per cent disease incidence was recorded in Badami taluk (19.91%) followed by Bagalkot (9.25%), Hunagund (9.25%), Jamakhandi (7.22%), Bilagi (6.99%) and Mudhol (4.08%).With respect to Vijayapur district, the results revealed that among five taluks surveyed maximum total per cent disease incidence was recorded in Sindagi (13.49%) followed by Basavana bagewadi (8.51%), Vijayapur (5.89%), Indi (4.15%) and Muddebihal (3.64%). Higher wilt disease incidence was noticed in orchards more than 5-6 years (24.28%), while it was less in orchards below 1-3 years (4.1%). Root knot nematode (up to 5 grade) and shot hole bore (up to 4 grade) association was noticed in orchards of more than 4-5 years.

The survey revealed that wilt incidence is in alarming stage in all pomegranate growing areas surveyed. The per cent of wilt incidence varied from locality to

locality. Farmers are using local planting material which is neither certified nor completely ensured that they are free from disease. Thus, initial inoculum through infected planting materials from one place to another probably attributes for the spread of the disease. The higher disease incidence in Bagalkot and Vijayapura in the present study may be due to the mono cropping of same susceptible varieties since many years. Most of the farmers are unaware of wilt complex disease. The lack of proper diagnosis for the disease and un awareness of proper management practices by farmers might have led to severity of wilt complex. The faster multiplication of nematode population in the soil probably due to congenial soil temperature and association of these nematodes along with the fungus might have aggravated wilt complex incidence which in turn results in severe loss to the farmers.

However, earlier surveys in Karnataka revealed the presence of *C. fimbriata* and *M. incognita* with an average disease incidence of 0.1 -33.3 per cent (Benagi *et al*., 2009), 8.69% (Jadhav and Sharma, 2009) and 5.69% (Somasekhara *et al*, 2009). Sonyal *et al*., 2015 reported that wilt incidence ranged from 22.3 to 45.2 per cent in different surveyed locations of six northern Karnataka districts. Somu (2017) carried out survey in major pomegranate growing districts of Karnataka to know the incidence of wilt during 2015-16, which revealed that the highest incidence of disease in Bagalkot district (15.27%) and least in Chitradurgra district (3.75%). Bagalkot and Vijayapura districts are traditional pomegranate growing belts where pomegranate is grown in large area as a sole crop. The higher incidence of pomegranate wilt disease in these districts may be due to the continuous cultivation of the crops since many years resulting in more build up of inoculum.

It was evident from the survey that among the commonly growing pomegranate cultivars, *viz.*, Bhagwa, Kesar, Ruby and Sindhur; the cultivar Bhagwa was found to be highly susceptible to *C. fimbriata*, root knot nematode and shot hole borer. Chaudhari *et al.*, 2016 screened four pomegranate cultivars/varieties against wilt disease of pomegranate under glass house condition. The maximum wilt disease incidence was recorded in the cultivar Bhagwa which was followed by Mridula, Arakta and G-137. Over all it was found that none of the variety showed resistance to wilt disease. Farmers are using local planting material which is neither certified nor completely ensured that they are from disease free orchards. Thus, initial inoculum

through planting material, accompanied with susceptible variety and prevailing congenial soil and climatic conditions help to aggravate the disease. Somu *et al.*, 2017 reported that the overall mean incidence of wilt in major pomegranate growing district of Karnataka was recorded 6.97 per cent on different cultivars such as Bhagwa, Ruby and Super Bhagwa. He also noticed that higher incidence (10.97%) in older orchards which attributed to build up of inoculum in the soil due to continuous cultivation of the crop year after year at same locations.

As per the survey carried out in pomegranate growing areas there was variation in per cent wilt incidence with respect to the soil type. The maximum incidence of wilt complex was observed in sandy loam soil (17.47%) followed by black soil (10.08%), sandy soil (9.81%) and red soil (5.85%). The wilt incidence, shot hole borer and root knot infection was high in black soil compared to red soil (Somu *et al.*, 2017). On the contrary Imran Khan (2017) reported that the maximum incidence of wilt complex disease was observed in red sandy soil (36.41%), followed by sandy loam soil (35.25%), red soil (33.15%) and black soil (28.50%).

The weather data of Bagalkot and Vijayapur district shows that the temperature ranges between 25-30̊ C with 55-60% humidity which is congenial for the fungus *C. fimbriata* for easy and rampant sporulation and spread of the disease. Somu *et al*., 2017 revealed that the maximum growth of *C. fimbriata* (81.00 mm) was obtained at 25^0C, whereas optimum range was 20^0C to 30^0C. Vijaya (2007) observed that maximum growth of *C. Paradoxa* obtained at 30^0C and optimum range is between 20 to 35^0C. A basic characteristic of soil fungi is their mycelial growth form and mycelial exploration through soil as influenced by soil physical characteristics since hyphae must ramify through the complex heterogeneous network of pores. The effects of physical conditions on hyphal spread are difficult to ascertain because of the geometric complexity of the pore networks (Vogel 1997). Many factors may modulate fungal growth in soils, such as nutrient availability, pH, aeration, and microbivory (Frey 1999).

During the survey root knot incidence was noticed up to grade 5 was higher. Most of the infected fields recorded root knot incidence more than 3 grade. In survey, in very few fields root knot infection was not noticed. The nematode population build up in the soil was probably due to steady increase in soil temperature. Association of

these nematodes along with fungus aggravated wilt complex incidence which results in severe loss to the farmers. The maximum population of root knot nematode (1260 J_2/200 cm^3 of soil), root galls (117/5 g roots) and egg masses (120/5 g roots) were recorded in the 52^{nd} meteorological week, when the maximum and minimum temperatures of air were 28.1^0C and 10.0^0C respectively (Walunj *et al.*, 2013).

The warm climate favours the development of root knot nematodes. Soil temperatures, in Bagalkot and Vijayapur districts, for most of the year, are suitable for nematode activity. The nematodes occur throughout the year where intensive agriculture is practiced. Nematodes generally take 5 to 15 years to develop; but once developed, it is almost impossible to eradicate them (Khan *et al*, 1993).

5.1.1 Symptoms of the disease caused by *C. fimbriata* and *M. incognita*

The typical symptoms of wilt complex of pomegranate results in complete wilting of plant and is characterized by the initial symptoms as yellowing and wilting of leaves on one to several branches. At times only one or two stems of the tree showed wilting and it took few weeks to some months for the entire tree to completely wilt. Wilt infected plants often revealed dried foliage and fruits being attached to the branches for many months. The xylem of the trunk turned brown to black with a star burst like pattern with blue strains on stem was noticed. Below ground symptoms like the dark black to greyish colour mycelial mat was observed on the root portion with characteristic fruity odour. In severely infected fields similar symptoms of wilt were earlier recorded by many scientists *viz.*, Somasekhara and Wali, 1999; Huang *et al.*, 2003; Sharma 2009; Khosla *et al*, 2013; Xu *et al.*, 2011; Jadhav and Sharma, 2009; Raja 2017, and Somu, 2017.

Root knot nematode associated roots showed large galls or knots throughout the root system of infected plants. In general plants aged more than four to five years were severely affected by root knot nematode. Plants infested severely by root knot nematode exhibited yellowing of foliage resulting in stunted plant growth. These plants produced less number of fruits or no fruits. In severe cases the galls were predominantly found on entire root system. The galls were white in colour turned light brown in colour and hardy when they become old. The symptoms observed in the present studies were more or less similar to those described by Kore and Mitkar,

1993 and Chaudhari *et al*., 2016. Perineal pattern is the most important morphological characters used for reliable species identification. The root knot nematode species on pomegranate were characterized by the presence of high, squarish dorsal arch that often continued a distinct whorl in the tail terminal area. The striae were smooth to wavy. Sometimes, zig zagged and distinct lateral lines. The observed characters were same with the descriptions given by Eisenback *et al*, (1981).

5.1.2 Isolation of pathogens associated with disease complex in pomegranate

5.1.2.1 Isolation of *C. fimbriata* from wilt affected samples

Standard tissue isolation was followed to isolate *Ceratocystis fimbriata* culture from diseased sample. The wilt fungus *C. fimbriata* grew well on potato dextrose agar and oat meal agar. As the growth progressed, production of endoconidia, aleurioconidia and perithecium was observed. Perithecia were black with a globose base. Ascospores exuded from the apex of the perithecium neck in a long coil which were small, hat shaped and hyaline. Conidiophores were septate and hyaline to dark greenish brown. Thick walled endoconidia were globose to oval, olive brown in colour. Aleurioconidia were thick walled, ellipsoildal or pyriform, golden-brown in colour, borne singly or in chain. These results are in conformity with Somasekhara and Wali (2000), Sharma *et al.* (2010), Sonyal *et al.* (2015), Chaudhari *et al*. (2016), Soni and Kanwar, (2016), Raja (2017) and Somu (2017).

5.1.2.2 Extraction of *M. incognita* from wilt affected samples

Soil samples collected from the field were brought to the laboratory. The known quantity (200 cc) of soil from each sample was processed by combined Cobb's sieving and Baermann's funnel technique and nematode population was estimated. The nematode population was maintained on Pusa Ruby variety of tomato. The present findings are in conformity with the work of Chaudhari *et al*. (2016).

5.1.3 Pathogenicity test

The pathogenicity of the *C. fimbriata* and *M. incognita* was proved by inoculating the pure culture of *C. fimbriata* and 1000 J_2 stage root knot nematodes to six month old pomegranate *cv* Bhagwa raised in sterile soil along with a control plant

without adding inoculum to sterile soil. The pathogen produced wilting symptoms after 45 days of inoculation. The reisolation of the fungus was made from the roots of wilted plant and was identical to original one with respect to cultural and morphological characters. The results are also in close agreement with Somasekhara (1999); Somasekhara and Wali (1999) ; Engelbrecht and Harrington (2005) ; Sonyal (2010) ; Khosla, (2013) and Chaudhari *et al*. (2016) who isolated *Ceratocystis fimbriata* from wilted pomegranate plant and Kumar (2008) who isolated *M. incognita* from *Coleus forskohlii* and proved the pathogenicity.

5.2 Interaction of *Ceratocystis fimbriata* and *Meloidogyne incognita* in causing wilt of pomegranate

In nature, plants are rarely exposed to the influence of single pathogen. Fawcett (1931) recognized that "nature does not work with pure culture" and that many plant diseases are influenced by associated organisms. Plant parasitic nematodes often play a major role in disease interactions as they contribute substantially to variability in crop growth and resulting heavy crop losses. It seems reasonable to expect that infection by one pathogen may alter the host response to subsequent infection by another (Zacheo, 1993).

Disease complexes involving nematode and fungi have gained momentum in the recent years. The data on interaction between *M. incognita* and wilt inducing fungi *C. fimbriata* on pomegranate revealed that that there was significant difference among the treatments. Simultaneous inoculation of *C. fimbriata* and *M. incognita* at 15 days after inoculation showed infection of 50.60% in tertiary branches, 36.67% in secondary branches and 45.6% in leaves which was on par with inoculation of *C. fimbriata* 15 days prior to inoculation of *M. incognita* which recorded infection of 29.3% in tertiary branches, 24.4% in secondary branches and 6.2 % in leaves. These two treatments found significantly superior over all the treatments. The same trend of wilt incidence increased at 30 days, 45 days and 60 days. At 90days after in simultaneous inoculation of *C. fimbriata* and *M. incognita*, inoculation of *C. fimbriata* 15 days prior to inoculation of *M. incognita* and inoculation of *M. incognita* alone recorded 100 per cent infection of secondary branches with respect to tertiary branches simultaneous inoculation of *C. fimbriata* and *M. incognita* and inoculation of *M. incognita* alone showed 100 per cent incidence. The individual and combined

inoculation of *C. fimbriata* and *M. incognita* showed characteristic wilted symptoms in the findings of Sonyal (2010) and Khan (2017). Under the right conditions *Ceratocystis fagacearum* can produce sporulation mats that emit “fruity” odors that, attract sap feeding nitidulid beetles. Transmission can take place when nitidulid beetles carrying spores from these mats move on to feed on fresh wounds of uninfected oak trees. *Ceratocystis fagacearum* enters oak trees through these fresh wounds and initially grows in the xylem vessels of the outer sapwood. Later in the infection cycle, hyphae are formed that penetrate the parenchyma cells and grow inter and intracellularly. During the final disease stage, when the tree is dying, sporulation mats are sometimes formed on red oak trees, forming a new primary infection source (Mara de Sain and Martijn, 2015).

Bergeson (1972) studied on nematode fungus associations in plant disease complexes and indicated that, in many cases, the nematode appears to be the primary pathogen. The role of nematodes has been established as wounding agents, host modifiers, rhizosphere modifiers, and resistance breakers. Nematode invade roots by penetrating directly into or between epidermal cells, and then it tunnels extensively through the cortex. On severely infested root systems, nearly all the feeder roots are damaged or destroyed. Injured cells leak their contents into the cavities produced by tunnelling thereby increasing the concentrations of amino acids, sugars, and other organic compounds in the rhizosphere.

Ramanath and Dwivedi (1981) determined the role of *Meloidogyne javanica* on the development of wilt caused by *Fusarium oxysporum* f. sp. *ciceri.* Plants grown in soil infested with *Fusarium* and *Meloidogyne* advanced the appearance of wilt by 15 days in chickpea cultivar T-3 than plants grown in soil infested by *Fusarium* alone. More than 56 per cent plants wilted by combined infection of both nematode and fungus compared to only 32 per cent plants wilted by interaction of fungus alone. Mani and Sethi (1987) studied the effect of combined inoculation of *Meloidogyne incognita, Fusarium oxysporum* and *Fusarium solani* on growth of chickpea cv. JG-62, which was found to be additive in nature. However, when nematode was established one week prior to fungus the resultant effects was more than additive. Occurrence of *Meloidogyne incognita* in combination with both the fungi not only increased the severity of diseases but also shortened the incubations period by 6 to11

days for disease expression when nematode preceded *Fusarium solani*. *Fusarium oxysporum* did not affect the nematode population significantly. Marimuthu, (1991) reported that the nematode may predispose the vine to *Phytophthora palmivora,* which is able to infect through wounds caused by *Meloidogyne incognita.* Umamaheshwari (1991) reported that inoculation of nematode prior to fungus reduced the incubation period for the appearance of wilt symptom by a week in a late wilt variety of chickpea K-850. The fungus did not affect the development of nematode in the roots. Krishna Rao and Krishnappa (1994) observed that inoculation *Meloidogyne incognita* along with *Fusarium oxysporum* f. sp. *cicero* resulted in 19.88 per cent wilt incidence when compared to 6.66 per cent with fungus alone. The nematode multiplication was adversely affected when fungus was inoculated prior to nematode. Simultaneous inoculation of nematode and fungus as well as nematode followed by fungus 15 days later, caused 100 per cent root knot disease and significant reduction in plant growth compared to the inoculation of fungus alone or fungus inoculation prior to nematode. The similar findings were observed by Sethamarai *et al.* (2006a).

Data on root and shoot parameters of interaction study revealed that there was significant reduction in fresh shoot weight, dry shoot weight, fresh root weight, dry root weight, shoot length and root length in all the treatments in comparison to uninoculated control.

Among the treatments inoculation of *M. incognita* alone, inoculation of nematode 15 days prior to inoculation of *C. fimbriata* and simultaneous inoculation of *C. fimbriata* and *M. incognita* recorded more than 50 galls (grade 4) while T4 recorded 25 to 30 galls (grade 3). There were no root galls in treatment with *C. fimbriata* alone and untreated control.

The plants with simultaneous inoculation of *C. fimbriata* and *M. incognita* adversely affected plant growth parameters like fresh and dry weight of shoot, fresh and dry weight of root, shoot and root length (28.13 g, 17.30 g, 23.33 g, 12.50 g, 7.16 cm and 65 cm respectively) per plant fallowed by (34.73 g, 20.53 g, 23.80 g, 14.40 g 7.90 cm and 87.33 cm respectively) compared to control. The same findings were observed by Sharma and Cerauskas (1985). He observed greater reduction in shoot and root weights in chickpea due to simultaneous inoculation of *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *ciceri* than by inoculation of either of them

alone. Singh *et al.* (1981) observed that simultaneous inoculation of *Meloidogyne incognita* and *Fusarium oxysporum* or inoculation of *Meloidogyne incognita* 10 days prior to fungus drastically reduced plant height and fresh shoot weight with high wilt incidence in crops. Singh *et al.* (1994) observed that inoculation of nematode alone, simultaneously with fungus and 10 days prior to the fungus drastically reduced the plant height and fresh shoot weight in french bean and also plants showed maximum wilt. Patel *et al.* (2000) observed interaction of *Fusarium oxysporum f. sp. ciceri* with *Meloidogyne incognita* on chickpea cv. Dahood yellow revealed that the organisms either individually or in combinations reduced plant height and fresh root and shoot weights. Akhtar Haseeb *et al.* (2007) studied simultaneous inoculation of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *pisi* inoculation prior to fungus significantly reduced the plant growth. Reproduction of nematode and galling on roots decreased with pre inoculation of fungus, while the infection of fungus increased in the presence of nematode. The least shoot and root length of pomegranate was recorded in the combined treatment inoculated with *C. fimbriata* and *M. incognita* followed by inoculation of giant culture of *C. fimbriata* which were on par with each other as compared to control (Sonyal *et al*., 2010 and Imran Khan, 2017).

5.3 *In vitro* evaluation of fungicides, nematicides and bioagents against *C. fimbriata* and *M. incognita in vitro*

5.3.1 *In vitro* evaluation of non systemic fungicides against *C. fimbriata*

In vitro evaluation of a fungicide provides useful preliminary information regarding its efficacy against pathogen within a shortest period of time and therefore, serve as a guide for further pot and field testing. Six non systemic fungicides were tested at three different concentrations in the laboratory against the *C. fimbriata*. Mancozeb 63% + Carbendazim 12% was found to be most effective and significantly superior over all other treatments which inhibited 100 per cent growth of the fungus. The next best treatment was mancozeb and captan which inhibited 95.45 and 96.33 per cent growth of the fungus respectively. Carbendazim is systemic fungicide with protective and curative action. Carbendazim works by inhibiting the development of fungi probably by interfering with spindle formation at mitosis (cell division). It is a broad spectrum systemic protective and curative fungicide. It inhibits the development of germ tubes, formation of appressoria and the growth of mycelia by

inhibiting cell division. Mancozeb inactivates the sulfhydryl groups of amino acids and enzymes of fungal cell, resulting in disruption of lipid metabolism, respiration and production of adenosine triphosphate. The captan reacts with sulfhydryl groups and reduces fungal spore germination, growth, and oxygen uptake. Somasekhara (2009) tested various fungicides and reported *C. fimbriata* was completely inhibited by the fungicides mancozeb and ziram. Sharma *et al.* (2010) observed complete inhibition of *C. fimbriata* by mancozeb (0.2%) and captan (0.2%). Sonyal (2010) reported that copper oxychloride was more effective than other fungicides at 0.1, 0.2 and 0.3 per cent against *C. fimbriata*. Apet *et al.* (2015) observed 77.07 per cent mycelial growth inhibited by captan against *C. paradoxa*. Chaudhari *et al.* (2016) reported that copper oxy chloride and mancozeb were found most effective at 0.2, 0.25 and 0.3 per cent against *C. fimbriata*. Raja (2017) reported that captan, mancozeb, ziram, thiram and zineb recorded the maximum inhibition of mycelial growth at all concentrations tested against *C. fimbriata*. Somu (2017) reported that cymoxanil + mancozeb, mancozeb and captan recorded the 100% inhibition of mycelial growth at all tested concentrations *viz.*, 0.10 %, 0.20 % and 0.30 %.

5.3.2 *In vitro* evaluation of systemic fungicides against *C. fimbriata*

Seven systemic fungicides were tested against *C. fimbriata* by poisoned food technique at three concentrations. The mycelial growth of the fungus was inhibited 100 per cent by hexaconazole and propiconazole at 0.025, 0.05 and 0.1 per cent concentrations. All the systemic fungicides inhibited the mycelia growth completely at 0.1 per cent concentration. In many fungi, ergosterol is essential to the structure of cell wall and its absence causes irrepairable damage to cell wall leading to death of fungal cell. Hexaconazole is ergosterol biosynthesis inhibitor, thereby inhibiting the growth and reproduction of plant fungal pathogens. It is a systemic triazole fungicide, with protective and curative action. Propiconazole also interfere with the biosynthesis of sterols in cell membranes. A similar study was reported for the effectiveness of triazoles, which inhibited the biosynthesis pathway in fungi (Nene and Thapliyal, 1993). The present findings are supported by earlier workers. Somasekhara (2009), Sharma *et al.* (2010) and Kishore and Bhardwaj (2011) observed complete inhibition of mycelial growth of *C. fimbriata* by carbendazim and propiconazole. Sonyal (2010)

observed complete inhibition shown by propiconazole and tricyclazole at all concentrations tested. Chaudhari *et al.* (2016) revealed that hexaconazole and tricyclazole completely inhibited colony growth of *C. fimbriata* at 0.05%, 0.1%, 0.15% concentrations. Balaganur (2016) found carbendazim and combi product of tebuconazole 50% + trifloxystrobin 25% WG were effective at all the concentrations tested. Raja (2017) noted 100 per cent inhibition by carbendazim, hexaconazole, thiophanate methyl, propiconazole and tebuconazole at all concentrations tested. Imran Khan (2017) reported that among systemic fungicides tested, cent per cent inhibition of mycelial growth was recorded in propiconazole followed by hexaconazole (94.65 per cent).

5.3.3 *In vitro* evaluation of bioagents against *C. fimbriata* through dual culture technique

Biological control offers an environmentally friendly and safe technology to control the plant pathogens. It is now widely recognized that biological control of plant pathogens using antagonistic fungi and bacteria is a distinct possibility for future and can be successfully utilized especially within the frame work of integrated disease management system (Muthamilan and Jeyarajan, 1996). Among the bio agents tested, *Trichoderma harzianum*, *Trichoderma* isolate 1 *and Trichoderma* isolate 5 recorded the maximum per cent inhibition of mycelial growth (90.00%). It was found to be significantly superior to the rest of the bioagents tested. This was followed by *Trichoderma* isolate 4 (77.08%), *Trichoderma virens* (72.30%), *Trichoderma* isolate 2 (64.31%), *Pseudomonas fluorescens* (56.76%), *Trichoderma* isolate 3 (48.94%) and *Paecilomyces lilacinus* (44.98%). Whereas, the minimum parasitic activity was noticed in case of *Pseudomonas putida* and *Bacillus subtilis* which inhibited 41.00 and 47.85 per cent of *C. fimbriata* colony. *Trichoderma harzianum* inhibits enzymes necessary for pathogens to penetrate plant surfaces. Dennis and Webster (1971) reported that mechanism involved in inhibition of the test fungus may be due to the release of antibiotic (viridian) produced by *T. virens.* Whipps (1992) reported that *T. harzianum* showed antagonistic behaviour towards *C. paradoxa*. Joshi (1999) reported that *T. viride* was found significantly superior which inhibited 73.50 per cent of mycelial growth of *C. paradoxa*. Another possibility for reduction in mycelial growth may be competition between *C. fimbriata* and *T. viride* for nutrition and other

growth factors. It was due to the penetration of the antagonistic hyphae into hyphae of the pathogen at the place of contact as confirmed by Mukherji *et al.*, (2000). Vijaya *et al.*, (2007) who reported the bio agents *T. longibrachiatum*, *T. koningii*, *T. hamatum*, *T. harzianum* and one bacterial antagonist *P. fluorescens* were completely inhibited *Ceratocystis paradoxa*, causing sett rot of sugarcane. Sonyal (2010) revealed that *T. harzianum* and *T. viride* showed maximum inhibition of the *C. fimbriata* (100%) followed by *P. fluorescens* (42.33%), where completely inhibited the perithecium production and could grow over the pathogen. Somu (2017) tested bioagents against *C. fimbriata* in which *Trichoderma harzianum*-55 recorded the maximum per cent inhibition of mycelial growth (76.00%). It was found to be significantly superior to the rest of the bioagents tested. This was followed by *Trichoderma viride*-27 (70.33%), *Trichoderma viride* (PDBC) (67.00%), *Trichoderma viride* (64.00%), *Trichoderma harzianum*-1 (54.67%), *Trichoderma harzianum*-2 (51.00%), *Pseudomonas fluorescens* (45.00%). The minimum parasitic activity was noticed in case of *Bacillus subtilis*-1 and *Bacillus subtilis*-2 which inhibited 40.67 and 41.67 per cent of *C. fimbriata* colony.

5.3.4 *In vitro* evaluation of nematicides against *M. incognita*

Nematicides were tested against number of juveniles emerged at 24, 48 and 72 hours interval against *M. incognita* in laboratory condition for their efficacy against the pathogen. Among the nematicides phorate (0.01 g/ml) was found significantly superior over all other nematicides in inhibiting the number of juveniles emerging capacity (1.59) at 24, 48 and 72 hours followed by carbosulfan (1.82 and 3.40), Carbofuran (2.01 and 3.33). Least inhibiting capacity was found in cartap hydrochloride (2.06 and 4.21) over the control. Phorate is an organophosphate and carbosulfan is a carbamate group chemical, which mainly effect on nervous system of nematodes by inhibiting acetylcholinesterase (AChE) enzyme a chemical messenger that function as neurotransmitter. The chemical which effect on cholinergic (*i.e.,* it mimics the action of neurotransmitter) system can have very dangerous effects which may lead to paralysis of the nematode. In a similar finding observed by Khan *et al.,* 2012 where carbofuran and phorate through root dip plus single soil application provided greatest suppression in galling (16−20%), egg mass production (18−22%) and soil population (27.5−58.2%) of *M. graminicola*. Mansoor (2006) reported neem

leaf, neem seed powder, neem oil cake and two nematicides *viz.*, carbofuran and phorate alone and in combination reduced the root knot development caused by *Meloidogyne incognita* in tomato. Highest reductions in the nematode infections and corresponding improvement in plant growth was noted in pots treated with neem oil cake combined with carbofuran.

5.3.5 *In vitro* evaluation of oil cakes against *M. incognita*

The oil cakes were tested against number of juveniles emerged at 24, 48 and 72 hours interval against *M. incognita* in laboratory condition for their efficacy against the pathogen. Among the oil cakes neem cake was found significantly superior over all other nematicides in inhibiting the number of juveniles emerging capacity at 24, 48 and 72 hours followed by pongamia cake and mustard cake. Least inhibiting capacity was found in jatropha cake. The specific chemical basis for the antinematicidal activity of neem remains obscure, although fractions containing steroids and terpenoid glycosides appear to be toxic *in vitro* to *M. incognita* (Akhtar, 2000). Nitrogen content of neem cake may also play significant role in reducing root knot nematode population in soil. Triterpene compounds in neem cake inhibit the nitrification process and provide more nitrogen in the form of ammonium to the plants for the same amount of nitrogen applied by the amendments (Akhtar and Alam, 1993). Therefore, application of oil cakes may be considered as the best option for root knot nematode not only because of its effectiveness and ease of availability, but also economic feasibility for the growers and environmental superiority.

Nematicidal properties of aqueous extracts of oil cakes or soil amended with oil cakes in the absence of plants have been proved to be challenging. Water soluble fractions of oil cakes extracted from neem, mahua, groundnut and castor were toxic to nematodes like *Hoplolaimus indicus*, *Rotylenchulus reniformis* and *Tylenchorynchus brassicae* and *M. incognita* (Khan *et al*., 1974). Similarly, the larval hatching of *M. incognita* was suppressed significantly by boiled extracts of mustard and cotton oil cake up to 99.92 and 99.38% in water. Eggs of *M. incognita* were found to be more vulnerable to oil cakes (neem, karanj, mahua, groundnut, cotton, linseed, sesamum and kokam) and fungicide (ceresan wet and aureofungin sol) treatment than larvae (Lanjeswar and Shukla, 1986). Neem cake extract was found to be most effective in killing *M. incognita* larvae (Gowda and Setty, 1978; Gowda and Gowda, 1999)

whereas, mustard cake extract proved to be most effective in controlling *Hoplolaimus indicus* (Deshmukh and Prasad, 1969). However, greater concentrations of oil cake extract shown best results due to the presence of higher nematotoxic compounds.

5.3.6 Effect of nematicides and oil cakes against *M. incognita*

Among the several methods of managing the plant diseases, soil amendment with organics is one of the effective methods. Amendments in the form of plant debris, green manures, farmyard manures, compost, oil cakes and fertilizers are known to improve crop productivity by improving nutrient status and soil tilth. Addition of amendments to soils might have increase microbial activities in soil to suppress diseases (Sivaprakasam, 1991).

Amendments of soil with decomposable organic matter is recognized as the most efficient method of changing soil and rhizosphere environment, thereby adversely affecting the life cycle of pathogens and enabling the plant to resist the attack of pathogens through better vigour or altered physiology. It was also reported that chemicals like ammonia (Khan *et al.*, 1974) and fatty acids (Sitaramaiah and Singh, 1978) liberated during the decomposition of neem cake could be one of the factors involved in nematode control.

In the current study four nematicides and five oil cakes were tested against *M. incognita* in pots and checked for the effect on gall index and plant growth parameters Among the treatments, application of neem cake and phorate either prior or after inoculation of nematodes to pomegranate plants showed less gall index, high fresh shoot weight, dry shoot weight, fresh root weight, dry root weight, shoot length and root length. High shoot and root weight were also observed in castor cake treated plants but the number of galls were also more. The efficacy of mustered cake was low compared to neem cake and phorate in case of nematode management. The neem cake itself contains formaldehyde (0.25%), which is responsible for nematode control (Sitaramaiah and Singh, 1978). Neem cake was more effective in reducing nematode population and improving tomato yield in pot experiments. Vijayalakshmi (2000) reported that aqueous extracts of neem seed and neem cake as root dip treatments were effective against *Meloidogyne incognita* infection in tomato. The castor cake though has showed good growth but it could not control nematode infection. The good growth may be nutrients supplied by the castor cake.

5.3.7 Efficacy of fungicides, bio agents, oil cakes and nematicides against *C. fimbriata* and *M. incognita* under pot and field condition

Protection of crop plants from disease causing agents has been the focal point of the scientists in dealing with plant pathogens. In depth analysis and realization about the startling features of microbial ecology have impelled workers to replace term control with management. No single method of pest and pathogen control has given a lasting solution. Prohibitive costs of chemicals and their adverse ecological impact has made diversions of research priorities from chemical methods to other alternatives. The successive shifting of priorities from chemical to cultural and further integrated management demonstrates the elasticity of scientific ideas (Khan and Reddy, 1993).

In vitro screening of fungicides, antagonists and nematicides provides preliminary information regarding their efficacy against *C. fimbriata* and *M. incognita* with a hope to utilize the promising fungicides, bio-agents and nematicides for management of pomegranate wilt under both pot and field conditions. Fungicides, bio agents and nematicides which found effective during *in vitro* were evaluated under pot culture.

Application of fungicides one week prior to inoculation of *C. fimbriata* and *M. incognita* to the pots revealed that there were no wilting symptoms up to 30 days. After 45th day gradual yellowing and wilting symptoms were noticed in difenoconazole, thiophanate methyl, mancozeb, pongamia cake, *P. fluorescence* treated plants. There is a significant increase in wilt symptoms at 60 days after treatment in both secondary and tertiary branches compared to control.

Maximum wilt incidence was seen in mancozeb treated plants which recorded 61.1% in secondary branches and 51.5% wilt incidence in tertiary branches and was on par with thiophanate methyl 30.6% and 51.3% in secondary branches and tertiary branches respectively. *T. virens* 30.6% and 50.9%, *P. fluorescence* 30.6% and 48.6%, pongamia cake 38.9% and 32.8%, difenoconazole 26.1% and 32.0%, carbosulfan 6.7% and 11.1%, Phorate 4.8% and 6.7% and neem cake 4.2% and 4.8% were next in order. Except these all other treatments *viz.*, tricyclazole (0.1%), propiconazole (0.1%), hexaconazole (0.1%), carboxin + thiram (0.1%), tebuconazole (0.1%) and

mancozeb + carbendazim (0.1%) were effective against wilt complex of pomegranate as they revealed zero per cent incidence.

Curative measures are taken 15 days after inoculation of *C. fimbriata* and *M. incognita* revealed that there was significant difference among the treatments of 15, 30, 45 and 60 days after drenching. Propiconazole @ 0.1% found effective which recorded 22.2% of secondary branches and 17.0% of tertiary branches infected which was on par with tebuconazole @ 0.1% which recorded 44.4% of secondary branches and 38.9% of tertiary branches infected. These two treatments found significantly superior over all the treatments. Tricyclazole @ 0.1 % which recorded 50.0% of secondary branches 32.4% of tertiary branches infected while, mancozeb + carbendazim @ 0.1% recorded 66.7% of secondary branches infected and tertiary 46.1% of tertiary branches infected. The cent per cent infection of secondary and tertiary branches infected was recorded in neem cake @ 50 g/pot, pongamia cake @ 50 g/pot and *P. fluorescence* @ 50 g/pot. In control 100.0 percentage of secondary branches and tertiary branches infected at 60 days.

The fungicides, bio agents, nematicides and Oil cakes that have found best under *in vitro* and pot culture are evaluated for their efficacy under field condition against both *Ceratocystis fimbriata* and *Meloidogyne incognita* infected field during 2018. Treatments were imposed by 10 litres of solution drenching at 15 days interval for 5 times.

Data obtained 90 days after drenching revealed that propiconazole @ 0.2% + phorate @ 18 g/ plant recorded 66.6 percentage of primary branches infected, 27.2 percentage of secondary branches infected and 44.7 percentage of tertiary branches infected which was on par with propiconazole @ 0.2% + neem cake @ 100 g/plant + *T. harzianum* @ 100 g/plant recorded 50.0 percentage of primary branches infected, 50.0 percentage of secondary branches infected and 60.0 percentage of tertiary branches infected and these two treatments found effective over all the treatments. This was followed by tebuconazole @ 0.2%, propiconazole @ 0.2%. The maximum percentage of primary secondary and tertiary branches infected was recorded in neem cake @ 100 g/plant. In control complete wilt incidence was observed in primary, secondary and tertiary branches. Propiconazole is absorbed by the assimilating parts of the plant the majority within one hour. It is translocated acropetally (upwards) in

the xylem. This systemic translocation contributes to good distribution of the active ingredient within the plant tissue and prevents it from being washed off. Propiconazole acts on the fungal pathogen inside the plant at the stage of first haustoria formation. In tebuconazole demethylase inhibitors interfere in the process of building the structure of fungal cell wall. Finally inhibit the reproduction and further growth of fungus. Carbendazim inhibits nucleic acid metabolism and protein synthesis. whereas mancozeb is a preventive fungicide has no effect once the infection establishes. Generally, mancozeb is more mobile in wet and sandy soil than in dry and organic rich soil.

The highest yield of 14.58 t/ha was recorded in propiconazole + phorate with net returns of Rs. 7,40,423 which was followed by propiconazole + neem cake + *T. harzianum* (12.99 t/ha) with net returns of Rs. 6,10,701, propiconazole (12.15 t/ha) with net returns of Rs. 5,97,455. Control recoded highest percentage of disease and no fruits were recorded in this treatment all the plants died before harvest. The findings of the present study are similar to study conducted by Somasekhara (2009) screened various fungicides against *C. fimbriata* and reported that propiconazole, boric acid and phosphoric acid were found effective against wilt pathogen. Sharma *et al*. (2010) reported that soil drenching of affected and adjacent healthy plants with carbendazim or propiconazole (0.2%) + chlorpyriphos (0.2%) has resulted in effective wilt management. Sonyal (2010) reported that under field condition propiconazole was significantly superior than all other treatments followed by difenoconazole and *T. harzianum* + *P fluorescens*. Khosla (2013) reported that triazoles such as tebuconazole, cyproconazole, propiconazole, difenoconazole and diniconazole provide excellent control some soil borne diseases including wilt. Raja (2017) conducted a field experiment on wilt of pomegranate caused by *Ceratocystis fimbriata* for two years. The result indicated that three drenching of propiconazole (0.2%), *T. virens* (diamond) (0.7 g/l) and *T. harzianum* (Th-R) (5 g/l) at an interval of 15 days showed the maximum disease control with higher mean fruit yield and cost benefit ratio. Somu (2017) reported that in the field experiment propiconazole @ 0.2%, propiconazole + difenoconazole @ 0.2%, tricyclazole @ 0.2% and tebuconazole @ 0.2%, four times at 15 days intervals showed the maximum disease control with higher fruit yield and net returns. As *C. fimbriata* and *M. incognita* are soil borne pathogens which survives long time in the soil.

Mycelia survives under adverse conditions within the plant host or as thick-walled aleurioconidia in the soil or in plant host or debris, which act as primary source of inoculum. Association of *M. incognita* produces root knots and galls throughout the root system of infected plants. The intensity of root knot nematode damage increased with increase in age of the plant. More number of females were found in a single compound gall. Severe infection resulting in the whole tree dying, causing severe yield losses leading to death of affected plants in a few weeks.

Bagalkot and Vijayapur districts are traditional pomegranate growing belts where pomegranate is grown in large area as a sole crop resulting in more build up of inoculum. The farmers of these districts are not practicing any type of cropping pattern. Improper management practices by farmers in the initial period of the crop also make it difficult to manage the wilt. As per the present recommendation, on observing first symptoms of wilt in the orchard farmers need to drench roots of infected plants and healthy plants surrounding the infected plants with chemicals or bio-agents; in case of root knot nematode infection along with chemicals nematicides or Oil cakes have to be applied. In the present study propiconazole @ 0.2% + phorate @ 18 g/ plant and propiconazole @ 0.2% + neem cake @ 100 g/plant + *T. harzianum* @ 100 g/plant followed by propiconazole @ 0.2% and tebuconazole @ 0.2 % gave best results in managing wilt complex disease. Moreover, infected plants are removed timely from field. The dead trees need to be removed and fresh planting is to be done after treating the soil with formalin. The prophylactic management practices have to followed by farmers to manage the wilt complex disease of pomegranate (Annon, 2017).

Future line of work

1. At present, association of *C. fimbriata* and *M. incognita* with respect to expression of symptoms and development of disease was studied. However, there is a need to study interaction of these pathogens at molecular level.

2. There is need to study alterations in host cell physiology and biochemistry brought by the interaction of these two pathogens.

3. Gene expression studies may be conducted to know how the pathogenesis of one pathogen influence on pathogenesis of another pathogen.

4. There is a need to develop schedule of plant protection measures for the integrated management of wilt complex.

5. The available root stocks of pomegranate may be screened for resistance to *M. incognita* and *C. fimbriata* for using them as resistant root stocks.

6. SUMMARY AND CONCLUSIONS

Pomegranate (*Punica granatum* L.) is a fruit bearing deciduous shrub belongs to family Lythraceae, which has been rated as an important cash crop among horticulture crops. This crop is prone to several diseases, among which wilt complex caused by *Ceratocystis fimbriata* Ellis and Halst. and *Meloidogyne incognita* is a potential threat to successful crop production. The information on the interaction of these two disease causing pathogens and their management is scanty. Hence looking to the economic importance of the crop, severity of the disease and future threat to the pomegranate cultivation, the present investigation was undertaken and results are summarized in this chapter.

The survey was conducted to know the incidence of *Ceratocystis fimbriata* and association of root knot nematode in pomegranate during 2016-17 and 2017-18 in Bagalkot and Vijayapur districts of northern Karnataka. Among two districts, maximum average per cent disease index of wilt was noticed in Bagalkot (8.53%) than Vijayapura (7.15%). Among 6 taluks of Bagalkot, maximum average wilt disease incidence was noticed in Badami (19.91%) followed by Bagalkot (9.25%). Among 5 taluks of Vijayapura districts surveyed average wilt disease incidence was noticed maximum in Sindgi (13.49%) followed by Basavana bagevadi (8.51%). The least disease incidence was observed in Muddebihal taluk (3.64%) of Vijayapura district followed by Mudhol taluk (4.08%) of Bagalkot district.

Among the pomegranate orchards maximum disease incidence was noticed in Neerbudihal village of Bagalkot district (71.12%) followed by Tanda village of Vijayapur district (26.8%). Lowest incidence was observed in Basarkod village of Vijayapur district (0.17%) followed by Kamatagi village of Bagalkot district (0.88%). The wilt incidence, root knot infestation and shot hole bore incidence were higher in orchards of 4-5 years old compared to orchards below 2-3 years.

The infected pomegranate plants show typical symptom of yellowing and drooping of foliage and drying of one or few branches which lead to the wilting of complete plant within 2-3 months. Wilt infected plants often revealed dried foliage and fruits being attached to the branches for many months. The xylem of the trunk

turned brown to black with a star burst like pattern and blue strain symptom from bottom to tip of stem.

The *Ceratocystis fimbriata* was isolated from infected tissues using carrot baits. The pure culture was maintained on potato dextrose agar at 25 ± 1^{0}C. The *Meloidogyne incognita* was extracted from infested roots though Baermann funnel method. *C. fimbriata* grew well on potato dextrose agar and oat meal agar. The mycelium was whitish grey in the beginning which later on changed to brown. As the growth progressed, production of endoconidia, aleurioconidia and perithecium was observed. Ascospores were exuded from the apex of the perithecium neck. The prevailing root knot nematode species on pomegranate (*Meloidogyne incognita*) were characterized based on the presence of perineal pattern. The male root knot nematode has long, thin cylindrical shape of body and the female was globose to saccate shape.

The pathogenicity test was carried out separately for both pathogens on six month old pomegranate *cv* Bhagwa and Koch's postulates were successfully proved where the pathogen took 45 days to express the symptoms.

Interaction study of *C. fimbriata* and *M. incognita* on pomegranate was carried out under pot culture. Test pathogens were inoculated by damaging tertiary roots of pomegranate. The observation taken 15 days after inoculation revealed simultaneous inoculation of *C. fimbriata* and *M. incognita* showed early wilting symptom followed by inoculation of *C. fimbriata* 15 days prior to inoculation of *M. incognita*. At 45 day complete wilting was observed in simultaneous inoculation of *C. fimbriata* and *M. incognita,* inoculation of *C. fimbriata* 15 days prior to inoculation of *M. incognita* and inoculation of *C. fimbriata* alone. The plants with simultaneous inoculation of *C. fimbriata* and *M. incognita* adversely affected plant growth parameters like fresh and dry weight of shoot, fresh and dry weight of root, shoot and root length per plant followed by Inoculation of *C. fimbriata* 15 days prior to inoculation of *M. incognita*.

Among non systemic fungicides, mancozeb 63% + carbendazim 12% was found to be most effective and significantly superior over all other treatments with respect to per cent inhibition of mycelial growth. Among systemic fungicides, the mycelial growth of the fungus was inhibited 100 per cent by hexaconazole and

propiconazole at 0.025% concentrations. While all other systemic fungicides inhibited the mycelia growth completely at 0.1 per cent concentration.

Among the bio agents tested, *Trichoderma harzianum*, *Trichoderma* isolate 1 *and Trichoderma* isolate 5 recorded the maximum per cent inhibition of mycelial growth. Whereas the minimum parasitic activity was noticed in case of *Pseudomonas putida, Paecilomyces lilacinus, Trichoderma* isolate 3 and *Bacillus subtilis.*

Among the nematicides phorate @ 0.01 g/ml and neem cake @ 0.1 g/ml were found effective over all other nematicides and oil cakes. It recorded significantly least numbers with respect to number of juveniles emerged. Among the treatments, neem cake and phorate applied plants before and after application of *M. incognita* showed higher fresh shoot weight, dry shoot weight, fresh root weight, dry root weight, shoot length, root length and less number of galls.

The fungicides, bio agents, nematicides and oil cakes were inoculated one week prior to inoculation of *C. fimbriata* and *M. incognita* and evaluated for efficacy against wilt complex. Among them tricyclazole (0.1%), propiconazole (0.1%), hexaconazole (0.1%), carboxin + thiram (0.1%), tebuconazole (0.1%) and mancozeb + carbendazim (0.1%) were effective against wilt complex of pomegranate as they revealed zero per cent incidence at pot condition. Where the fungicides, bio agents, nematicides and oil cakes were inoculated fifteen days after inoculation of *C. fimbriata* and *M. incognita* at 60 days after drenching propiconazole @ 0.1% and tebuconazole @ 0.1% found significantly superior over all the treatments.

Experiment was conducted during 2017-18 for the management of wilt complex of pomegranate by using fungicides, bio agents, nematicides, oil cakes and their combination. These were applied at15 days interval for five times. Propiconazole @ 0.2% + phorate @ 18 g/ plant and propiconazole @ 0.2% + neem cake @ 100 g/plant + *T. harzianum* @ 100 g/plant were found superior in controlling the disease complex with high fruit yield and net returns compared to other treatments.

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Appendix I: Meteorological data recorded during experimental period at the meteorological observatory of the Main Horticultural Research & Extension Centre, UHS Bagalkot.

Month	Temp. (°C)		Average Temp (°C)	RH (%)		Average RH (%)	Maximum Rainfall (mm)
	Max	Min		Max	Min		
June 2017	37.6	21.3	28.11	98.3	34.9	68.62	98
July 2017	35.6	20.8	27.17	94.5	39.9	70.57	23.5
August 2017	36	20.2	27.12	95.7	37.6	71.41	32.5
September 2017	35.3	20	26.81	99.5	39.8	76.15	105
October 2017	34	14.3	26.47	99.9	26.7	74.42	70
November 2017	35.4	11.0	23.94	100	28.4	66.53	6
December 2017	33.2	8.1	22.49	99.9	17.4	64.47	6
January 2018	34	9.3	22.64	99.5	11.8	56.91	1
February 2018	36.2	8.1	24.23	98.7	7.4	48.19	18.5
March 2018	39.6	13.8	28.27	94.9	9.1	45.91	6.5
April 2018	42.2	18.0	30.97	94.5	8.1	47.37	39
May 2018	42.4	20.9	31.18	97.5	10.0	53.24	32.5

Appendix II. Prices of inputs for management of pomegranate wilt complex

Sl. No.	Particulars	Price (Rs)	Price/ha (Rs)
1	Pomegranate planting materials	25 /plant	1850
2	FYM	10 /kg	250000
Cost of fertilizers/kg			
3	Urea	8.00 /kg	5813
4	SSP	7.24 /kg	
5	MOP	16.04 /kg	
6	Labour wages	400	
7	Harrowing and deep ploughing		6000
8	Planting		720
9	Weeding		6400
10	Harvesting		8000
11	Irrigation		14826
12	Miscellaneous		7000
Total			**300609**
Cost of chemicals, bio agents, nematicides and organic oil cakes			
13	Difenoconazole	2600 /l	192400
14	Tricyclazole	1600 /kg	118400
15	Propiconazole	1000 /l	74000
16	Thiophanate Methyl	900 /kg	66600
17	Hexaconazole	360 /l	26640
18	Carboxin + Thiram	1100 /kg	81400
19	Tebuconazole	1700 /l	125800
20	Mancozeb +Carbendazim	500 /kg	37000
21	*Trichoderma harzianum*	100/kg	7400
22	Phorate	92/kg	6808
23	Neem cake	25/kg	1850
Total			**738298**

INVESTIGATION ON ASSOCIATION OF *Ceratocystis fimbriata* Ell. and Halst. AND *Meloidogyne incognita* (Kofoid and White) Chitwood IN CAUSING WILT OF POMEGRANATE AND IT'S MANAGEMENT

MADHUSHRI S. KERAKALAMATTI 2018 Dr. R. K. MESTA
Major Advisor

ABSTRACT

Pomegranate (*Punica granatum* L.), one of the important commercial horticulture crops of Karnataka, is highly threatened by the wilt complex caused by *Ceratocystis fimbriata* and *Meloidogyne incognita*. To know the incidence and severity of the disease, a survey was conducted during 2016-17 and 2017-18 in Bagalkot and Vijayapur districts of northern Karnataka. Among two districts, average per cent disease index of wilt was noticed maximum in Bagalkot (8.53%) than Vijayapura (7.15%). In Bagalkot district, average wilt disease incidence was maximum in Badami taluk (19.91%). In Vijayapura district average wilt disease incidence was maximum in Sindgi taluk (13.49%). The wilt incidence, root knot infestation and shot hole bore incidence were higher in orchards of 4-5 years old compared to orchards below 2-3 years. The *Ceratocystis fimbriata* was isolated from infected tissues using carrot baits. The pure culture was maintained on potato dextrose agar at 25 ± 1^0C. The prevailing root knot nematode species on pomegranate was characterized based on the perineal pattern. Interaction study of *C. fimbriata* and *M. incognita* on pomegranate was carried out under pot culture, which revealed that simultaneous inoculation of *C. fimbriata* and *M. incognita* showed early and highest wilting symptoms and galls, followed by inoculation of *C. fimbriata* 15 days prior to inoculation of *M. incognita.* These two treatments adversely affected plant growth parameters *viz.,* fresh and dry weights of roots, shots and root and shoot length. *In vitro* studies recorded that, non systemic fungicides *viz.,* mancozeb 63% + carbendazim 12% at 0.025%, concentrations; systemic fungicides *viz.,* hexaconazole and propiconazole at 0.025% concentrations and 3 isolates of bio agent *Trichoderma spp,* were effective against *C. fimbriata*. Phorate @ 0.01 g/ml and neem cake @ 0.1 g/ml were effective against *M. incognita*.

Pot culture studies revealed propiconazole (0.1%) and tebuconazole @ 0.1% were effective against *C. fimbriata*, while phorate @ 9 g/pot and neem cake @ 50 g/pot were effective against *M. incognita.* Under field conditions, propiconazole @ 0.2% + phorate @ 18 g/ plant and propiconazole @ 0.2% + neem cake @ 100 g/plant + *T. harzianum* @ 100 g/plant were found effective in controlling the disease complex with high fruit yield (19.70 and 17.55 kg) and net returns (` 7,40,423 and 6,10,701) compared to other treatments.

ದಾಳಿಂಬೆಯ ಸೊರಗು ರೋಗದಲ್ಲಿ *ಸೆರಾಟೋಸಿಸ್ಟಿಸ್ ಫಿಂಬ್ರೀಯಾಟಾ* ಎಲ್ಲ್. ಮತ್ತು ಹಾಲ್ಸ್ಟ್. ಮತ್ತು *ಮೆಲ್ಲೈಡೋಗೈನ್ ಇನ್‌ಕಾಗ್ನಿಟಾ* (ಕೊಫ್ಮೈಡ್ ಮತ್ತು ವೈಟ್) ಚಿಟ್‌ವುಡ್ ರೋಗಾಣುಗಳ ಸಾಂಘಿಕ ಪಾತ್ರ ಮತ್ತು ರೋಗ ನಿರ್ವಹಣೆಯ ಅಧ್ಯಯನ

ಮಧುಶ್ರೀ ಶಿ. ಕೆರಕಲಮಟ್ಟಿ **2018** **ಡಾ. ಆರ್. ಕೆ. ಮೇಸ್ತ**
ಮುಖ್ಯ ಸಲಹೆಗಾರರು

ಸಾರಾಂಶ

ಕರ್ನಾಟಕದ ಪ್ರಮುಖ ವಾಣಿಜ್ಯ ತೋಟಗಾರಿಕೆ ಬೆಳೆಯಾದ ದಾಳಿಂಬೆ *(ಪುನಿಕಾ ಗ್ರಾನಾಟಮ್)*ಯು ಇತ್ತೀಚಿನ ದಿನಗಳಲ್ಲಿ *ಸೆರಾಟೋಸಿಸ್ಟಿಸ್ ಫಿಂಬ್ರಿಯಾಟಾ* (ಶಿಲೀಂಧ್ರ) ಮತ್ತು *ಮೆಲ್ಲೈಡೋಗೈನ್ ಇನ್‌ಕಾಗ್ನಿಟಾ* (ಸಸ್ಯ ಜಂತುಹುಳ) ರೋಗಾಣುಗಳ ಸಾಂಘಿಕ ಸೋಂಕಿನಿಂದಾಗಿ ಸೊರಗು ರೋಗದ ತೀವ್ರ ಬಾಧೆಗೆ ತುತ್ತಾಗಿದೆ. ಈ ರೋಗದ ತೀವ್ರತೆಯನ್ನು ತಿಳಿಯಲು ಉತ್ತರ ಕರ್ನಾಟಕದ ಬಾಗಲಕೋಟೆ ಮತ್ತು ವಿಜಯಪುರ ಜಿಲ್ಲೆಗಳಲ್ಲಿ 2016–17 ಮತ್ತು 2017–18ರ ಅವಧಿಯಲ್ಲಿ ಸಮೀಕ್ಷೆಯನ್ನು ನಡೆಸಲಾಯಿತು. ಇದರನುಸಾರವಾಗಿ ಈ ಎರಡು ಜಿಲ್ಲೆಗಳ ಪೈಕಿ, ಸರಾಸರಿ ಶೇಕಡಾ ರೋಗದ ಸೂಚ್ಯಂಕವು ವಿಜಯಪುರ ಜಿಲ್ಲೆ (7.15%)ಗಿಂತ ಬಾಗಲಕೋಟದ (8.53%) ಜಿಲ್ಲೆಯಲ್ಲಿ ಗರಿಷ್ಠ ಪ್ರಮಾಣದಲ್ಲಿ ಕಂಡುಬಂದಿದೆ. ಎರಡು–ಮೂರು ವರ್ಷಗಳ ಕಡಿಮೆ ಬೆಳವಣಿಗೆ ಇರುವ ತೋಟಗಳಿಗೆ ಹೋಲಿಸಿದರೆ ನಾಲ್ಕು–ಐದು ವರ್ಷದ ಬೆಳವಣಿಗೆ ಇರುವ ತೋಟಗಳಲ್ಲಿ ಸೊರಗು ರೋಗ, ಬೇರುಗಂಟು ರೋಗ ಮತ್ತು ಕಾಂಡದ ಗುಂಡು ರಂಧ್ರಕೊರಕಗಳ ಬಾಧೆ ಹೆಚ್ಚಾಗಿ ಕಂಡುಬಂದಿದೆ. ಗಜ್ಜರಿಯ ಬೇಟಿಂಗ್ ಪದ್ಧತಿಯ ಮೂಲಕ ಸೋಂಕಿತ ಅಂಗಾಂಶಗಳಿಂದ *ಸೆ. ಫಿಂಬ್ರೀಯಾಟಾ*ವನ್ನು ಬೇರ್ಪಡಿಸಿ, ಆಲೂಗಡ್ಡೆ ಡೆಕ್ಸ್ಟ್ರೋಸ್ ಅಗಾರ್‌ನಲ್ಲಿ 25±1^{0}ಸೆ. ಉಷ್ಣಾಂಶದಲ್ಲಿ ರೋಗಾಣುವನ್ನು ಬೆಳೆಸಲಾಯಿತು. ದಾಳಿಂಬೆಯಲ್ಲಿ ಕಂಡುಬರುವ ಬೇರುಗಂಟು ಜಂತುಹುಳುವಿನ ಪ್ರಭೇದವನ್ನು ಪೆರಿನಿಯಲ್ ಪ್ಯಾಟರ್ನ ಮೂಲಕ ಧೃಡಪಡಿಸಲಾಯಿತು. *ಸೆ. ಫಿಂಬ್ರೀಯಾಟಾ* ಮತ್ತು *ಮೆ. ಇನ್‌ಕಾಗ್ನಿಟಾ* ರೋಗಾಣುಗಳ ಸಾಂಘಿಕ ಸೋಂಕು ಕ್ರಿಯೆಯ ಅಧ್ಯಯನವನ್ನು ಕುಂಡದಲ್ಲಿ ಬೆಳೆಸಿದ ದಾಳಿಂಬೆ ಸಸಿಗಳಲ್ಲಿ ಕೈಗೊಳ್ಳಲಾಯಿತು. ಏಕಕಾಲಕ್ಕೆ *ಸೆ. ಫಿಂಬ್ರೀಯಾಟಾ* ಮತ್ತು *ಮೆ. ಇನ್‌ಕಾಗ್ನಿಟಾ* ರೋಗಾಣುಗಳನ್ನು ಹಾಕಿರುವ ಸಸಿಗಳಲ್ಲಿ ಸೊರಗು ರೋಗವು ಅತೀವೇಗವಾಗಿ ಮತ್ತು ಹೆಚ್ಚಿನ ಲಕ್ಷಣವನ್ನು ಹಾಗೂ ಬೇರುಗಂಟುಗಳನ್ನು ಉಂಟುಮಾಡಿತು. ನಂತರದ ರೋಗ ತೀಕ್ಷ್ಣತೆಯು *ಮೆ. ಇನ್‌ಕಾಗ್ನಿಟಾ*ವನ್ನು ಹಾಕುವ 15 ದಿನ ಮೊದಲು *ಸೆ. ಫಿಂಬ್ರೀಯಾಟಾ* ಹಾಕಿದ ಗಿಡಗಳಲ್ಲಿ ಕಂಡುಬಂದಿತು. ಈ ಎರಡೂ ಉಪಚಾರಗಳು ಸಸ್ಯಬೆಳವಣಿಗೆಯ ಮೇಲೆ ಅಂದರೆ ಬೇರು ಮತ್ತು ಚಿಗುರುಗಳ ತಾಜಾ ಮತ್ತು ಒಣ ತೂಕ ಹಾಗು ಅವುಗಳ ಬೆಳವಣಿಗೆಯ ಮೇಲೆ ಪ್ರತಿಕೂಲ ಪರಿಣಾಮ ಬೀರುವುದು ಕಂಡುಬಂದಿತು. ಪ್ರಯೋಗಾಲಯದಲ್ಲಿ ಪರೀಕ್ಷಿಸಿದ ಸಂಪರ್ಕ ಶಿಲೀಂಧ್ರನಾಶಕಗಳಲ್ಲಿ, 0.025% ರಾಸಾಯನಿಕ ಸಾಂದ್ರತೆಯಲ್ಲಿ ಮ್ಯಾಂಕೋಜೆಬ್ 63% + ಕಾರ್ಬನ್‌ಡಾಜಿಮ್ 12%; ಅಂತರವ್ಯಾಪಿ ಶಿಲೀಂಧ್ರನಾಶಕಗಳಲ್ಲಿ, 0.025% ರಾಸಾಯನಿಕ ಸಾಂದ್ರತೆಯಲ್ಲಿ ಹೆಕ್ಸಾಕೊನಾಜೋಲ್ ಮತ್ತು ಪ್ರೋಪಿಕೊನಾಜೋಲ್ ಮತ್ತು 3 ವಿವಿಧ ಟೈಕೊಡರ್ಮಾ ಜಾತಿಯ ಜೈವಿಕ ಶಿಲೀಂಧ್ರನಾಶಕಗಳು *ಸೆ. ಫಿಂಬ್ರೀಯಾಟಾ* ವಿರುದ್ಧ ಹೆಚ್ಚು ಪರಿಣಾಮಕಾರಿಯಾಗಿ ಕಂಡು ಬಂದವು. ಹಾಗೆಯೇ ಫೋರೆಟ್ 0.01 ಗ್ರಾಂ/ಮಿ.ಲೀ. ಮತ್ತು ಬೇವಿನ ಹಿಂಡಿ 0.1 ಗ್ರಾಂ/ಮಿ.ಲೀ. ಸಾಂದ್ರತೆಯಲ್ಲಿ *ಮೆ. ಇನ್‌ಕಾಗ್ನಿಟಾ* ವಿರುದ್ಧ ಪರಿಣಾಮಕಾರಿಯಾಗಿವೆ.

ಕುಂಡಗಳಲ್ಲಿ ಬೆಳೆಸಿದ ಸಸಿಗಳ ಮೇಲೆ ಮಾಡಿದ ಪರೀಕ್ಷೆಯಲ್ಲಿ ಪ್ರೋಪಿಕೊನಾಜೋಲ್ 0.1% ಮತ್ತು ಟೆಬುಕೊನಾಜೋಲ್ 0.1% ಶಿಲೀಂಧ್ರನಾಶಕಗಳು *ಸೆ. ಫಿಂಬ್ರೀಯಾಟಾ* ವಿರುದ್ಧ ಪರಿಣಾಮಕಾರಿಯಾಗಿ ಕಂಡು ಬಂದಿತು. ಹಾಗೆಯೇ ಫೋರೆಟ್ 9 ಗ್ರಾಂ/ಗಿಡ ಮತ್ತು ಬೇವಿನ ಹಿಂಡಿ 50 ಗ್ರಾಂ/ಗಿಡ ಗಳು *ಮೆ. ಇನ್‌ಕಾಗ್ನಿಟಾ* ವಿರುದ್ಧ ಪರಿಣಾಮಕಾರಿಯಾಗಿವೆ. ಕ್ಷೇತ್ರ ಪ್ರಯೋಗದಲ್ಲಿ ಪ್ರೋಪಿಕೊನಾಜೋಲ್ 0.1% + ಫೋರೆಟ್ 18 ಗ್ರಾಂ/ಗಿಡ ಮತ್ತು ಪ್ರೋಪಿಕೊನಾಜೋಲ್ 0.1% + ಬೇವಿನ ಹಿಂಡಿ 100 ಗ್ರಾಂ/ಗಿಡ + ಟ್ರೈ. ಹರ್ಜೀಯಾನಮ್ 100 ಗ್ರಾಂ/ಕುಂಡ ಹಾಕಿದ ಗಿಡಗಳು ಇತರ ಉಪಚಾರಗಳಿಗೆ ಹೋಲಿಸಿದರೆ ಹೆಚ್ಚಿನ ಇಳುವರಿ (19.70 ಮತ್ತು 17.55 ಕೆಜಿ.) ಮತ್ತು ನಿವ್ವಳ ಲಾಭ (` 7,40,423 ಮತ್ತು 9,10,201)ವನ್ನು ದಾಖಲಿಸಿದವು.