STUDIES ON WILT COMPLEX IN CUCUMBER INCITED BY *Fusarium oxysporum* f. sp. *cucumerinum* (OWEN) AND *Meloidogyne incognita* (KOFOILD AND WHITE) CHITWOOD

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AUGUST, 2017

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DEPARTMENT OF PLANT PATHOLOGY
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CERTIFICATE

This is to certify that the thesis entitled '"**STUDIES ON WILT COMPLEX IN CUCUMBER INCITED BY *Fusarium oxysporum* f. sp. *cucumerinum* (OWEN) AND *Meloidogyne incognita* (KOFOILD AND WHITE) CHITWOOD**" submitted in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE) in PLANT PATHOLOGY** to the college of Agriculture, Shivamogga. University of Agricultural and Horticultural Sciences, Shivamogga is a bonafide record of research work carried out by **Mr. KOLI GANAPATI, ID NO. MA1TAE0134** (koliganapati@gmail.com) during the period of study in this university under my guidance and supervision and no part of the thesis has previously formed the basis for the award of any other degree, diploma, associateship, fellowship or any other similar titles.

Shivamogga
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STUDIES ON WILT COMPLEX IN CUCUMBER INCITED BY *Fusariumoxysporum* f. sp. *cucumerinum*(OWEN) AND *Meloidogyne incognita* (KOFOILD AND WHITE) CHITWOOD.

KOLI GANAPATI

ABSTRACT

Cucumber is one of the important vegetable crops,affected by many soil borne pathogens. Among them, Root- knot nematode and *Fusarium oxysporum* f. sp. *cucumerinum (FoC)* are major pathogens. The present study was undertaken with various aspect of cultural and morphological studies, *invitro* and *in vivo* evaluation of chemicals, bio-agents and botanicals revealed that, maximum radial growth and dry mycelial weight of *FoC* was recorded in Potato dextrose agar (90 mm) and Potato dextrose broth (326 mg) respectively. Among the nine cucumber varieties screened against wilt complex under polyhouse condition, none of the varieties showed resistant reaction.Sambar Southe and Uttam showed moderately susceptible reaction to wilt complex and Green long showed susceptible and highly susceptible reaction to root knot nematode. *In vitro* evaluation of fungicides against *FoC,* recorded hundred per cent inhibition of mycelial growthin carbendazim at alltested concentrations (0.05, 0.10 and 0.15 %). Among bio-agents, *T. viride*-II showed maximum per cent inhibition of *FoC*(72.00 %), whereas,*P. Lilacinus* showed higher juvenile mortality of*M. Incognita* (61.33%). Among seven plant extracts, turmeric (68.35 %) and garlic clove extract (65.19 %) at 15 per cent showed maximum inhibition of mycelia growth of*FoC.* The field evaluation of fungicides, bio-agents and soil amendments against wilt complex indicated that, combined application of neem cake at 200 g/m^2+ *P. lilacinus* at 50g/m^2 during sowing showed higher plant growthparameters and less nematode population with least RKI. Whereas, Carbendazim (0.1%) and Carbofuran (3G @ 0.3g a.i/m^2) showed less per cent wilt incidence.

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ಫ್ಯುಸೇರಿಯಂ ಆಕ್ಸಿಸ್ಪೋರಮ್ ಮತ್ತು *ಮೆಲಾಯಿಡೊಗೈನೆ ಇಂಕಾಗ್ನೈಟಾ* ದಿಂದ ಉಂಟಾಗುವ ಸೌತೆಕಾಯಿ ಬೆಳೆಯ ಸೊರಗುರೋಗ ಸಂಕೀರ್ಣದ ಕುರಿತು ಅಧ್ಯಯನ

ಕೋಲಿ ಗಣಪತಿ

ಸೌತೆಕಾಯಿ ಒಂದು ಪ್ರಮುಖ ತರಕಾರಿ ಬೆಳೆಯಾಗಿದ್ದು ಅನೇಕ ರೋಗಗಳಿಗೆ ತುತ್ತಾಗುತ್ತಿದೆ. ಅವುಗಳಲ್ಲಿ ಬೇರುಗಂಟು ಜಂತುಹುಳು ಮತ್ತು ಫ್ಯುಸೇರಿಯಂ ಸೊರಗು ಸಂಕೀರ್ಣವು ಪ್ರಮುಖ ರೋಗವಾಗಿದೆ. ಇದರಿಂದಾಗಿ ಸೌತೆಕಾಯಿ ಉತ್ಪಾದನೆಯಲ್ಲಿ ಹೆಚ್ಚಿನ ನಷ್ಟ ಉಂಟಾಗುತ್ತಿದೆ. ಆದ್ದರಿಂದ ಸೌತೆಕಾಯಿ ಬೇರುಗಂಟು ಜಂತುಹುಳು ಮತ್ತು ಫ್ಯುಸೇರಿಯಂ ಸೊರಗು ಸಂಕೀರ್ಣ ರೋಗದ ಕುರಿತು 2016–17 ರ ಮುಂಗಾರು ಹಂಗಾಮಿನಲ್ಲಿ ಸಸ್ಯರೋಗ ಶಾಸ್ತ್ರ ವಿಭಾಗ, ಕೃಷಿ ಮಹಾವಿದ್ಯಾಲಯದಲ್ಲಿ ಅಧ್ಯಯನವನ್ನು ಕೈಗೊಳ್ಳಲಾಗಿತ್ತು. ಅದರಲ್ಲಿ ವಿವಿಧ ಕೃತಕ ಆಹಾರ ಮಾಧ್ಯಮ ಮತ್ತು ದ್ರಾವಣಗಳಲ್ಲಿ ರೋಗಕಾರಕ ಶಿಲೀಂಧ್ರ ಬೆಳವಣಿಗೆಯನ್ನು ಪರಿಶೀಲಿಸಿದಾಗ ಗರಿಷ್ಠ ಬೆಳೆವಣಿಗೆಯು ಆಲೂಗಡ್ಡೆ ಡೆಕ್ಟ್ರೊಸ್ ಅಗಾರ್ (90 ಮೀ.ಮೀ.) ಮತ್ತು ಗರಿಷ್ಠ ಒಣ ತೂಕವು ಆಲೂಗಡ್ಡೆ ಡೆಕ್ಟ್ರೊಸ್ ದ್ರಾವಣದ (326 ಮೀ.ಗ್ರಾಂ) ಮೇಲೆ ಕಂಡುಬಂದಿತ್ತು. ಒಂಬತ್ತು ವಿವಿಧ ಸೌತೆಕಾಯಿಯ ತಳಿಗಳನ್ನು ಬೇರುಗಂಟು ಜಂತುಹುಳು ಮತ್ತು ಫ್ಯುಸೇರಿಯಂ ಸೊರಗು ಸಂಕೀರ್ಣದ ತೀವ್ರತೆಯನ್ನು ಎದುರಿಸುವ ಶಕ್ತಿಗಾಗಿ ಪಾಲಿ ಮನೆಯಲ್ಲಿ ಬೆಳೆಯಲಾತು. ಅವುಗಳಲ್ಲಿ ಯಾವುದೇ ತಳಿಯು ರೋಗ ನಿರೋಧಕ ಶಕ್ತಿ ಹೊಂದಿಲ್ಲದಿರುವುದು ಹಾಗೂ ಸಾಂಬಾರ್ ಸೌತೆ ಮತ್ತು ಉತ್ತಮ್ ತಳಿಗಳು ಸಾಧಾರಣ ರೋಗ ನಿರೋಧಕ ಶಕ್ತಿ ಹೊಂದಿದ್ದು ಇನ್ನುಳಿದ ಏಳು ತಳಿಗಳು ಅತಿಯಾದ ರೋಗದ ತೀವ್ರತೆಗೆ ತುತ್ತಾಗಿರುವುದನ್ನು ಗಮನಿಸಲಾಯಿತು. ಪ್ರಯೋಗಾಲಯದಲ್ಲಿ ರೋಗಾಣುವಿನ ನಿಯಂತ್ರಣಕ್ಕೆ ವಿವಿಧರಾಸಾಯನಿಕ ಶಿಲೀಂಧ್ರನಾಶಕಗಳನ್ನು ಪರಿಶೀಲಿಸಿದಾಗ, ಶೇ.100 ರಷ್ಟು ಕಾರ್ಬೆಂಡೈಜಿಮ್ ಎಲ್ಲಾ ಪರೀಕ್ಷಿತ ಪ್ರಮಾಣದಲ್ಲಿ ಅತ್ಯಂತ ಪರಿಣಾಮಕಾರಿಯಾಗಿರುವುದು ಕಂಡು ಬಂದಿತು. ಅದರಂತೆ ಜೈವಿಕ ಶಿಲೀಂಧ್ರನಾಶಕಗಳ ಪರಿಶೀಲನೆಯಲ್ಲಿ *ಟ್ರೈಕೋಡರ್ಮಾ ವಿರಿಡೆ* ಶೇ.72.00 ರಷ್ಟು ಶಿಲೀಂಧ್ರವನ್ನು ನಾಶ ಮಾಡುವುದರಲ್ಲಿ ಪರಿಣಾಮಕಾರಿಯಾಗಿರುವುದನ್ನು ಗಮನಿಸಲಾಯಿತು. ಹಾಗೂ ಜಂತುಹುಳು ಮರಣ ಪರೀಕ್ಷೆಯಲ್ಲಿ *ಪಾಸಿಲೊಮೈಸಿಸ್* (ಶೇ.61.33) ಪರಿಣಾಮಕಾರಿಯಾಗಿರುವುದು ಕಂಡು ಬಂದಿರುತ್ತದೆ. ಏಳು ವಿವಿಧ ಬಗೆಯ ಸಸ್ಯಸಾರಗಳನ್ನು ಪ್ರಯೋಗಾಲಯದಲ್ಲಿ ಪರಿಶೀಲಿಸಿದಾಗ ಶೇ.15 ರ ಅರಿಶಿಣ (ಶೇ.68.35) ಮತ್ತು ಬೆಳ್ಳುಳ್ಳಿ (ಶೇ.65.19) ಸಸ್ಯಸಾರಗಳು ಹೆಚ್ಚಿನ ಪರಿಣಾಮಕಾರಿಯಾಗಿರುವುದನ್ನು ಗುರುತಿಸಲಾಯಿತು. ನಂತರರೋಗಗ್ರಸ್ಥ ತಾಕಿನಲ್ಲಿ ಬಿತ್ತನೆಯ ಸಮಯದಲ್ಲಿ ಬೇವಿನ ಹಿಂಡಿ ಮತ್ತು *ಪಾಸಿಲೋಮೈಸಿಸ್ ಲಿಲಾಸಿನಸ್* ಶಿಲೀಂಧ್ರವನ್ನು ಮಣ್ಣಿಗೆ ಬೆರೆಸುವುದರ ಮೂಲಕ ಸಸ್ಯ ಬೆಳವಣಿಗೆಯಲ್ಲಿ ಮತ್ತು ಜಂತುಹುಳುವಿನ ಸಂತತಿ ಕಡಿಮೆ ಮಾಡುವಲ್ಲಿ ಪರಿಣಾಮಕಾರಿಯಾಗಿರುವುದನ್ನು ಗಮನಿಸಲಾಯಿತು. ಕಾರ್ಬೆಂಡೈಜಿಮ್ ಹಾಗೂ ಕಾರ್ಬೋಫ್ಯೂರಾನ್ ಸೊರಗು ರೋಗ ತೀವ್ರತೆಯ ಕಡಿಮೆಮಾಡಿರುವುದನ್ನು ದಾಖಲಿಸಲಾಯಿತು.

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INTRODUCTION

I INTRODUCTION

Cucumber (*Cucumis sativus* L.) is a native of India,widely cultivated plant in the gourd family cucurbitaceae, which includes squash. It is popularly known as "Kakdi" in hindi. Commercial production of cucumber is usually divided into two types. *viz.,* Slicing cucumbers for fresh consumption and pickling cucumbers are produced for processing into pickles. Cucumber is a well known crop throughout the country due to its versatility used as food and medicine. It is also known for its edible fruits because it is delicious, crispy, high in nutrients, low in calories and excellent source of fiber needed for a healthy digestive system. (Floraceli *et al.* 2008) Compared with other vegetables, cucumber occupies fourth place in the world next to tomato, cole crops and onion.

In India, cucumber production is around 678.15 thousand tones with an area of 43.28 thousand ha (National horticulture Board database, 2015) Cucumber family consists of about 118 genera and 825 species (Rai *et al*., 2008).

It is a warm season crop cannot tolerate cold and frost. Cucumber requires minimum temperature of 18 ^{o}C for seed germination, 20 ^{0}C for growth and development with pH of 5.5 to 6.7 for better development. In Sandy loam soils and heavy soils limitable for the crop with gives high yield. Cucurbits share about 5.6 per cent of the total vegetable production of India.

Cucumber is affected by many different fungal, bacterial, viral and nematode diseases. Among fungal diseases *viz*., anthracnose, belly rot, downy mildew, *Fusarium* wilt, gummy stem blight, Scab, *Cercospora* leaf spot are the major one. Amomg bacterial diseases like, Angular leaf spot and Bacterial wilt and viral diseases like, Cucumber Mosaic Virus (CMV), Papaya Ring spot Virus (PRSV), Watermelon Mosaic Virus (WMV), Zucchini Yellow Mosaic Virus (ZYMV) are causes loss. However, Root- knots nematode and *Fusarium* wilt is a major complex disease which cause heavy loss in cucumber production though this disease not recognize easily with complex. The root-knot nematode, soil borne pathogen was first discovered them as "vibrios" on cucumber roots in a greenhouse (Berkeley, 1855) and in Holland by Beijerinck (1883) and are Root-knot > 3000 plants including cucurbits have been recorded as hosts (Sasser, 1977).

The damage caused by root knot nematode is much higher in tropical and subtropical countries (Taylor and Sasser, 1978) and they reduce crop yields considerably both qualitatively and quantitatively (Sasser and Freckman, 1986). Although over 90 species of *Meloidogyne* have been described to date, four species, *viz., M. incognita, M. javanica, M. arenaria* and *M. hapla* are of particular economic importance to vegetable production (Taylor and Sasser, 1978). *M. incognita* is more dominant, accounting for approximately 64 per cent of the total population of the root-

knot nematodes occurring in tropical countries (Sasser, 1979). The menace in cucurbits cultivation is root- knot nematodes and they were responsible for huge annual losses of marketable fruits and vegetables.

M. incognita infection on cucumbers, resulted changes in carbohydrate and protein metabolism and an increase of free amino acids in galls. Incidence of *M. incognita* in Punjab was quantified at 58 per cent and causing per cent annual yield loss for cucumbers plant on-open fields (Anwar and McKenry, 2012). *Meloidogyne* spp, were considered to be the most important parasites of cucumber and caused yellow foliage, unthrifty growth, small slow growing fruits, poor yield, heavy root galling, root decay and reduced root system (Krishnaveni and Subramanian, 2005). *F. oxysporum* f. sp. *cucumerinum* is the most common pathogen on cucumber plants causing *Fusarium* wilt on cucumber and reduced the yield (Ogura, 1992). *F. oxysporum* Schlechtend *is* a common soil borne plant pathogen with a worldwide distribution. As a species, it probably causes more economic damage to agricultural crops than any other plant pathogens Within the species, there is a high level of host specificity with over 122 described formae speciales and races capable of causing vascular wilt disease of many agricultural crops.

Disease complex situation in agricultural crop systems are very common in nature. It has long been shown that fungal pathogens of crop plants interact with plant parasitic nematodes leading to increased disease severity (Atkinson, 1892). There have been a number of reports of disease complexes involving many crops where in plant nematodes were found interacting with fungal or bacterial pathogens. However, reports of disease complexes involving *M. incognita* and *F. oxysporum* f. sp. *cucumerinum*. Since, wilt complex disease causes high yield loss, efforts in the recent past has been initiated on the management of this wilt complex by studying the cultural and morphology of *Fusarium*, management in through host resistance, bio-agents, botanicals and chemicals. However, information with regard to the occurrence and distribution of root-knot nematode on cucumber, availability of tolerant or resistant cultivars and an effective, management of root-knot nematode and *Fusarium* wilt in cucumber are lacking. Keeping the deficit of research in view, a study was undertaken with the following objectives.

1) To study the cultural and morphological characteristics of the *F. oxysporium* f. sp. *cucumerinum.*
2) Screening of cucumber varieties for disease resistance against wilt complex.
3) *In vitro* evaluation of chemicals, botanicals and bio agents against *F. oxysporium* f. sp. *cucumerinum* and *M. incognita.*
4) Management of wilt complex disease under field condition.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

Cucumberis subjected to infect by many soil borne pathogens. Among them, root-knot nematode *M. incognita* and *F. oxysporum* f. sp. *cucumerinum* believed to be associated with wilt complex disease. *F. solani* and *M. incognita* rank high among serious soil borne pathogens affecting cucumber plants and cause root rot and root-knot diseases respectively (Kader *et al,* 2015).

Plant parasitic nematodes interact with different groups of plant pathogens *viz*., fungi, bacteria and other nematodes. However, the interactions of pathogens with nematodes, the wilt inducing fungi and plant parasitic nematodes cause more damage in all the crops throughout the world (Franel and Wheeler, 1993). Not much work has been done on wilt complex disease and the pathogens and literature available as follows.

2.1 Economic Importance

Increase in nematode density and subsequent reduction in yield or other pathogenic effects are directly influenced by the initial density of nematodes in soil (Wallace, 1961; Oostenbrink, 1966). Measurable damage occurs only when population density exceeds certain limit. Thus, for integrated pest management programmes ultimate goal of sampling is relating to numbers and kinds of nematodes to crop performance as well as evaluation and selection of management practices (Barker, 1985).

One of the basic requirements in managing nematodes is to find out the economic importance of the nematode on particular crop based on population densities and distribution, nature and amount of damage besides the losses in yield. (Sasser, 1979).

The pathogen in plant debris survived more than a year in soil, but the propagules on their own survived only four months. Cucumber and soyabean crops were more favorable for dispersal of the pathogen than tomato crops. The fungus spread further in soyabean fields than in those cropped to cucumbers, but the population was greater in the latter. Wilt damage to cucumber was most severe n the first year and decreased in the subsequent year, although the population of the pathogen gradually increased. Later the population remained stable cause damage to the crop was severe. These results indicated that the cucumber wilt pathogen colonizes roots of both host and non host plants in order to survive (Ogura, 1992).

2.2 Pathogenicity

Huda and Zakaria (2014) they were taken 83 *Fusarium* isolates from different *F.* isolats in vegetable crops and they found that, among rotting tissues of nine vegetable crops, namely, cucumber (*Cucumis sativus*),tomato (*Lycopersicon esculentum*), okra (*Hibiscus esculentus*), loofah (*Luffa acutangula*), bitter gourd

(*Momordica charantia*), moringa (*Moringa olifel*), brinjal (*Solanum melongena*), long bean (*Vigna sesquipedalis*) and red chilli (*Capsicum annuum*). The species identified were *F. oxysporum* (22 isolates), *F. semitectum* (19 isolates), *F. solani* (19 isolates), *F. proliferatum* (14 isolates), *F. pseudocircinatum* (four isolates), *F. sacchari* (two isolates), *F. equiseti* (two isolates) and *F. verticillioides* (one isolate) From pathogenicity test, only 21 isolates were found to be pathogenic, causing vegetable rot on their host. The present study showed that *F.* species are prevalent on vegetable crops and the species might be pathogenic or epiphytic.

Forty isolates of *F. oxysporum* isolated from wilting tomato plants were inoculated to four tomato cultivars to examine pathogenic reactions. Isolation rates of *F. oxysporum* f. sp. *lycopersici* races 1 and 2, and *F. oxysporum* f. sp. *radicis-lycopersici* were 3.5, 24.5 and 57.5 % respectively (Kim-Jongtae *et al.,* 2001).

2.2.1 Symptomotology

Michail *et al.* (1989) found that *F. oxysporum* f. sp. *cucumerinum* and *F. oxysporum* f. sp. *niveum* were to be externally and internally seed born in cucumber and watermelon, respectively. Each pathogen caused severe symptoms of wilt on the affected plants.

Ogura (1992)studied the dispersal and survival ability of *F. oxysporum* f. sp. *cucumerinum* in soil. He reported that, the pathogen in plant debries survived more than one year in soil, but propagules on their own survived only four months. Species of *Fusarium* are responsible for vascular wilt ad cause crown , Root rot and fruit rot of of squash and pumpkin (Zitter, 1996) in addition to it reduced in seed germination (Shakir and Mirza, 1992).

Noling and Dunn (1997) typical symptoms of nematode injury can involve both above ground and below ground plant parts. Foliar symptoms of nematode infestation of roots generally involve stunting and general unthriftiness, premature wilting, and slow recovery to improved soil moisture conditions, leaf chlorosis (yellowing), and other symptoms characteristic of nutrient deficiency. An increased rate of ethylene production, thought to be largely responsible for symptom expression in tomato, has been shown to be closely associated with root-knot nematode root infection and gall formation. Plants exhibiting stunted or decline symptoms usually occur in patches of non uniform growth rather than as an overall decline of plants within an entire field.

Punja and Parker (2000) reported that disease could develop at 17°C and 24°C but not at 32°C. Infection of seedlings can occur within the first four weeks of growth. Wounding of roots and low temperatures during seedling development and fruit load on mature plants enhance disease severity.

Cerkauskas *et al.* (2001) observed foliar chlorosis on the lower foliage of stem and root rot affected cucumber plants. The basal stem tissue developed a yellow

butt discoloration with superficial rot, followed by advanced stages of stem disintegration accompanied by the production of white buff fungus mycelium and orange spore masses externally and yellowish or reddish brown discoloration of vascular tissue that extended up to 5-6 cm.

Punja *et al*. (2001) identified the pathogen as *F. oxysporum* f. sp. *radicis cucumerinum*. The crops like Muskmelon (*Cucumis melo*), squash (*Cucurbita Pepo*), watermelon (*Citrullus vulgaris, C. lanatus*), and grourd (*Luffa aegyptiaca)* developed symptoms on root and stem similar to those on cucumber when inoculated by root dip method.

Moreno *et al*. (2001) observed that, stem lesions extended up to 10 to 12 cm above the crown in affected plants. In the advanced stages, abundant orange sporodochia were observed on crown and tem lesions without vascular discoloration. The causal pathogen was identified as *F.oxysporum* f. sp. *radicis cucumerinum.*

Punja *et al.* (2001) reported the wilt symptom of muskmelon as initially yellowing and wilting of leaves followed by plants death prior to flowering. The crown area of diseased plants had dark brown lesions that progressed into lateral branches for distances of 45 -75 cm. Some lesions also extended into the developing fruit, which frequently became infected and rotted stem lesions were a accompanied by dark brown exudates and necrosis extended tin to the cortical and vascular regions.

Martinez *et al*. (2003) observed symptoms such as yellowing, necrotic steaks on the stems and vascular discoloration finally wilting of plants.

Egenl *et al.* (2005) observed symptoms like partial wilting and vascular discoloration which were typical *Fusarium* wilt symptoms and the suspected causal agent was *F. oxysporum* f. sp. *niveum* in water melon in water melone.

2.3To study the cultural and morphological characteristics of the *F. oxysporum* f. sp. *cucumerinum*

Fusarium taxonomy creates confuse and different classification systems have been reported (Nelson, 1991). Morphology, shape, size, conidia and pigmentation production are based on media and fluctuation in environmental conditions (Cherbawy *et al*, 2009).

2.3.1 Cultural and morphological characteristics

Major (1923) studied the cultural characteristics of *F. solani* (isolated from wilted lupins) and *F. orthoceras* from asters on various natural and synthetic media and observed that the type of growth varied on different media, from complete absence (*F. solani* on Richard's media). Coloration varied broth in intensity and in the actual colour produced.

Prasad (1949) studied thirty three strains of *F. solani* and *F. cucurbitarieae* which were found to differ from each other in culture type, late of growth pingmentation and size of macroconida.

Venkataraman (1955) observed that culture of *Fusarium* wilt of muskmelon produced fluffy mycelium with sparse number of conidia differing with 'wild type' strain having abundant sporulation.

Booth (1971) studied that, considered spore morphology was the major character in the identification of *Fusarium*. Conidia produced were as simple or polyphialidic slime spores or as enteroblastic spores. Chlamydospores were formed as the resting spores. Conidia might occur as 0-1 septate, pyriform, fusoid to oval microconidia as straight or curved, 0-10 or more septate macroconidia and the size varied from 17-35 x 3.5µm to 135-200 x 12.5-20µm.

Potato dextrose agar, Richard's agar and Czapek's agar provided maximum growth and sporulation of *F. oxysporum* f. sp. *niveum* (Jhamaria, 1972).

Nelson *et al.* (1983) reported that, Microconidia are one or two celled, and are the type of spore most abundantly and frequently produced by the fungus under all conditions. It is also the type of spore most frequently produced within the vessels of infected plants. Macro conidia are three to five celled, gradually pointed and curved toward the ends. These spores are commonly found on the surface of plants killed by this pathogen as well as in sporodochia like groups. Chlamydospores are round, thick-walled spores, produced either terminally or intercalary on older mycelium or in macroconidia.

Smith *et al.* (1988) reported that, in solid media culture, such as potato dextrose agar (PDA), the different special forms of *F. oxysporum* can have varying appearances. In general, the aerial mycelium first appears white, and then may change to a variety of colors - ranging from violet to dark purple - according to the strain (or special form) of *F. oxysporum*. If sporodochia are abundant, the culture may appear cream or orange in color.

Sowmya (1993) studied four isolated of panama wilt pathogen (*F. oxysporum* f. sp. *cubense*) on different nutrient media and observed maximum growth and sporulation of the pathogen on Potato sucrose agar and Richard's media.

Venkataraman (1995) observed that monoconidial lines of the linseed wilt pathogen isolated from different linseed growing regions differed in their cultural and morphology charcters with marked diversity in virulence.

Jeswani *et al.* (1997) demonstrated that single spore isolated form single strain also varied among themselves with regard to growth pattern, segmentation and capacity of selecting metabolic products. Tsay (1998) The pathogen of *Fusarium.* wilt of wax

gourd (*Benincasa hispida*) was observed in Taiwan . The pathogen produces three types spores. Microconidia are slightly sickle shaped, mostly 3-5 septate, and 20-45 µx 2.5-5µ m.

Pandey *et al.* (2011) reported that, maximum growth of the pathogen was obtained on Richard's medium (205.5 mg) followed by Nash and Synder (138.9 mg), Czapek's medium (136.5 mg) and Potato dextrose medium (130.6 mg), whereas it was least in modified Asthana and Hawker's medium (42.3 mg) and Glucose Asparagine medium (46.6 mg). The sporulation was excellent on the Richard's medium followed by Potato dextrose medium and Asthana and Hawker's broth the medium. Microconidia were formed on Joffe's medium and macroconidia on Glucose Asparagine medium. Chlamydospore formation was fair on oatmeal medium, Nash and Synder, Potato dextrose and Czapek's medium. Those media which represented bestin enhancing the mycelial growth, exhibited good results in chlamydospore formation.

Duan *et al*. (2004) worked on morphology of *Fusarium* and based on the colony morphology the isolated were divided into three groups. The aerial hyphe of group white and villiform and the colony colour was white light violet. The aerial hyphae of group II were white wadding with a light violet colony colour. The aerial hyphae of group III were not well developed and the colony colour was white or light violet.

Susan (2005) divided different *F. oxysporum* f. sp. *cubense* isolates, according to their morphology, into three morphological types, namely sporodochial, cottony and slimy pinnotal, out of this sporodochial type was the most dominant morphological type.

Kulkarni and Sumitra (2006) worked on diffetent media among them, ten solid media showed maximum radial growth of *F. oxysporum* was observed on potato dextrose agar followed by Richard’s agar. The least mycelial growth was observed is potato carrot agar and malt extracts. Mycelium was pink in potato dextrose agar and Sabourd’s agar.

Khan and Khan (2012) studied the effect of five culture media on mycelia growth of *F. oxysporum* f. sp. *ciceri.* The pathogen produced maximum growth on Potato dextrose agar followed by Richard’s and Czapek’s medium.

Majdah and Altuwaijri (2015), reported that, Potato Dextrose medium was the best medium for linear and amount of growth for all tested isolates, whereas, rate of growth varied between the different tested isolates with the different tested media.

2.4 Screening of cucumber varieties for disease resistance

Plant parasitic nematodes are known to break the disease resistance of crop variety to a given pathogen. Endoparasitic nematodes such as root-knot and cyst nematodes have long been known as primary pathogens due to their ability to

predispose plants to infection by secondary pathogens such as several species of *Fusarium, Phytophthora* and *Rhizoctonia*. However, a vast majority of nematode infections involved especially root-knot nematode andwilt inducing fungi (Francl and Wheeler, 1993).

Phukan and Bharali (1996) reported that, six cucumber varieties tested in pot experiment against *M. incognita* only Poinsette was moderately resistant and the rest were susceptible, or susceptible (Pura snyog, khira-90, EX 173934, priya and 20-3-3-1).

Krishnaveni and Subramanian (2005) reported that, after inoculated with 0, 10, 100, 1000, 10 000, 100 000 juveniles per plant and plant showed reduction in the plant growth, yellowing and dropping of leaves and also significantly reduction in growth and yield and finally they got 100000 nematode per plot.

Ozarslandan and Elekcioglu (2003) genotypes are evaluated under controlled conditions. To know resistance of twenty eight cucumber, twenty six tomato and sixteen pepper (*Capsicum annuum*) cultivars to *M. javanica* race-1 and *M. incognita.* race-2 were All cucumber cultivars showed susceptible to *M. javanica* and *M. incognita* and all tomato cultivars were susceptible to *M. Incognita*, except three genotypes showed (144 RN, N F1 and 1077) resistant to *M. javanica*. All pepper cultivars were resistant to *M. javanica* but were susceptible to *M. incognita* and also showed that all pepper cultivars remained resistant to *M. javanica*.

Praveen and Nanjegowda, (2004) screened cucumber varieties evaluated against *M. incognita* among varietiesthe Poinsette was moderately resistant and proved to possess factors for immunity to *M. incognita.*

Bansa *et al.* (2004) studied combined effect of root-knot nematode, *Meloidogyne javanica* and wilt pathogen, *F. udum* in ten wilt resistant/tolerant accessions of pigeonpea. Presence of *M. javanica* with *F. udum* increased wilting from 8 to 33 per cent in KPL-44, 15 to 0 per cent in AWR-74/15, 25-50 per cent in ICP 8859 and ICPL 89049 and 15-50 per cent in ICP 12745. Whereas, other five accessions wilting did not increase much in presence of nematodes. The plant weight, fresh shoot and root weight and dry shoot and root weight were significantly lower in combined inoculations of nematode and fungus compared to nematode or fungus alone. The root-knot index varied from 3.00 to 4.50. In these accessions in both treatments having nematode alone and nematode fungus together. The lowest root-knot index was observed in KPL 43 (1.50) and GPS 33 (1.75).

Akhtar *et al.* (1998) evaluated 33 cultivars of chickpea against *M. incognita* and *F. oxysporum* f. sp. *ciceri* disease complex and they found cultivars like BGM-547, RSJ-823 and RSJ-865 were found resistant against disease complex. The root-knot index and disease index varied from 0.5 to 0.75 and 5.6 to 10.00 per cent, respectively.

However, cultivars *viz.*, PUSA-372, CSJ-103, CSJ-353, CSJ-313, CSJ-390 and CSJD-125 were shown moderately resistant against both the pathogens.

Haseeb *et al.* (2005) evaluated 33 cultivars of lentil for resistance, against *Meloidogyne incognita* and *F. oxysporum* f. sp. *lentis* complex. Among 33 cultivars, only six cultivars *viz.*, L-45-97, L-4147, L-4666, HUL-60, LL-887 and PL-01 were found resistant to moderately resistant against disease complex. The root-knot index and disease index varied from 0.5 to 1.50 and 10.0 to 29.70 per cent, respectively

Fifteen cultivars and lines of cucumber (*Cucumis sativus*; Ps-29033, Rubah, Super Monarch, Royal 21445, Sina (Rs 24189) F1, Gb, Luna F1 Hybrid, Spark, 2201 Rz F1, Vikima-982, Danito F1, Zenubi, Vilmorin, Super Dominus and Hamadan), were evaluated for resistance to *Meloidogyne incognita* race 1 under greenhouse conditions at 22-32°C. The results indicated that all the evaluated cultivars and lines were susceptible to *M. incognita* race 1. (Moosavi *et al*., 2006)

The relative susceptibility of cucumber (*Cucumis sativus*) cultivars Alzaeem, Royal Sluis, Alnems and Hybrid Prince as well as squash (*Cucurbita pepo* var. *melopepo*) cultivars Arlika, Alexander and Alexander Hybrid to *Meloidogyne javanica* was investigated in greenhouse tests Only Alzaeem and Arlika cultivars were tolerant to nematode (Elgawad *et al*., 2007)

De *et al*. (2009) the eight *Cucumis* germplasms were selected for evaluation of their resistance to the root-knot nematodes *Meloidogyne incognita*. The results showed that the two *Cucumis* wild relatives were highly resistant to *Meloidogyne incognita*, and the six varieties of cucumber were all susceptible

Markova and Yankova (2010) conducted an experiment at Maritsa Vegetable Crops Research Institute to determine the response to root-knot nematodes (*Meloidogyne* spp.) in three vegetable crops (tomato, cucumber and pepper) grown in mid-early production in unheated cultivation facilities was determined. The highest percentage of infested plants 64.40% and index of infestation 32.82 per cent were established in tomato variety Belle. In cucumber variety Mirey the percentage of infested plants is 36.20 per cent and index of infestation 20.67 per cent. The lowest values of investigated indicators are in pepper variety Piruet 4.82 per cent and 12.19 per cent.

Aboulipour *et al*. (2011) studied the reaction of 15 cucumber cultivars to the root-knot nematode, *M. javanica*, the growth factors of the host plants and nematode reproduction were used with different methods. Results showed that all the greenhouse cultivars used in the experiment were susceptible to *M. javanica* and only two local cultivars from Isfahan (Chambar and Dastgerdi) recognized as tolerant and the amount of vitamin - C, Calcium and Potassium minerals as well as leaf area index decreased in infected plants.

De You *et al*. (2011) inoculated seedlings of cucumber with *M. incognita* in greenhouse. Progenies from interspecific cross between cultivated cucumber and sour cucumber were screened and an introgression line ILs-10-1 was identified with resistance to the root-knot nematode, *M. incognita* following resistance evaluation, morphological observation and molecular characterization. The results showed that the ILs-10-1 displayed stable resistance to *M. incognita*.

Sung *et al.* (2012)The study was conducted to evaluate the resistance of commercial cucurbit cultivars (21 cultivars of cucumber, 9 cultivars of watermelon, 7 cultivars of oriental melon, and 2 cultivars of melon) to powdery mildew and root-knot nematode. One cucumber cultivar, 'Baegbongdadagi' was moderately resistant and the others were susceptible to root-knot nematode. In case of watermelon, 'Dalgonakkul' was resistant and the others were moderately resistant or susceptible to root-knot nematode. All examined oriental melon and melon cultivars were susceptible to root-knot nematode.

Tariq *et al.* (2013) the 15 cultivars of cucumber (*Cucumis sativus*) were tested for their response to *Meloidogyne incognita*. No single cultivar showed resistence or highly resistence to *Meloidogyne incognita* except Long Green was the only cultivar which was found resistant against *M. incognita* as it showed minimum galls (8.2) and reductions in growth parameters. Mehran, Mirage, Thamin-II, and Royal Sluis cultivars were highly susceptible as evident by maximum galls (>100). Similarly, the cultivars Green Wonder, Cucumber Citriolo and Poinsett weremoderately susceptible (31–70 galls) while Babylon, Cobra and Falcon- 560 (71–100 galls) were susceptible and reductions in growth parameters of these cultivars were less than those in highly susceptible cultivars. Four cultivars, Marketmore, Dynasty, Pioneer-II, and Summer Green, were moderately resistant (11–30 galls).

Majdah and Altuwaijri (2015) Isolated from wilted cucumber plant samples collected from eight different localities in Egypt. These isolates were pathogenic to the susceptible cucumber cultivar "Bi*et al*pha". Isolate 3 was the most virulent, followed by isolate four, while the least pathogenic was isolate 5.

2.5 *In vitro* evaluation of chemicals, botanicals and bio agents against pathogens

2.5.1 In vitro evaluation of fungicides

Kapoor and Kumar (1910) reported that Captan was least toxigenic to *F. oxysporum* and *F. solani* infecting tomato compared to Carbendazim.

Kalra and Sohi (1984) reported that, the system fungicide fundicides *viz*., benomyl, carbendazim and systemic completely inhibited growth o *F. oxysporum* in *vitro*, Difolatan, Dithane M045 and Thiram reduced its considerable but blitox proved almost ineffective.

Narendreappa *et al.* (1995) evaluated different fungicides, among seven fungicides inhibited growth of *F. oxysporum* f. sp. *cepae* tested under in *vitro* condition. Benlate perfomed best at 250 ppm followed by carbendazim, thiram and vitavax at 2000 ppm.

Verma and Dohroo (2002) reported that, the efficacies of different fungicides were determined in *vitro* against *F. oxyporum* f. sp. *pisi* by poisoned food technique. Among the systemic fungicides Carbendazimj, Kri-Benomyl and TBZ were found most effective, giving complete inhibition of the mycelia growth at 25 ppm and higher concentrations followed by Topaz.

Bardia and Rai (2007) also showed per cent inhibition of mycelia growth of *F. oxysporum* f. sp, *cumini* by carbendazim.

Banyal *et al.* (2008) reported that, out of eight fungicides evaluated against *F. oxysporum* under in *vitro* condition. carbendazim have been shown to completely inhibit the mycelia growth of in Richard medium.

Sangeetha and Jahagirdar (2013) in *vitro* screening of fungicides, bioagents and botanicals were taken up to identify an effective molecule against all the three pathogens. *In vitro* studies revealed that Mancozeb, Carbendazim, Thiophanate methyl, Hexaconazole, Propiconazole, Carbendazim + Mancozeb and Carboxin + Mancozeb were more effective in inhibiting the mycelial growth of all the three pathogens.

Ravichandran and Hegde (2015) evaluated six combi-product fungicides, four contact fungicides and four systemic fungicides at three concentrations against *F. oxysporum* f. sp. *ciceri* causing wilt of chickpea. Among combi products, Carbendazim 12% + Mancozeb 63% (Saaf) was effective in all concentrations (0.1, 0.2 and 0.3%) with cent per cent inhibition and least inhibition (21.85%) was recorded in Zineb 68% + Hexaconazole 4%WP (Avatar) @ 0.1% concentration. In contact fungicides, Copper oxychloride was effective with 90.0 % inhibition @ 0.3% concentration and minimum inhibition (44.58%) was in Zineb @ 0.1% concentration. Among systemic fungicides Carbendazim, Tebuconazole recorded cent per cent inhibition at all concentrations, (0.015, 0.075 and 0.1%) where as less inhibition (82.08%) was in the Difenconazole @ 0.1 per cent.

2.5.2 In vitro evaluation Botanicals

Arya *et al.* (1995) studied the antifungal activity of the extracts of various plant species against *F. pallidaorseum* and reported inhibitory effect of extracts of garlic bulbs and Bignonia leaves on the mycelia growth of *F. pallidoroseum.*

The effect of aqueous extracts and oil of neem (*Azadiracha indica* on four soil borne pathogens, *F. oxysporum, Rhizoctonia solani,Sclerotium rolfsii,* and *Sclerotinia sclerotiorum*, which incite wilt and rot in gram was studies, Neem oil showed maximum inhibitory effect, The germination of gram seeds was inhibited at higher concentrations of oil. Oil trated seeds sown in soil infested with the pathogens singly and intermixed produced disease free seedlings where as all the seedling from untreated seeds exhibited disease symptoms (Singh *et al*., 1980).

Thirty one plants belonging to Asteraceae family (Rai and Acharya, 1999) were tested against the cowpea wilt pathogen, *F. oxysporum* f. sp. *ciceris*. Oaer *et al.* (2003) evaluated the pectplytic impact of Allium cepa " Akugum 12" against two *Fusairum* isolates FOC 6 and FOC 8. in addition *A. sativum* was known to act as antifungal activity (Sahavaraj *et al.*, 2006).

Kishore (2007) studied different botanicals agaist *Fusarium* showed that, Among the 11 plant extracts evaluated against *F. oxysporum* f. sp. *dianthi* clove oil found most effective (100%) followed by garlic extract . chilli and neem were less effective and *Clerodendrum inermae,Pongamia pinnata* and *Tridax procumbens* were completely failed to inhibit the mycelia growth.

A study was conducted to gauge the effect of bio control agents and neem based formulation on wilt of carnation. Out of the neem formulations used achook was found to be most effective in managing the disease with 89.63 per cent disease control followed by neemazal. While amongst the bio control agents *T. harzianum* was found to be superior over *Trichoderma viridae* and *Gliocladium virence* in managing the disease (Sunita Chandel and Manica Tomar, 2008).

2.5.3 In vitro evaluation bioagents

Vasudeva (1949) noticed that, the pigeon pea wilt fungus, *F. oxysporum* f. sp. *udum* was adversely affected by *Bacillus subtilis,* which is commonly presented in soil.

Mishra and Goud (1972) reported that, all the strains of *P. fluorescens* isolated from banana rhizospere had significant inhibitory action on the growth of *F. oxysporum* f. sp. *cubense*. Among the strains pfm of *P. fluorescens* had higher inhibitory action than other strain.

Saravana *et al.* (2004) reported that, all the strains of *Pseudomonas fluorescens* isolated from banana rhizpshere had significant inhibitory action on the growth o f *F.*

oxysporum f. sp. *cubense*.Among the strains Pfm *Pseudomonas fluorescens* had higher inhibitory action than other strain.

Thangavelu *et al*. (2004) studied different *Trichoderma* spp. in Tamilnadu and he concluded that, among eleven *Trichoderma* sp. Isolated from different parts of Tamilnadu *T. harzianum* (Th-10) was found to be effective in inhibiting the mycelia growth of *Fusairum* in *invitro* , and recorded the inhibition zone of 1.4 cm.

Chawla and Gangopadhyay (2009) reported that, maximum inhibition of mycelia of *F. oxysporum* f. sp. *cumini* was recorded in presence of *P. fluorescens* (84.2%) followed by *T. viride* -16 and *T. harzianum* -10. among four bacterial bio-agnts and indigenous isolated *Pseudomonas fluorescesns* was most effective followed by *P. fluorescens* PGPR isolate.

Pandey *et al.* (2011) reported that, *T. viride* completely inhibited the mycelaial growth of *F. oxysporum* f. sp. *udum* in dual culture method and was found significantly superior over bacterial bioagent *P. fluorescens* and *P. aeruginosa.*

Rani *et al.* (2009) evaluated six *Trichoderma* and four *Pseudomonas* isolated against *F.* wilt of chilli. Among six *Trichodrma* isolates, maximum inhibition was noticed in *Trichoderma viridae* (indigenous) followed by *T. fluorescens.*

In dual culture a significant reduction was observed in untreated seeds in uninoculated soil was 40 % in coated seeds in inoculated soil was 6.25 % in filtrate treated seeds in inoculated soil was 12.5 % and untreated seeds in inoculated soil was 87 %. Per cent disease control was calculated to be 86.99% for antagonist filtrate treated seeds in infected soil and 93.62 % for antagonist coated seeds in infected soil over untreated seeds in *Fusarium* infested soil taken as check so the strongly suggest that *Trichoderma s*p. can be exploited for the biological control of soil borne pathogens (*F. oxysporum* f. sp. *cucumerinum)* (Srivastava., 2016)

2.5.3.1 Nematode juvenile mortality test

Anita and Selvaraj (2010) revealed that, *T. viride* and *P. lilacinus* were the most effective species against *M. hapla.* The egg parasitic fungus completely parasitized the eggs and depleted the eggcontent. The mortality of the juveniles increased with increase in the concentration andexposure to fungal culture filtrates and their resultindicated that these fungi were highlyeffective in reducing the population of root-knot nematodes in soil.

Ashoub and Amara (2010) studied different bacteria biocontrol agents against second stage juveniles and reported that, bacteria have a greatly significant effectiveness for suppressing *M. incognita*and*B.thuringiensis,P. fluorescens, R. leguminosarum* can achieve *M. incognita* juveniles' mortality to 100% at 72 hrs.

Samina and Reddy (2012) studied *In vitro* Evaluation of Bio-agents against the Root-Knot Nematode. Also there was a positive correlation between incubation period and larval mortality with an increase in incubation period there was increase in larval mortality. At 24 hrs, all the bio-agents recorded maximum larval mortality ranging from 39.33 to 52.33 per cent compared to 63.33 per cent (chemical check).

At 48 hrs also, all the bio-agents recorded highest larval mortality ranging from 44.66 to 62.00 per cent than 72.00 per cent (chemical check) and Larval mortality at 72hrs ranged from 49.66 to 68.33 per cent compared to 79.33 per cent. *T. viride* and *T. harzianum* were more effective in increasing larval mortality.

Belay *et al.* (2015) they studied different biocontrol agents and plant extrats against *M .incognita* (second stage juveniles) and reported that, T. *harzianum.* Inhibited egg hatching of nematode and resulted 84.67-100% mortality of the J2 juveniles of *M. incognita.*

2.6 Management of wilt complex disease

Certain bacterial and fungal bio-control agents are being used to control a wide range of plant parasitic nematodes (Hallamann *et al.* and Meyer *et al.* 2001).

Narendreappa *et al.* (1995) Use of disease free planting material and preplanting dipping in Carbendzim (0.2 %) for 45 minutes and application of lime and neem cake at 1kg /plot before planting or application of urea (200 g per plant plus sugarcane trash mulch) at 5 and 7 months after planting, Carbendazim corn injection with 2 % solution (3ml/plant) or drenching of chemical (0.2%) at 5,7 and 9 months after plating could also be followed for effective control of panama disease in the field.

Haseeb *et al.* (2005) Carbofuran was highly effective against nematode, carbendazim against fungus, *A. indica* seed powder against both the pathogens and both the bioagents were moderately effective against both the pathogens.

Neog and Chauhan (2012) Conducted field experiment evaluated the efficacy of neem (*Azadirachta indica*) cake and mustard oil cake (1.0 litre/ha each) against root-knot nematode on cucumber and green gram. Both oilcakes enhanced the yield, and reduced the root-knot index and final nematode populations.

Indra and Bishnoi (2012) conducted a study on cowpea (variety- RMG 268) and Mungbean (RG 268) among of them recorded significantly higher grain yield and reduced root-knot population over control in cowpea.

Singh and Thumar (2012) reported that *Allium schoenoprasum, Aeglemar melo, Annona squamosa, Azadirachta indica, Calotropis procera, Ficusracemous, Ficus religiosa, Moringa olifera, Pongamia glabra* and *Vitexnegunda* for the management of *M. incognita* on bottle gourd. Among all these botanicals *C. procera* proved to be best in improving the plant growth, development and reducing host infestation followed by

A. indica than other treatments. *T. harzianum* strain ESALQ-1306 was assessed for its potential biological control against *Meloidogyne incognita* race 4 under *in vitro*and greenhouse conditions (Gabriel Moura Mascarin *et al*. 2012)

Zakaria *et al*. (2013) reported that, maximum reduction in gall formation, female numbers, egg-mass production, developmental stages and final population of juveniles in soil in plants treated with bioagents in combination. Even plant growth i.e. length of shoot and root, fresh and dry weight of shoot and root, number of leaves, flowers, fruits and weight of fruits per plant were significantly high compared to control.

Somasekhara *et al.* (2013) evaluated organic amendments for the management of root-knot nematode (*Meloidogyne incognita*) infesting cucurbits in field condition. On bitter gourd, bottle gourd and cucumber the soil application of neem cake at 30 g/plant reduced root-knot nematode incidence and also increased yield.

MATERIAL AND METHODS

III MATERIAL AND METHODS

The present investigation on wilt complex disease of cucumber and its management was carried out during 2016-17 at Department of Plant Pathology, College of Agriculture, University of Agricultural and Horticultural Sciences, Shivamogga.

Field experiment was conducted at Budigere village of Shivamogga distinct during *Kharif* 2016. Studying of cultural and morphological characterstics of *F. oxysporum* f. sp. *cucumerium,* evaluation of botanicals, bio-agents, and chemicals under *in vitro* for the managementand screening of cucumber varieties for disease resistance was conducted. The details of the material used and the methodology followed during the course of investigation are detailed here under.

3.1. General laboratory procedure

3.1.1 Cleaning of glassware

All the glassware used in the studies were cleaned by washing with detergent solution followed by rinsing several times in water.

3.1.2 Sterilization

All the glassware used in the studies were sterilized in an autoclave at 121 °C at 15 PSI pressure for 15 minutes and kept in hot air oven at 60 ºC for one hour. Both solid and liquid media were sterilized at 121 ºC at 15 PSI pressure for 15 minutes.

3.2 Isolation and maintenance of the pathogens

The cucumber plants showing typical symptoms of *Fusarium* wilt was collected from infected field. Standard tissue isolation technique was followed after thorough surface sterilisation of infected plant parts with 1.0 per cent sodium hypochlorite solution for 30 seconds and washed separately in distilled water twice to remove the traces of sodium hypochlorite, if any and then transferred to sterilized Petri plates containing Potato Dextrose Agar (PDA) media.

The Petriplates were incubated at room temperature (27± 1 ^{0}C) and observed periodically for the growth of fungal colonies. The pure colonies which developed from the bits were transferred to PDA slants and incubated at 27± 1 ^{0}C for 15 days.

3.2.1 Identification of the pathogens

The cultures were identified based on spore morphology and colony characters, referring to description by Booth (1971).

3.2.2 Hyphal tip isolation

This method was followed for maintaining pure culture. Dilute spore suspensions (8- 10 macro conidia/ml) of the isolated pathogen (*F. oxysporum* f. sp. *cucumerinum*) were prepared in sterile distilled water. One ml of such suspension was

spread uniformly on two per cent water agar plates such plates were incubated at 27± 1 ºC and periodically observed for the germination of spores under the microscope. Hyphae germination from each end cell of the single spore was traced and marked with the ink on the reverse side of the petri plates. Then tip of mycelia disk was cut and transferred to PDA slants with the help of cork borer under aseptic conditions and incubated at temperature of 27± 1 ºC for 10 days. Later, the mycelial bits of the fungus were placed in the centre of petri plates containing potato dextrose agar medium and incubated at room temperature for 10 days. No saltation or sectoring was observed in the culture and it was concluded that, it was a pure culture of the fungus. Such cultures were used for further studies.

3.2.3 Taxonomic position of F. oxysporum f. sp. *cucumerinum*

Kingdom : Fungi

Phylum : Ascomycota

Class : Sordariomycetes

Sub class : Hypocreomycetidae

Order : Hypocreales

Family : Nectriaceae

Genus : *Fusarium*

Species : *oxysporum*

Sub species : *cucumerinum*

3.2.4 Maintenance of the cultures

The fungal pathogen was sub cultured on PDA slants and allowed to grow at room temperature (27 to 30 ºC) for 10 days and such slants were preserved in a refrigerator at 5˚C and reviewed once in 30 days.

3.2.5 Mass multiplication of the pathogen

Maize grain medium was prepared in order to get maximum inoculum production of the fungus. About 100g of maize grain was taken in 500 ml flasks and for that 10 g of dextrose was added and water added. It was watered to 20 per cent of its weight and sterilized. The pure culture of *F. oxysporum.* f. sp. *cucumerinum*was inoculated separately to the flask under aseptic condition and incubated at 27± 1 ºC for 15 days. The flasks were shaken on alternate days to get uniform growth. The giant culture so obtained was use to incorporate in the soil developing for preparing sick soil for further studies.

3.2.6 Extraction of root-knot nematode

Soil and root samples from 5 to 10 spots were collected randomly with from root zone of infected cucumber crop. Later, a composite sample of 200 cc soil and 5 g roots were put in a polythene bag.

3.2.7 Extraction of nematode population from soil

- Soil sample of 200 cc was washed thoroughly and processed using combined "Cobb's sieving and Baermann's funnel method (Ayoub, 1977) as given below
- Two hundred cc of soil was taken in 1000 ml beaker and sufficient quantity of water was added to make soil solution.
- This was stirred thoroughly and allowed to stand for heavier particle to settle down.
- Then the soil solution was passed through a set of sieves of 100, 250, 325 and 400 mesh size, respectively.
- Residue from 325 and 400 mesh sieves were collected and poured, cover a tissue paper spread on a wire gauge and placed on Baermann's funnel.
- Level of water in the Baermann's funnel was maintained to keep the tissue paper wet and left undisturbed for 48 hrs.
- After incubation of 48 hrs, the volume of suspension was made to 200 ml, out of which 10 ml was pipetted out and used for counting of various plant parasitic nematodes present, nematode population from this was finally estimated for 200 cc soil.

3.2.8 Estimation of nematode population in root samples

Nematode populations in 5 gm of roots were estimated by root incubation method (Ayoub, 1977) as explained below.

3.2.9 Collection of culture of root-knot nematode

Culture of root-knot nematode collected from infected soil was maintained at greenhouse belonging to AICRP (Nematodes), University of Agriculture Horticultural Sciences, Shivamogga. Nematode culture was maintained in glass house and single egg mass was separated of the gall from nematode infected plant and kept in a dish containing distilled water for hatching. Later, hatched larvae were used for inoculating to cucumber grown in sterilized soil for buildup of the single egg mass culture of *M. incognita*.

Procedure:

- Roots were washed with tap water to remove adhered soil particles.
- Washed roots were cut into small bits of 2.5 cm and split longitudinally. These were then placed over tissue paper spread on a wire gauge and kept in a petri plate.
- Level of water was maintained in Petri plate and left undisturbed or 48 hrs.
- Later, the suspension in the Petri plate was collected and observed for nematodes using stereo binocular microscope.

3.2.10. Maintenance and multiplication of root-knot nematode

3.2.10.1 Soil sterilization

1. Red sandy loam soil free from lumps and stones was collected, sieved and mixed thoroughly with compost in 6:1 proportion.

2. Cleaned soil mixture was transferred to a cement tank and four per cent formalin (4%) was added (for every 9" soil bed) for sterilization.

3. Soil bed was made airtight by covering with polythene sheet.

4. Polythene sheet was removed after 48 hrs of fumigation and then the soil was spread to facilitate escape of toxic fumes, if any.

5. Sterilized soil was tested to+ confirm that no living nematodes exist in it.

6. Soil was stored in clean polythene bags for further use in the investigation.

3.2.11 Identification of root-knot nematode species

1) The roots infested with root-knot nematode were washed

2) The females were dissected out from well-developed galls of the root under stereo-binocular microscope and transferred to petriplate 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 drop of glycerine 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 perennial pattern as described by Chitwood (1949).

3.2.12 Taxonomic position of nematode

Phylum : Nematoda

Class : Phasmidia (Secernentia)

Order : Tylenchida

Sub order : Tylenchina

Sub family : Heteroderoidea

Family : Heteroderidae

Genus : *Meloidogyne*

Species : *incognita*

3.2.13 Main morphological characters:

Life cycle: 25-28 Days.

Body: Elongated in larvae and male, typically saccate, spheroid with a distinct neck in female.

Stylet: Male: Strong with rounded knob

Female: more slender than in males or larvae but with strong basal knobs.

Oesophagous : With very large median bulb followed by a short isthumus.

Excretory pore: Often seen with part of excretory tube in the area between posterior stylet knobs and oppose to median bulb.

Vulva and anus: Female: Typically opposite to neck and surrounded by a pattern of fine lines resembling human finger prints.

Spicules: Very near the terminus of males

Second stage juvenile: Measures 415-484 µm in length, 22-27 µm inthickness, tail less pointed, rectum not detailed.Female is swollen 445-765 µm long and 275-520 µm indiameter, spheroid, and perennial pattern with whorl of striae around tail tip.

Male: Measures 1000-2000 µm long and 100-200 µm diameter, lateral field with 6-8 Lateral incisures.

Parasitism and habitat: Endoparasitic on numerous plants. All stages found inside the roots and in soil around the roots.

3.3 Pathogenicity

The pathogenicity test was conducted for *F. oxysporum.* f. sp. *cucumerinum*isolate on cucumber seedlings. The soil was sterilised with four per cent formalin (4%) and filled in the cups, and sterilised soil consisting of soil, sand and FYM

in the ratio of 3:1:1. Further, the sick soil was made by inoculating the giant culture of *Fusarium oxysporum.* f. sp. *cucumerinum*to the sterile soil at rate of 20 g/kg of soil and 1000 nematodes. A control treatment was maintained without adding the inoculum.

Observations were made regularly for the appearance and development of wilt symptoms. After symptom development, the associated fungus was re-isolated and confirmed with original culture of *F. oxysporum* f. sp. *cucumerinum*.

3.4 To study the cultural and morphological characteristics of *F. oxysporum* f. sp. *cucumerinum*

3.4.1 Cultural studies

3.4.1.1 Growth characters on solid media

The cultural characters of studied*F. oxysporum* f. sp.*cucumerinum* on five different non synthetic/semi-synthetic and two synthetic solid and liquid media.

1) *Non synthetic or semi synthetic media*
   i. Potato dextrose agar
   ii. Host leaf extract agar
   iii. Sabouraud's dextrose agar
   iv. Corn meal agar
   v. V8 juice agar
   vi. Oat meal agar
2) *Synthetic media*
   i. Richard's agar
   ii. Czapek's Dox agar

All the media were sterilized at 1.1 kg/cm^2 pressure for 15 min. To carry out the study, 20 ml of each of the medium was poured in 90 mm Petriplates. Such petriplates were inoculated with 5 mm disc cut from periphery of actively growing culture and incubated at 27±1 ºC. Each treatment was replicated thrice. Observations were taken when the fungus covered complete Petriplate in any one of the media. The colony diameter was recorded. The fungus colony colour and margin were also recorded. The data on radial growth was analyzed statistically. The composition of each medium used is furnished below.

I. Potato dextrose agar

Peeled potato	200 g
Dextrose	20 g
Agar-agar	20 g
Distilled water	1000 ml (volume to make up)

Two hundred gm of peeled potato was cut into small bits and boiled in distilled water and extract was collected by filtering through muslin cloth. Dextrose 20 g and agar- agar powder 20 g each were dissolved in potato extract and the final volume was made up to 1000 ml with distilled water. Later, it was sterilized at 1.1 kg /cm^2 pressure for 15 min and preserved for future use.

II. Host leaf extract agar

Healthy cucumber leaves	200 g
Agar	15 g
Distilled water	1000 ml

Two hundread of cucumber leaves were boiled in 500 ml of water for 30 minutes. Extracts was collected by filtering through muslin cloth. The agar was melted in 500 ml of water. Both the solutions were mixed and the volume was made to 1000 ml and sterilized at 1.1 kg /cm^2 pressure for 15 min.

III. Sabourauds dextrose agar

Dextrose	40.0 g
Neo peptone	10.0 g
Agar -agar	20.0 g
Distilled water	1000 ml.

All the ingredients one by one were dissolved in 400 ml distilled water. Agar was dissolved separately in 500 ml distilled water and mixed with the above solution to make up the volume was made to one liter before sterilization.

IV. Czapeck's dox agar

Sucrose	30.00 g
Sodium nitrate ($NaNO_3$)	2.00 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.00 g
Magnesium sulphate ($MgSO_4\ 7H_2O$)	0.50 g
Ferric chloride ($FeCl_3\ 6H_2O$)	0.01 g

Potassium chloride (KCl)	0.50 g
Agar - agar	20.00 g
Distilled water	1000 ml

Agar - agar was melted in 500 ml distilled water. The other ingredients were dissolved in remaining 500 ml of distilled water. The two solutions were mixed thoroughly and the volume was made up to one litre. The medium was sterilized at 1.1 kg /cm^2 pressure for 15 minutes at 121 ºC.

VI. Corn meal agar

Corn meal	60 g
Agar	15 g
Distilled water	1000 ml

Sixty grams of powdered corn meal was placed in clean muslin bag and tied. The bag was steamed in 500 ml of distilled water in a beaker for one hour. Agar was melted separately in 450 ml of distilled water. The boiled corn meal was then strained into the melted agar and the volume was made to 1000 ml and then sterilized at 1.1 kg/cm^2 pressure for 15 min.

VII.V8 juice agar

V8 juice	8.3 g
L-asparagine	10 g
Yeast extracts	2 g
Calcium carbonates	2 g
Glucose	2 g
Agar-agar	20 g
Distilled water (to makeup)	1000 ml

V8 juice agar powder of 44.3 g obtained from Hi media was suspended in 1000 ml distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

VIII. Richard's agar

Sucrose ($C_6H_{12}O_6$)	20.00 g
Potassium nitrate (KNO_3)	2.00 g
Potassium dihydrogen phosphate (KH_2PO_4)	5.00 g
Magnesium sulphate ($MgSO_4.7H_2O$)	2.50 g
Ferric chloride ($FeCL_3.6H_2O$)	0.02 g

Agar -agar	20.00 g
Distilled water	1000 ml.

All the ingredients except potassium dihydrogen phosphate was dissolved separately in 450 ml of distilled water. Agar melted in 500 ml distilled water was mixed with the above solution. Potassium dihydrogen phosphate was dissolved separately in 50 ml water and mixed and final volume of 1000 ml was made-up by addition of distilled water and autoclaved.

XI. Oat meal agar

Oat meal powder	40 g
Agar - agar	20 g
Distilled water	1000 ml

The oat meal powder was dissolved in 500 ml of distil water and agar was melted in 500 ml of distilled water separately. Both the solutions were mixed thoroughly. Then the volume was made up to one litre and sterilized at 1.1 kg/cm^2 pressure for 15 min.

3.4.1.2 Growth characters on liquid media

The liquid media used were same as that of solid media. The composition and preparations of different liquid media used were the same as that of solid media except that the agar-agar was not added. Hundred ml of the medium was added to each of 250 ml flask. All the flasks were sterilized at 1.1 kg /cm^2 pressure for 15 min. Inoculum disc of five mm size was inoculated to all flasks and incubated at 27±1^{0}C for 10 days. Each treatment was replicated thrice. The mycelial mat was harvested; dried in hot air oven at 60-65 ^{0}C and dry mycelial weight was recorded.

3.4.2. Morphological studies

3.4.2.1 Spore shape and size

Spores of *F. oxysporum* f. sp. *cucumerinum* were taken from the culture and mounted on a clean glass slide. Spores were mixed with lactophenol thoroughly in order to obtain a uniform spread, on which cover slip was placed. The average size of the spore was calculated. Microphotographs were taken to show the typical spore morphology of the pathogen.

3.5Screening of cucumber varieties for disease resistance

A pot experiment was conducted during 2016 at glass house, Department of Plant Pathology, UAHS, Shivamogga to screen the reactions of nine varieties *viz*., Sambar Southe, Dharwad green, Green long, White long, Ranebennur local, Uttam, Malini, Harini and Khushi against wilt complex, *F. oxysporum* f. sp. cucumerinum and *M. incognita.* Seeds were sown in earthen pots, containing sterile pot mixture (soil: sand

in 2: 1). After ten days of their establishment, 1000 J_2 hatched juvenilesof *M. incognita* and 30 g giant culture of *F. oxysporum* were inoculated into the individual pots in the root zone. The observation on wilting was taken after 45 days by using 1-9 scale given by nematode Nene *et al.* (1981) and during incidence was calculated by using the following formula

Infection (%)	Rating scale	Reaction
No wilt	1	Resistant (R)
10% or less wilted	2-3	Moderately Resistant (MR)
11-20% wilted	4-5	ModeratelySusceptible (MS)
21-50% wilted	6-7	Susceptible (S)
51% and more wilted	8-9	Highly Susceptible (HS)

$$\text{Per cent wilt incidence} = \frac{\text{Number of plants wilted}}{\text{Total number of plants}} \times 100$$

Further, root-knot index and number of galls per root systems were estimated by using 0-5 scale (Taylor and sasser, 1978) .

Grade	Description	Reaction
0	No galls	Immune
1	1-2 galls / root system	Resistant
2	3-10 galls / root system	Moderately resistant
3	11-30 galls / root system	Moderately susceptible
4	31-100 galls / root system	Susceptible
5	>100 galls/root system	Highly susceptible

3.6 *In vitro* evaluation of chemicals, botanicals and bio-agents against pathogens

3.6.1 Evaluation of fungicides against F. oxysporum f. sp. *cucumerinum*

Five systemic and four systemic fungicides and four combi fungicides were tested using poisoned food technique under *in vitro* conditions. The non systemic fungicides evaluated at 0.1, 0.2 and 0.3 per cent concentrations whereas, systemic fungicides evaluated at 0.05, 0.10 and 0.15 per cent concentrations. The details of the fungicides evaluated are given here under.

3.6.1.1 Poisoned food technique

The poisoned food technique (Shravelle, 1961) was followed to evaluate the efficacy of fungicides in inhibiting the mycelial growth of *F. oxysporum*f. sp. *cucumerinum*. The fungus was grown on PDA medium for 12 days prior to setting up the experiment. The PDA medium was prepared and melted. The fungicidal suspension was added to the melted medium to obtain the required concentrations on commercial formulation basis of the fungicide. Twenty ml of poisoned medium was poured in each sterilized Petriplates. Suitable check was maintained without addition of fungicide. Mycelial disc of 5 mm was taken from the periphery of 12 days old colony was placed in the center of Petriplates and incubated at 27 ±1° C for 12 days and three replications were maintained for each treatment. The diameter of the colony was measured in two directions and average was recorded. Per cent inhibition mycelial growth of the fungus was calculated by using the formula by Vincent (1947).

$$I = \frac{(C-T)}{C} \times 100$$

Where,

I = Per cent inhibition

C = Radial growth in control

T = Radial growth in treatment (fungicide/ botanicals/bioagents)

List of chemicals

a) Systemic fungicides

Sl. No.	Fungicides	Trade Name	Concentrations
1	Carbendazim 50 WP	Bavistin	0.05, 0.10 and 0.15 per cent
2	Difenconazole 25 EC	Score	0.05, 0.10 and 0.15 per cent
3	Hexaconazole 5 EC	Contaf	0.05, 0.10 and 0.15 per cent

4	Propiconazole 25 EC	Tilt	0.05, 0.10 and 0.15 per cent
5	Thiophanate methyl 70 WP	Roko	0.05, 0.10 and 0.15 per cent

b) Non systemic fungicides

Sl. No.	Fungicides	Trade name	Concentration
l	Chlorothalonil	Kavach 75 WP	0.1, 0.2 and 0.3 per cent
2	Copper oxy-chloride	Blitox 50 WP	0.1, 0.2 and 0.3 per cent
3	Mancozeb	Indofil M-45 75 WP	0.1, 0.2 and 0.3 per cent
4	Captan	Captaf 50 WP	0.1, 0.2 and 0.3 per cent

c) Combi fungicides

Sl.No.	Combi fungicides	Trade name	Concentration (%)
1	Vitavax power	Thiram 37.5 + Carboxin 37.5	0.1, 0.2 and 0.3
2	Nativo 75% WG	Tebuconazole 50%+ trifloxystrobin 25%	0.1, 0.2 and 0.3
3	Saaf	Carbendazim 12% + Mancozeb 63%	0.1, 0.2 and 0.3
4	Merger	Tricyclazole 18% + Mancozeb 62 %	0.1, 0.2 and 0.3

3.6.2In vitro evaluation of bio-agents

3.6.2.1 In vitro evaluation of bio-agents against *F.oxysporum* f. sp. *cucumerinum*

The efficacy of seven bio-agents was tested against *F. oxysporum* f. sp. *cucumerinum* for radial growth inhibition on the potato dextrose agar media using dual culture technique under *in vitro* condition.

Bioagents were evaluated for their efficacy through dual culture technique. The bio agents and the test fungus were inoculated side by side on a single petridish containing solidified PDA medium. Four replications were maintained for each

treatment with one control by maintaining only pathogen separately. They were incubated for 12 days. The diameter of the colony of both bioagents and the pathogen was measured in two directions and average was recorded. Per cent inhibition of growth of the test fungus was calculated by using the formula of Vincent (1947).

List of bio-agents used against F. *oxysporum*are mentioned below

1. *Trichoderma harzianum* - I
2. *Trichoderma viride* - II
3. *Trichodermaharzianum*- I
4. *Trichoderma viride* -II
5. *Pseudomonas fluorescens*
6. *Bacillus subtillis*

3.6.2 .2 In vitro evaluation of bioagents against juveniles of M. incognita

culture for the nematode inoculum

Egg masses were handpicked using sterilized forceps from heavily infected roots of root-knot cucumber plants washed in distilled water and then placed in 15 mesh sieves (8 cm in diameter) containing crossed layer of tissue paper and placed in Petri dishes having water just deep enough to contact the egg masses which can help in juvenile hatching that can be used in experiment.

J

The fungal stock culture filtrate was diluted and each dilution was replicated three times, in addition to control treatment (distilled water). Fifty freshly hatched juveniles of *M .incognita* (J_2) in 0.8 ml of distilled water were added to each treatment, and all Petri dishes kept at room temperature (18 to 20 ºC). Observations were made after 24, 48 and 72 incubation hours. Dead (unmoved) juveniles in each petri dish were counted under a stereomicroscope to calculate the per cent mortality of the nematode. After 72 hrs *M. incognita* juveniles were collected from each treatment and kept for 24 hrs in distilled water to ensure nematode immobility.

$$\text{Per cent juvenile mortality} = \frac{\text{No. of dead}}{\text{Total nematode}} \times 100$$

List of bio-agents used

1) *Pseudomonas fluroscens*
2) *Trichoderma viride*
3) *Paecilomyces lilacinus*

4) Bacillus subtilis

3.6.3 In vitro evaluation of botanicals against F. oxysporum f. sp. *cucumerinum*

The present investigation was carried out to evaluate the extracts of seven plant species to know the presence of fungitoxicant properties against *F. oxysporum* f. sp. *cucumerinum* under *in vitro* condition.

3.6.3.1 Preparation of plant based products

Fresh healthy plant parts of 100 g (leaves/bulb/rhizome) as indicated below were collected from field were washed with distilled water and air dried and crushed in 100 ml of sterile water. The crushed product was tied in muslin cloth and collected the filtrate. The prepared solution gave 100 per cent, which was further diluted to required concentrations of 5 per cent 10 per cent and 15 per cent. The extracts were tested against *F. oxysporum* f. sp. *cucumerinum* on the PDA using poisoned food technique under *in vitro* condition as described earlier. The per cent inhibition of growth of the test fungus was calculated by using the formula of Vincent (1947).

List of plant extracts used under *in vitro*condition

Sl. No.	Botanicals	Scientific name	Plant parts used
1.	Turmeric	*Curcurma longa*	Rhizomes
2.	Eucalyptus	*Eucalyptus globules* L.	Leaf
3.	Pongamia	*Pongamia pinnata*	Leaf
4.	Marigold	*Tagetes erecta*	Leaf
5.	Neem	*Azadirachta indica* Juss	Leaf
6.	Garlic	*Allium cepa* L.	Cloves
7.	Tulasi	*Ocimum sanctum* L.	Leaf

3.7 Management of wilt complex disease under field condition

To know the effectiveness of different chemicals, bioagents and soil amendments against cucumber wilt complex was carried out in field at Budigere village of Shivamogga district during *Kharif* 2016.

Design - RCBD

No. of Replication - 3

No. of Treatments -12

Variety - Green long

Treatments	Details
T_1	Application of Neem cake at 200g /m^2 during sowing
T_2	Application of *P. fluroscence* at 50g/m^2 alone during sowing
T_3	Application of *T. harzianum* at 50 g. m^2 during sowing
T_4	Soil application of *P. lilacinus* alone at 50g/ m^2 during sowing
T_5	T_1+T_2
T_6	T_1+T_3
T_7	T_1+T_4
T_8	Carbofuran at 0.3 g a.i/m^2 during sowing
T_9	Carboxil and Thiram 3g/l of water
T_{10}	Carbendazim(0.1%) and Carbofuran.(3G @ 0.3g a .i/m^2)
T_{11}	Cow urine (10 per cent/l)
T_{12}	Control

3. 7.1 Observations recorded

3.7.1.1 Growth parameters of the host

1) Fresh and dry weight of shoot(g)
2) Fresh and dry weight of root(g)
3) Root length and
4) Yield (kg / plot)

3.7.1.2 Nematode parameters

1) Nematode population in soil (200 cc) and root (5 g)
2) Galls per root system
3) Root-knot index

3.7.1.3 Per cent wilt incidence

3.7.1.4 Statistical analysis experimental data

The experimental data collected were analyzed statistically for its significance of difference by the normal statistical procedure adopted for completely randomized design and randomized block design and interpretation of data was carried out in accordance with Walter (1997). The level of significance used in 'F' and 'T' test was P=0.05 and P=0.01. Critical differences were calculated wherever 'F' test was significant.

EXPERIMENTAL RESULTS

IV EXPERIMENTAL RESULTS

Experimental results with respect to the cultural and morphology of *F. oxysporum* f. sp. *cucumerinum*, screening of varieties against disease resistance for wilt complex disease, *in vitro* evaluation of chemicals, botanicals, bio-agents and against *F. oxysporum* f. sp. *cucumerium* and *M. incognita* and management of *Fusarium* wilt complex disease by using bioagnets, chemicals and organic amendments as individual and combined application under field condition have been presented here under**.**

4.1 Isolation, identification and proving the pathogenicity of *F. oxysporum* f. sp. *cucumerinum* and *M. incognita*

4.1.1 Symptomatology

Investingations on visual observations of wilting of cucumber plants were recorded at various stages of the crop growth in pot culture. Wilt symptoms were noticed 30 days after sowing. Wilt affected plants showed various symptoms *viz.,* drooping of lower leaves, yellowing, interveinal chlorosis, stunted growth and ultimately death of entire plants and galls seen in root system The plants showed two types of wilting symptoms *viz.,* complete wilting and partial wilting. The affected plants showed vascular discoloration(plate 1).

4.1.2 Isolation and identification of the pathogen

Standard tissue isolation technique was followed to isolate causal agent from thecucumber stem portion and typical wilt symptom and root-knot root of cucumber.Isolations and sub culturing were repeated several times to obtain pure culture and yield a species of *Fusarium*. Identification of the pathogens was carried out based on morphological characters of the fungus and nematode(plate 2).

4.1.3 Pathogenicity

Pathogenicity test was conducted by following artificial inoculation of soil with *F.oxysporum* f. sp. *cucumerinum* by giant culture prepared by using maize and sand and soil 1:4 ratio in pots. The first symptom was observed at about 30th day after sowing, where primary leaves showed epinasty, by 35th day these leaves showed chlorosis in the interveinal areas with the veins remaining green. In advanced stages the diseased leaves were shriveled and finally the plant wilted by 40th day. Symptoms due to wilting of plants in the pots inoculated with *Fusarium* culture were similar to that of plants wilted in the main field. Reisolation of the pathogen from collar region of plant was made and pathogenic culture obtained was compared with original culture of *F. oxysporum* f. sp. *cucumerinum* and was similar with regard to all morphological characters on PDA and thus the pathogen was identified as *F. oxysporum* f. sp. *cucumerinum* (plate 4).

A. *Fusarium* and *Meloidogyne* infected plant

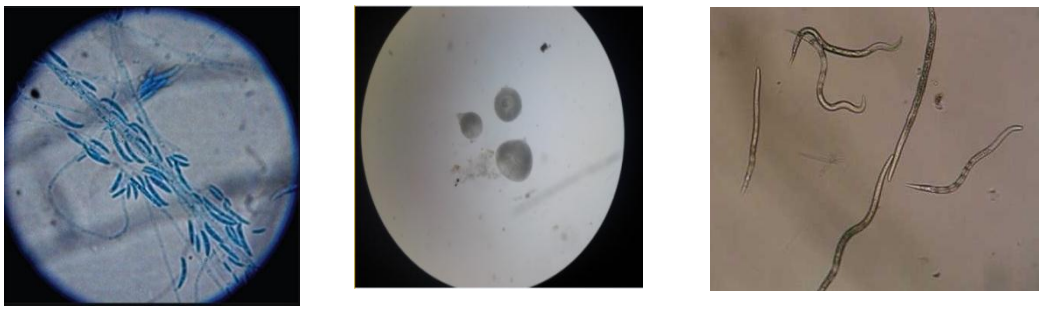
B. Vascular discoloration on stem

C. Galls with vascular discoloration

D. Root-knot galls on root

Plate 1. Symptomatology of wilt complex disease (A - D)

Plate 2: Pure Culture of *F. oxysporium* f. sp. *cucumerinum*



A. Macro Conidia **B. Female with eggs** **C. Nematode Juveniles**

Plate 3. Microphotographs of *F. oxysporium* f. sp. *cucumerinum* (A) and *M. incognita* (B – C)

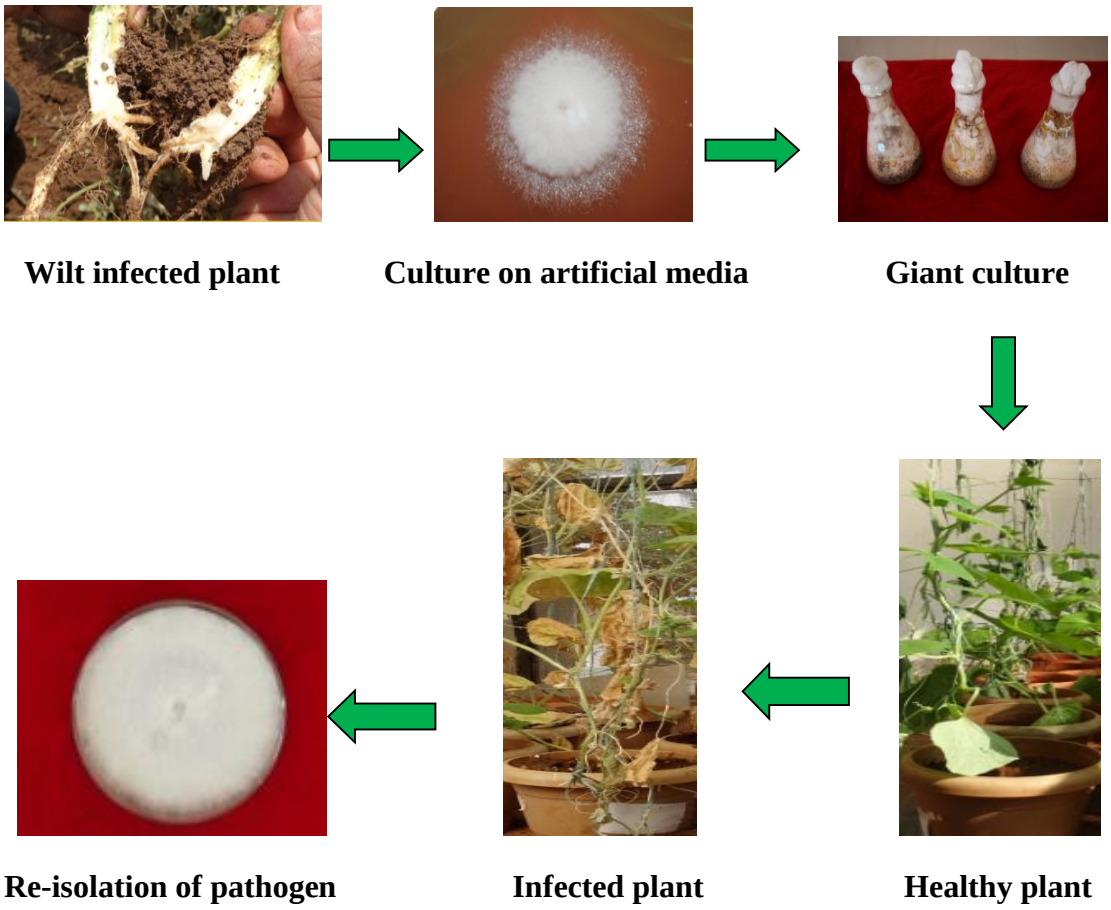


Plate 4. Proving the pathogenicity of *F. oxysporum* f. sp. *cucumerinum* under Glass house

4.1.4 Culture on Potato dextrose agar

Growth and sporulation of *F. oxysporum* f. sp. *cucumerinum* studied on PDA indicated that, the fungus produced white cottony mass consisting of septate, profusely branched hyaline mycelium. It produced three types of asexual spores viz., microconidia and macroconidia AND chlymadospores. Microconidia were produced in chains later detached and were small, elliptical or curved, unicellular or with one or two septa and measured 2.0-10.0 x 0.64 – 3.20 µ m in size. Macroconida were long curved (fusoid) pointed at the tip and knocked at the base, thin walled with two to six septa and measured 6.0-21.0 x 1.0-5.0 µ m in size.(plate 3)

4.2 Cultural and morphological characteristics of *F. oxysporum* f. sp. *cucumerinum.*

Diversity in cultural and morphological characters of *F. oxysporum* f. sp. *cucumerinum* was studied in different solid and liquid media as described in material and methods. The inoculated plates were incubated at room temperature for seven days and the colony diameter, dry mycelial weight, colony characters and sporulation of the fungus were recorded and the results obtained are presented in the table 1.

4.2.1 Growth of F. oxysporum f. sp. cucumerinum on different solid media

The results presented in the table 1 and plate 5revealed that, the different solid and their interaction on increase or decrease in radial growth of *F. oxysporum* f. sp. *cucumerinum* differed significantly.

The fungus showed differences in growth and cultural characteristics on different solid media tested maximum radial growth was observed on potato dextrose agar (90 mm) and richard's media (86.53 mm) followed by v_8 juice agar (84.62 mm),host extract agar (76.74 mm),corn meal agar (59.67 mm), czapecks (Dox) agar (55.80 mm), and oat meal agar (52.23 mm) whereas, least radial growth was recorded in sabouraud's dextrose agar (45.77 mm). hense potato dextrose agar media is superior over compare to all media.

With respect to sporulation, the maximum sporulation was found in Richards agar, potato dextrose and host leaf extract followed by Sabourdsdextrose agar, corn meal agar, V8 juice agar, oat meal agar.

In potato dextrose agar media, and Richards agar media, the fungus produce abundant mycelia growth, dull white in color with pink at centre and white margin, uniformly flat growth, rough margin with compact mycelia. Based on maximum growth, potato dextrose was chosen for to study the effect of growth on solid and liquid med ia, morphological studied in lab.

Table.1. Cultural and morphological characteristics of *F. oxysporum* f. sp. *cucumerinum* on different solid media

Sl. No	Media	Growth character	Radial growth (mm)	Colour of mycelium
1	Potato dextrose agar	Abundant mycelial growth	90.00	White with pink at centre
2	Sabouraud's agar	Moderately mycelial growth, flat edges, smooth margin	45.77	Pure white, pink centre
3	Host leaf extract agar	patchy growth of mycelium	76.74	White colour mycelia
4	V8 juice agar	Moderate mycelial growth	84.62	Pinkish cottony growth
5	Czapecks dox agar	Abundant mycelial growth	55.8	White to pink centre
6	Corn meal agar	Cottony growth, raised and rough growth	59.67	Pinkish white with white centre
7	Richard's agar	Abundant mycelial growth with smooth margins	86.53	White with pink margins
8	Oat meal agar	Moderate mycelial growth, cottony white growth	52.23	White cottony growth
		S.Em±	**0.46**	
		C.D at 1%	**1.91**	

4.2.2 Effect of different liquid media on the growth of F. oxysporum f. sp. cucumerinum

The data presented in the table 2 and plate 6 revealed that, there was significant difference among the different liquid media on growth of *F. oxysporum* f. sp. *cucumerinum.*

Among the isolated and different liquid media statistically significant differences were obtained. Maximum mean dry mycelial weight of *F. oxysporum* f. sp. *cucumerinum* was recorded in potato dextrose broth (326.40 mg) which was found to be significantly superior over all the tested broths. Next best basal media was Richard's broth followed by corn meal broth and least growth was recorded in Sabouraud's dextrose agar media (166.53 mg).

4.3 Screening of cucumber varieties for disease resistance

Nine popular cucumber varieties were screened under polyhouse conditions in order to evaluate the promising variety having desired phenotype characters for tolerance / resistant against *M. incognita* and *F. oxysporum* f. sp. *cucumerinum.* Wilt complex disease and the data are presented in table 3 and plate 7 and 8.

The results revealed that, the Green long variety has showed the maximum per cent wilt incidence of 52.50 per cent followed by White long (47.60 %) and Ranebennure Local (46.80 %). Whereas, minimum per cent wilt incidence was recorded in variety Sambar Soudhe (24.00%) followed by Dharwad Green (26.60 %).

The results indicated that, the cucumber variety Green Long recorded maximum number of galls per root system and root knot index (118.40 galls/ root system and RKI of 5 respectively) followed by Harini (83.80 galls/root system and RKI of 3.67) and Khushi (75.30 galls/ root system and RKI of 4). Whereas, minimum number of galling and RKI was observed in Sambar southe variety (23.60 galls/ root system and RKI of 2.67) followed by Uttam (26.30 galls/ root system and RKI of 3.67).

Based on disease reactionSambar Southe and Uttam showed moderately susceptible reaction to wilt complex and Green long variety showed susceptible reaction to wilt incidence and highly susceptible reaction to root knot nematode.

4.4 *In vitro* evaluation of chemicals, botanicals and bio-agents against pathogens

4.4.1 In vitro evaluation of fungicides against F. oxysporum f. sp. cucumerinum

Screening of fungicides was done against *F. oxysporum* f. sp. *cucumerinum* under laboratory condition by following poisoned food technique as described in "Material and Methods".

Table 2. Effect of different liquid media on the growth of *F. oxysporum* f. sp. *cucumerinum*

Sl. No	Media	Mean dry mycelial weight (mg)
1	Potato dextrose broth	326.40
2	Sabourauds dextrose broth	166.53
3	Host leaf extract broth	304.20
4	V8 juice broth	218.53
5	Czapecks dox broth	200.80
6	Corn meal broth	227.20
7	Richard's broth	312.10
8	Oat meal broth	171.20
	S. Em ±	**1.27**
	C.D at 1%	**5.24**

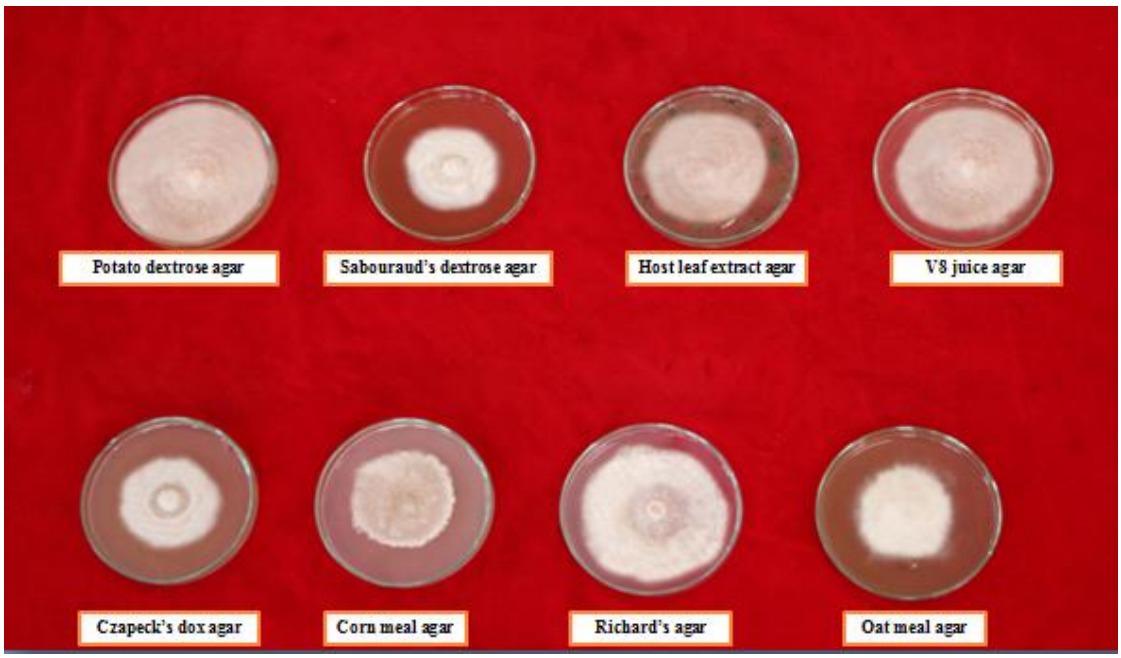


Plate 5. Cultural and morphological characteristics of *F. oxysporum* f. sp. *cucumerinum* on different solid media.

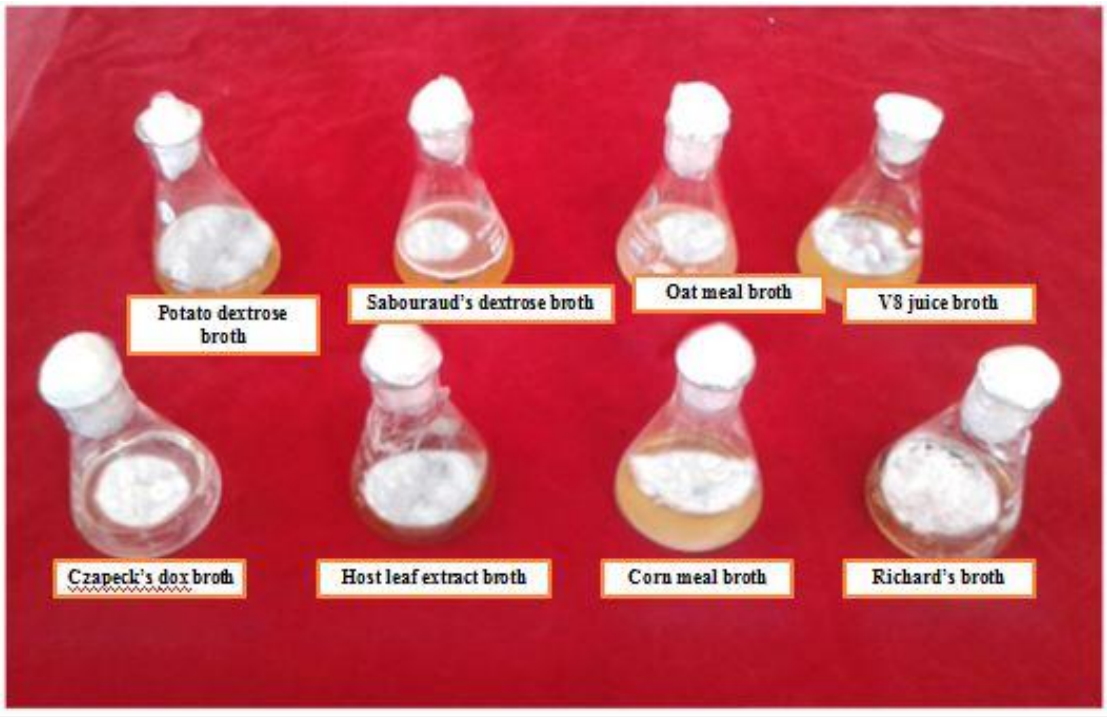


Plate 6. Cultural and morphological characteristics of *F. oxysporum* f. sp. *cucumerinum* on different liquid broth.

Table 3. Reaction of cucumber varieties against wilt complex disease

Varieties	Wilt incidence (%)	Rating scale	Disease reaction	No of galls/root system	Root knot index (RKI)	Disease reaction
Sambar Southe	24.00 (29.35)*	6	MS**	23.60	2.67	MS
Dharwad Green	26.60 (31.06)	7	MS	73.50	3.67	S
Green Long	52.50 (46.46)	9	S	118.40	5.00	HS
White Long	47.60 (43.65)	7	MS	74.10	4.00	S
Ranebennur Local	46.80 (43.19)	7	MS	51.90	3.33	S
Uttam	35.60 (36.65)	7	MS	26.30	2.33	MS
Malini	31.80 (34.34)	6	MS	74.40	4.00	S
Harini	37.20 (37.60)	7	MS	83.80	3.67	S
Khushi	34.40 (35.93)	7	MS	75.30	4.00	S
S. Em ±	0.14	-	-	-		-
CD @ 0.01	0.57	-	-	-		-

*Figures in parenthesis are arc sine transformed values

**HS-Highly Susceptible, S-Susceptible, MS- Moderately Susceptible

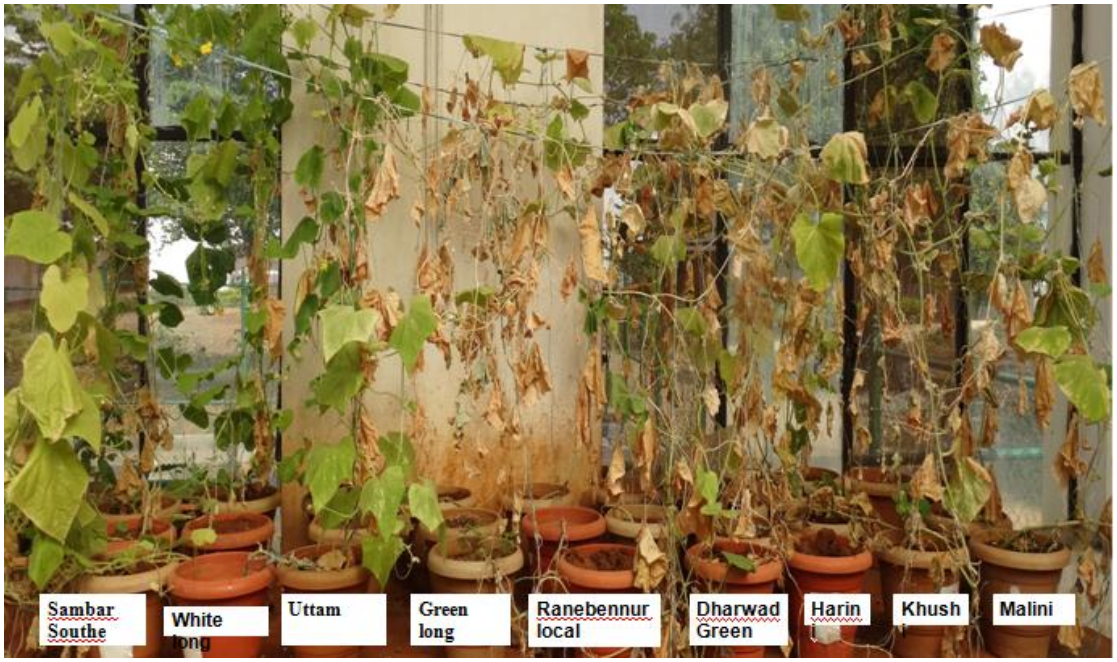


Plate 7. Reaction of cucumber varieties against wilt complex disease in glass house condition

Plate 8. Wilting and galling reaction of different varieties of cucumber screened against wilt complex disease

Five systemic fungicides, four non-systemic fungicides and four combi-products were evaluated against *F. oxysporum* f. sp. *cucumerinum* in laboratory at three concentrations by poisoned food technique.

4.4.1.1 In vitro evaluation of systemic fungicides against F. oxysporum f. sp. cucumerinum

Data with respect to inhibition of mycelial growth of *F. oxysporum* f. sp. *cucumerinum* at three concentrations of five systemic fungicides were recorded and per cent inhibition is presented in table 4 and plate 9.

It was observed that, fungicides, concentrations and their interaction differed significantly with respect to inhibition of the mycelial growth of *F. oxysporum* f. sp. *cucumerinum.*

Among five systemic fungicides, irrespective of concentration maximum per cent inhibition of growth of *F. oxysporum* f. sp. *cucumerinum* was observed in Carbendazim (100%) which was significantly superior to all other fungicides followed by hexaconozole (98.15 %), propiconozole (98.15 %), and difenconazole (84.69 %). The least per cent inhibition of fungus was recorded in thiophanate methyl (75.43 %) at 0.05 per cent concentration.

At 0.15 per cent concentration, significantly maximum per cent inhibition was noticed in carbendazim (100 %), hexaconozole (100 %), propiconozole (100 %) which are on par, followed by defenconazole (93.70%). The least inhibition of mycelia growth was significantly noticed in thiophanate methyl (78.89 %).

At 0.1 per cent concentration, significantly maximum per cent inhibition was noticed in carbendazim (100 %), hexaconozole (100 %) and propiconozole (100 %), which are on par, followed by difenconazole (82.22 %) significantly least inhibition of mycelia growth was significantly noticed in thiophanate methyl (75.19 %).

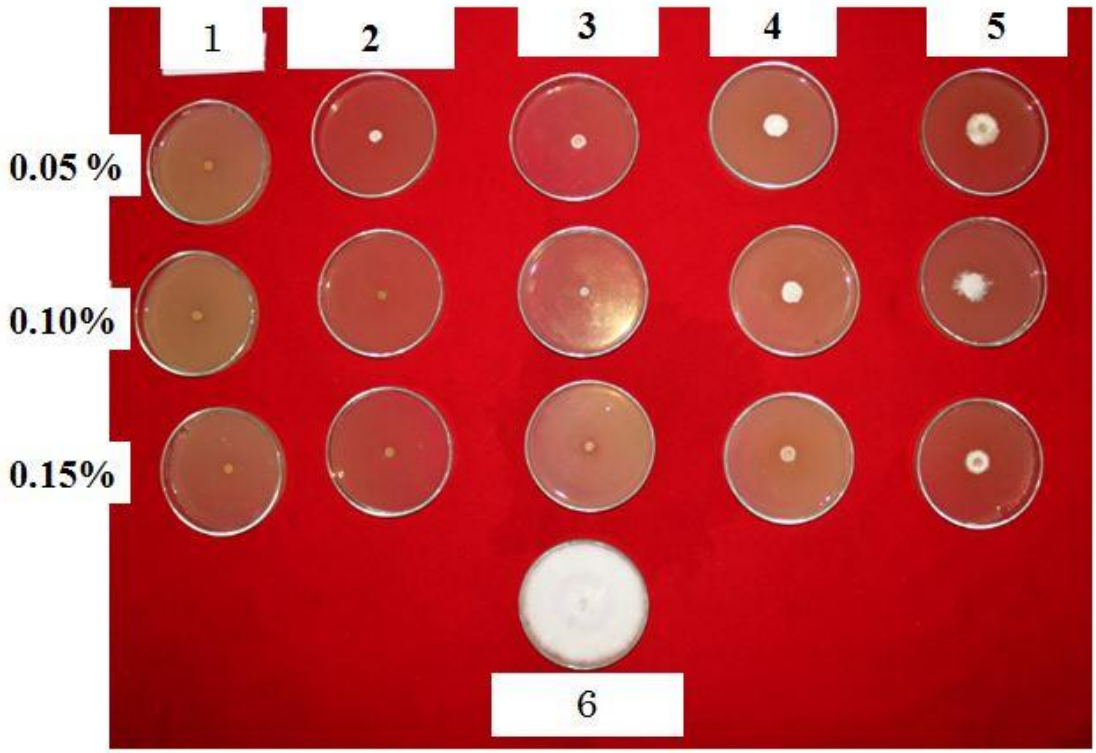
At 0.05 per cent concentration, significantly maximum per cent inhibition was noticed in carbendazim (100 %), followed by hexaconozole (94.44 %), propiconozole (94.44%) which are on par, The least inhibition of mycelia growth was significantly noticed in thiophanate methyl (72.22 %).

Irrespective of all systemic fungicide concentrations, carbendazim (100 %), hexaconozole and propiconozole were found to be the best in inhibiting the mycelia myceal growth followed by difenconazole where as the least mycelial inhibition was noticed in thiophanate methyl as the concentration of fungicides concentration increases the inhibition of mycelia growth also increases.

Table 4.*In vitro* evaluation of systemic fungicides against*F. oxysporum* f. sp. *cucumerinum*

Sl. No.	Fungicides	Per cent inhibition			Mean
		Concentration (%)			
		0.05	0.10	0.15	
1	Carbendazim 50% WP	100.00 (90.05)*	100.00 (90.05)	100.00 (90.05)	100.00
2	Hexaconozole 5% EC	94.44 (76.41)	100.00 (90.05)	100.00 (90.05)	98.15
3	Propiconozole 25 % EC	94.44 (76.41)	100.00 (90.05)	100.00 (90.05)	98.15
4	Difenconazole 25 % EC	78.15 (62.16)	82.22 (65.10)	93.70 (75.51)	84.69
5	Thiophanate methyl 70 %WP	72.22 (58.22)	75.19 (60.15)	78.89 (62.68)	75.43
		S. Em±		CD at 1%	
	Fungicides (F)	0.18		0.69	
	Concentrations (C)	0.14		0.54	
	F x C	0.31		1.20	

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



1. Carbendazim
2. Hexaconazole
3. Propiconazole
4. Difenconazole
5. Thiophanate methyl
6. Control

Plate 9. *In vitro* evaluation of systemic fungicides against *F. oxysporum* f. sp *cucumerinum*

4.4.1.2 Evaluation of non-systemic and combi- product fungicides

In vitro evaluation of non-systemic and combi-product fungicides was conducted with respect to inhibition of mycelial growth of *F. oxysporum* f. sp. *cucumerinum* at different concentrations of non-systemic fungicides and combi-product as explained in "Material and Methods" and the data presented in table 5 and plate 10.

The efficacy of four non-systemic and four combi-product fungicides on inhibition of mycelial growth of *F. oxysporum* f. sp. *cucumerinum* was carried out, among non systemic and combi-product fungicides,

At 0.3 per cent concentration significantly maximum growth inhibition was noticed in copper oxycloride (100%), carbendazim 12% + mancozeb 63 % (100 %), carboxyl 35.5 + thiram 35.5 (100.00 %) followed by tebuconazole 50 %+ trifloxystrobin 25% (93.89%), captan (76.11%) and chlorothalonil (74.07%) Significantly least per cent inhibition was recorded in mancozeb (66.11%).

Similarly at 0.2 per cent concentration, the maximum inhibition was noticed in copper oxycloride (100 %), carbendazim 12% + mancozeb 63% (100 %), tricyclazole 18% + mancozeb 62 % (100 %) which was significantly superior over all other treatments. Significantly least per cent inhibition was recorded in mancozeb (56.48%).

At 0.1 percent concentration, the maximum inhibition was noticed in carbendazim 12% + mancozeb 63% (100 %), followed by tebuconazole50%+ trifloxystrobin 25% (92.78 %) thiram 37.5 + carboxin 37.5 (90.59 %), copper oxycloride (68.48 %) which are significantly superior over all other treatments, Significantly least per cent inhibition was recorded in mancozeb (30.56 %).

Irrespective of all non systemic combi product fungicides carbendazim 12% + mancozeb 63% (100 %) was significantly found to be the best in inhibiting the mycelia growth, followed by tebuconazole50 % + trifloxystrobin 25 % (91.85 %), carboxyl 35.7 % and thiram 37.5 % (91.14 %).

The data from the table revealed that, the efficacy of different non systemic fungicides and combi fungicides, concentrations and their interaction on per cent inhibition of mycelial growth of *F.oxysporum* f. sp. *cucumerinum* differed significantly.

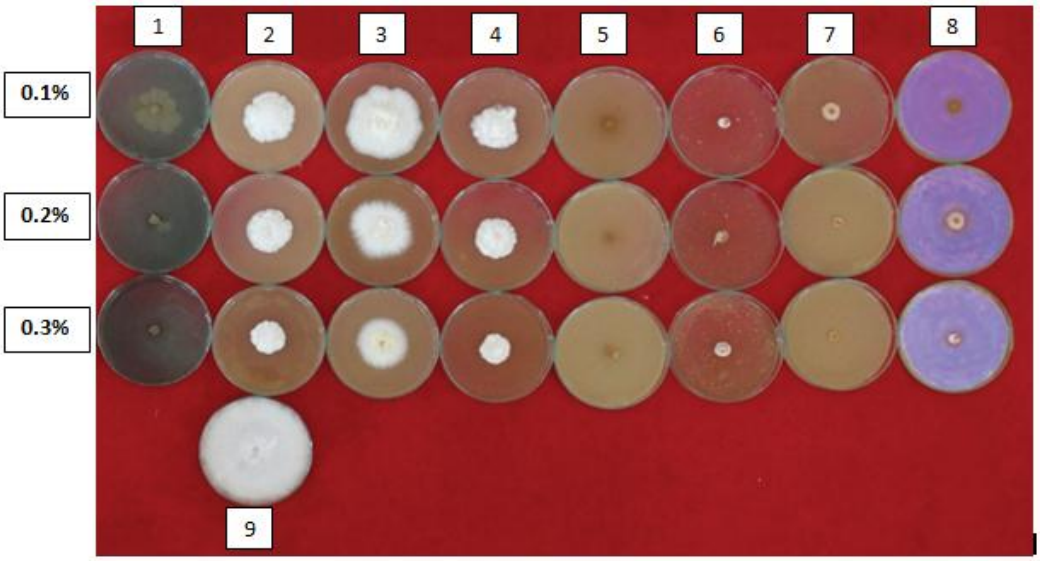
4.4.2 In vitro evaluation of botanicals against F. oxysporum f. sp. cucumerinum

As plant extracts are cost effective and are available cheaply and in plenty, hold promise for their utilization in increasing crop production and in disease management. Therefore an effort was made to know the efficacy of botanicals on inhibition of pathogens. The antifungal activity of seven plant extracts was evaluated

Table 5. ***In vitro*** **evaluation of non systemic and combi fungicides against*F. oxysporum*** **f. sp.** ***cucumerinum***

Sl. No	Fungicides	Per cent inhibition			Mean
		Concentration (%)			
		0.3	0.2	0.1	
1	Copper oxy-chloride 50 % WP	100.00 (90.05)*	100.00 (90.05)	68.48 (55.87)	89.49
2	Chlorothalonil 75 % WP	74.07 (59.42)	66.19 (54.47)	59.78 (50.66)	66.68
3	Mancozeb 75 % WP	66.11 (54.43)	56.48 (48.75)	30.56 (33.57)	51.05
4	Captan 50 % WP	76.11 (60.77)	71.63 (57.85)	66.56 (54.70)	71.43
5	Carbendazim 12 % + Mancozeb 63 %	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00
6	Tricyclazole 18 % + Mancozeb 62 %	100.00 (90.05)	100.00 (90.05)	88.52 (70.07)	96.17
7	Trifloxystrobin 25 % + Tebuconazole 50 %	93.89 (75.74)	92.78 (74.47)	88.89 (70.56)	91.85
8	Carboxin 37.5 %+ Thiram 37.5 %	100.00 (90.05)	94.81 (78.64)	90.59 (72.18)	91.14
		S.Em±		**CD at 1%**	
	Fungicides (F)	0.16		0.60	
	Concentrations (C)	0.10		0.37	
	F x C	0.28		1.04	

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



1. **Copper oxy-chloride**
2. **Cholorothalonil**
3. **Mancozeb**
4. **Captan**
5. **Carbendazim**
6. **Tricyclazole + Mancozeb**
7. **Tebuconazole + Trifloxystrobin**
8. **Carboxin + Thiram**
9. **Control**

Plate 10. *In vitro* evaluation of non-systemic and combi fungicides against *F. oxysporum* f. sp. *cucumerinum*

against *F. oxysporum* f. sp. *cucumerinum* at three concentrations in the laboratory by using Poisoned food technique as detailed in Material and Methods. The experimental results obtained are presented in the table 6 and plate 11.

At 5 per cent maximum per cent inhibition of mycelia growth was noticed in eucaluptus (28.70 %) which was on par with tulasi (28.41%), followed by neem (23.09 %), pongemia (19.90 %), garlic (17.72 %), turmeric (10.16 %), where as significantly least per cent inhibition was recorded in marigold (9.61%). At 5 per cent eucalyptus (28.70%), tulasi (28.41%) and were superior over all other plant extracts.

At 10 per cent, maximum per cent inhibition of mycelial growth was noticed in turmeric (50.85 %), followed by tulsi (37.78 %), eucalyptus (30.60 %), neem (27.93 %), pongemia (27.01 %) and marigold (25.16 %), which was on par with garlic (25.04%). At 10 per cent turmeric (50.85 %) was superior over all other plant extracts.

At 15 per cent, maximum inhibition of mycelial growth was noticed in turmeric (68.35 %), garlic (65 %) followed by eucalyptus (51.00 %), marigold (42.70 %), tulsi (42.22%), whereas, significantly least per cent inhibition was recorded in neem (35.11%). At 15 per cent turmeric (68.35%) was superior over all other plant extracts.

4.4.3 In vitro evaluation of bio-agents

4.4.3.1 Evalutation of bio-agents against F. oxysporum f. sp. cucumerinum

Efficacy of fungal and bacterial bioagents was studied under *in vitro* condition by following dual, culture method as described in "Materal and Methods" and the results are presented in table 7 and plate 12.

There was significant difference among all the tested bioagens. *T. viride*-II (72.00 %) was significantly superior and recorded maximum inhibition of mycelia growth followed by *T. viride-I* (68.22 %) *T. harzianum*-I (64.89 %), *T. harzianum*-II (60.96%), *P. fluorescence* (50.52 %). Whereas, least mycelia inhibition was recorded *in B. subtilis* (49.67 %).

4.4.3.2 Effect of bioagents on juvenile mortality of M. incognita.

The results presented in table 8, indicated that, all the bioagents tested showed maximum juvenile mortality. Also, there was a positive correlation between incubation period and larval mortality. Filtrates of biocontrol was tested *in vitro* for their nematicidal action on *M. incognita*. Data indicated that various biocontrol at their different concentrations were highly deleterious to the nematode. In general, juvenile mortality increased with increase in exposure period. No nematode mortality was recorded in control (distilled water).

Table 6. *In vitro* evaluation of botanicals against*F. oxysporum* f. sp. *cucumerinum*

Botanicals	Per cent inhibition			Mean
	Concentration (%)			
	5	10	15	
Neem (Leaves)	23.09 (28.73)*	27.93 (31.92)	35.11 (36.35)	28.71
Turmeric (Rhizomes)	10.16 (18.59)	50.85 (45.51)	68.35 (55.80)	43.12
Eucalyptus (Leaves)	28.70 (32.41)	30.60 (33.60)	51.00 (45.60)	36.77
Tulasi (Leaves)	28.41 (32.22)	37.78 (37.94)	42.22 (40.55)	36.14
Pongamia (Leaves)	19.90 (26.50)	27.01 (31.33)	49.41 (44.68)	32.10
Garlic (Clove)	17.72 (24.90)	25.04 (30.03)	65.19 (53.87)	35.98
Marigold (Leaves)	9.61 (18.04)	25.16 (30.11)	42.70 (40.82)	25.82
	S.Em±		**CD at 1%**	
Botanicals (B)	0.23		0.87	
Concentrations (C)	0.15		0.57	
B x C	0.39		1.50	

*Figures in parenthesis are arc sine transformed values

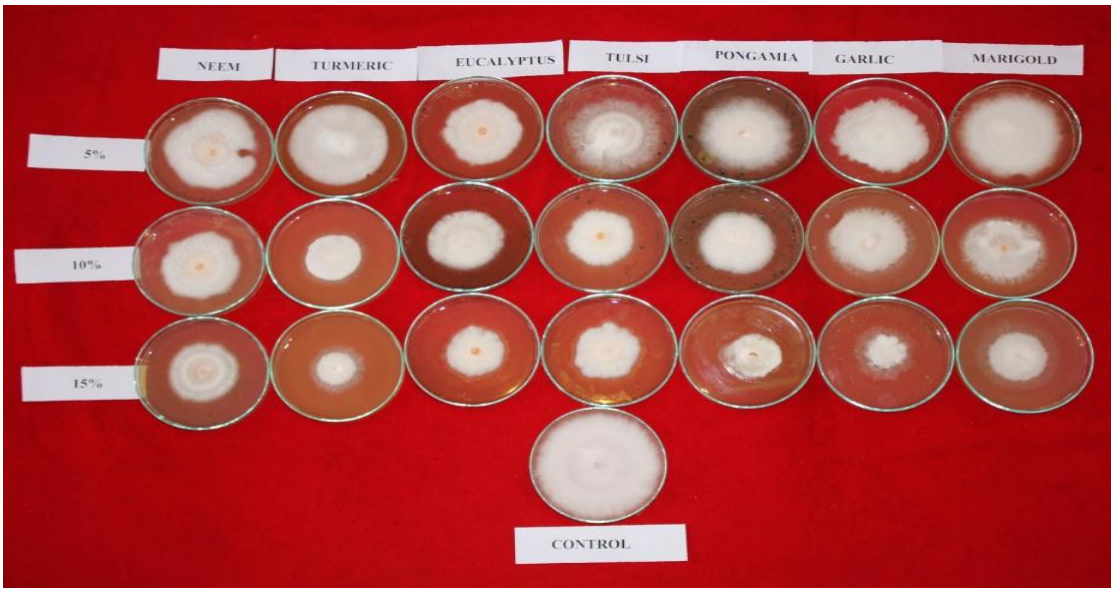


Plate 11. ***In vitro*** **evaluation of botanicals against** ***F. oxysporum*** **f. sp.** ***cucumerinum.***

Table 7. *In vitro* evaluation of bio-control agents against*F. oxysporum* f. sp. *cucumerinum*

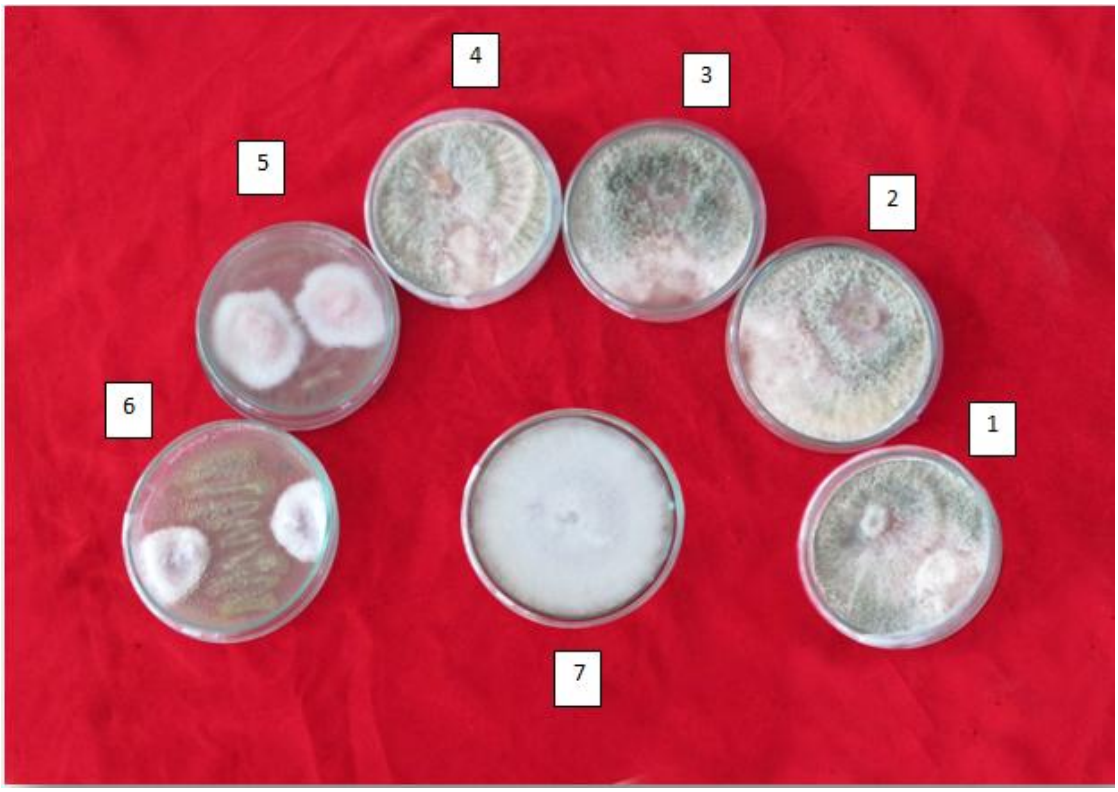
Sl. No.	Bio-control agents	Mean per cent inhibition (%)
1	*T. harzianum- I*	64.89 (53.69)*
2	*T. harzianum- II*	60.96 (51.36)
3	*T. viride-I*	68.22 (55.72)
4	*T. viride-II*	72.00 (58.08)
5	*P. fluorescens*	52.52 (44.83)
6	*B. subtillis*	49.67 (46.47)
7	Control	00
	S. Em±	0.13
	CD at 1%	0.54

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

Table 8: Effect of bio-control agents on juvenile mortality of *M. incognita*

Sl. No.	Treatments	Juvenile mortality (%)			Mean
		24 hrs	48 hrs	72 hrs	
1	*T. harzianum*	24.00 (28.89)*	54.67 (47.70)	62.00 (51.99)	46.89
2	*P. lilacinus*	46.00 (42.73)	66.67 (54.77)	71.33 (57.71)	61.33
3	*B. subtilis*	44.67 (41.96)	48.67 (44.26)	55.33 (48.09)	49.56
4	*P. fluorescens*	40.00 (39.24)	51.33 (45.79)	48 .67 (44.26)	46.67
5	Control (Sterile distilled water)	0.00 (0.00)	4.00 (11.54)	7.33 (15.71)	3.77
		S. Em±		**CD at 1%**	
	Bio-agents (B)	0.60		2.34	
	Hour (H)	0.47		1.81	
	BxH	1.04		4.06	

*Figures in parenthesis are arc sine transformed values



1) *T. harzianum I*
2) *T. harzianum II*
3) *T. viride I*
4) *T. viride II*
5) *B. subtilis*
6) *P. fluorescens*
7) Control

Plate 12. *In vitro* evaluation of bio-agents against *F. oxysporum* f. sp. *cucumerinum.*

At 24 hrs, all the bio-agents recorded larval mortality ranging from 24 % to 40 % than the control (0 %). Maximum juvenile mortality was observed in *B. subtilis* (44.67 %)followed by *P. lilacinus* (46.00 %). Whereas, least juvenile mortality observed in *T. harzianum* as compare to control.

At 48 hrs also, all thebioagents recorded highest larval mortality ranging from 48.00 to 66.00 per cent than 4.00 per cent (control).

Larval mortality at 72 hrs ranged from 48.67 to 71.33 per cent compared to 7.33 per cent (control). Overall, the different bioagents tested, *P.lilacinus* was more effective in increasing larval mortality than others.

4.5Disease management of wilt complex in cucumber under field condition

The experiment was conducted in randomized block design with three replications during 2016 in the farmer field of Budigere village (Shivamogga taluk) on disease management of cucumber wilt complex disease presented in plate 13.

The bio-agents (*T. viride, P. fluorescens* and *P. lilacinus*), organic amendment (neem cake) and chemicals (carbofuran and carbendazim) are evaluated against wilt complex in cucumber. The experiment and treatments were imposed as explained in Material and Methods.

4.5.1 Plant parameters

The results of the trial conducted on efficacy of various treatments on plant parameters *viz*., plant fresh and dry shoot weight, root length, fresh and dry root weight and yield of cucumber. Infected by *F. oxysporum* f. sp. *cucumerinum* and *M. incognita* were analyzed and are presented in table 9.

4.5.1.1 shoot weight

a) Fresh shoot weight

The effect of treatments on the fresh shoot weight of cucumber plant was recorded at the time of harvest. The fresh shoot weight of cucumber was recorded at the time of harvest and was varied from 113.86-365.65 g. The maximum fresh shoot weight was recorded T7 (neem cake + *P lilacinus* (365.62g) followed by T6 (neem cake +*T. harzianum*) (350.37 g) followed by T5 (neem cake +*P. fluorescens)* (333.99g). Whereas,the minimum fresh weight was recorded in untreated check (113.86 g). All the treatments were significantly superior over untreated check. However, treatment which received significantly superior over all other treatments and significantly higher fresh shoot weight.

In general T7 (neem cake + *P. lilacinus)*treated plants were recorded significantly higher fresh shoot weight compared to other treatments.

b) Dry shoot weight

Effect of treatments on the dry shoot weight of cucumber plants were recorded at the time of harvest dry weight of shoot was recorded at the time of harvest ranged 38.07 to 141.49 g. The maximum dry shoot weight was recorded in T7 (neem cake + *P. lilacinus)*followed by T6 (neem cake + *T. harzianum*)(139.26 g), T5 (neem cake + *P. fluroscense*) (130.47 g), The minimum dry weight was recorded in case of untreated check (38.07 g). However, the treatment which received T7 (neem cake + *P. lilacinus*) was significantly superior over all other treatments. The untreated check recorded least dry shoot weight.

In general, carbendazim T7 (neem *cake + P. lilacinus)*treated plants were recorded significantly higher dry shoot weight compared to other treatments.

4.5.1.2 Root length and yield

The observation on root length of cucumber plants were recorded at the time of harvest. The whole root system of the uprooted plants was washed thoroughly and the lengths were measured by using a scale.

At the time of harvest, all the treatments recorded significantly better root length. The maximum root length was observed T7 *(*neemcake + *P. lilacinus*)(33.05 cm) which were superior over all the treatments. Among the treatments maximum root length (33.05 cm) was observed in T7 (neemcake + *P. lilacinus*) followed by T6 (neemcake + *T. harzianum*) (32.17 cm), T5 (neem cake + *P. fluroscense*) whereas, least root length observed in control (0.17cm).

The maximum fruit yield per plot was recorded in T7 (neemcake + *P. lilacinus*) (2.56 kg) treatment and the least fruit yield per plot was observed in control (0.77 kg). All the treatments were significantly superior over untreated check. However, the treatments which received T7(neemcake + *P. lilacinus)* was recorded significantly higher yield compared to other treatments followed by T6 (neemcake + *T. harzianum)* (2.39 kg per plot).

4.5.1.3 Fresh and dry root weight

a) Fresh root weight

The effect of treatments on the fresh root weight of cucumber plant was recorded at the time of harvest. Root weight of cucumber was recorded at the time of harvest and was ranged from 8.07 to 125.49 g. The fresh root weight was significantly higher in untreated check (8.07 g).

The maximum fresh root weight was recorded in T7 (Neem Cake + *P .lilacinus)* (8.07 g) treatment and the maximum Fresh root weight was observed in control (125.49 g). All the treatments were significantly superior over untreated

Table 9. Effect of chemicals, bio-agents and soil amendments on plant parameters

Treatments	Shoot		Root		Root length (cm)	Yield (kg/plot)
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)		
T_1- application of Neem cake at 200g/m^2 during sowing	312.50	104.77	16.63	6.30	26.82	1.77
T_2 -Application of *P. fluroscence* at 50g/m^2 alone during sowing	231.86	85.36	25.17	9.84	24.99	1.36
T_3- Application of *T. harzianum* at 50 g. m^2 during sowing	282.66	92.00	79.79	17.60	25.83	1.73
T_4- Soil application of *P. lilacinus* alone at 50g/ m^2 during sowing	333.99	122.76	11.39	4.40	29.99	1.95
T_5- T_1+T_2	344.55	130.47	11.19	4.19	30.89	2.17
T_6 -T_1+T_3	350.37	139.26	10.38	3.63	32.17	2.39
T_7 -T_1+T_4	365.65	141.49	8.07	3.22	33.05	2.56
T_8 -Carbofuran 3 g a.i.m^2 during sowing	321.70	109.16	15.17	5.68	28.07	1.79
T_9 –Carboxil and Thiram 3g/l of water	133.40	54.57	93.19	35.81	22.3	0.94
T_{10}- Carbendazim(0.1%) and Carbofuran.(3G @ 0.3g a .i/$m^{2)}$	148.18	60.64	84.29	23.78	23.69	1.32
T_{11}- Cow urine (10 per cent)	127.72	42.08	111.50	41.66	22.91	1.11
T_{12}- Control	113.86	38.07	125.49	44.09	18.09	0.87
S.Em±	0.78	0.63	1.04	0.67	0.17	0.03
CD at 5%	2.27	1.84	3.03	1.96	0.50	0.10

check. However, the treatments which received T7 (neem cake + *P. lilacinus*) was recorded significantly higher Fresh root weight compared to other treatments followed by T6 (neem cake + *T. harzianum)* (10.38 g).

In general, control plants recorded significantly higher fresh root weight compared to other treatments.

b) Dry root weight

The maximum dry root weight was recorded in T7(neem cake + *P. lilacinus)* (3.22) treatment and the maximum Fresh root weight were observed in control (125.49 g). All the treatments were significantly superior over untreated check. However, the treatments which received T7(neem cake + *P. lilacinus)* was recorded significantly higher Dry root weight compared to other treatments followed by T6 (neem cake + *T. harzianum)* (3.63 g).

In general, control plants were recorded significantly higher dry root weight compared to other treatments.

4.5.2 Nematode population in final root and soil

Observation on *M. incognita* population in soil (200 cc) and root (5 g) was recorded at harvest. The final population of *M. incognita* in soil was lowest in T7 (neem cake + *P. lilacinus)*(230.43 in soil and 129.20 in root), followed T6 (neem cake + *T. harzianum*) (306.23 in soil and 161.63 in root), followed by T5 (neem cake + *P. fluroscense*) (347.37 in soil and 176.33 in root), whereas maximum population observed in control (893.40 in soil and 583 in root) (table 10).

4.5.2.1 Number of galls and root-knot index

Among the treatments, least galls are recorded in T7(neem cake + *P. lilacinus*) with 25.60 per cent galls per root system with Root-knot index with 2.00 which was significantly superior over other treatments followed by T6 (neem cake + *T. harzianum*) (29.43 per cent galls per root system with root-knot index with 2.33) where as maximum gall per root system observed in cow urine (162.57) galls per root system with 4.67 root gall index) followed by control (181.50 per cent galls per root system with Root-knot index with 5).

4.5.3 Wilt incidence

The plants treated with T9 (carbendazim at 0.1% and carbofuran 3 g a.i.m^2) recorded very less wilt incidence of 1.37 per cent compare to maximum wilt incidence of 64.43 per cent in control treatment. The next best treatment was plant inoculated with T9 (thiram 37.5 + carboxin 37.5 3g/l of water) (5.87 %) followed by T6 (neem cake + *T. harzianum)* (16.07 %).

Table 10. Effect of fungicides, bio-agents and soil amendment on wilt complex disease of cucumber under field condition during *Kharif* 2016

Treatments	Nematode population		Number of galls/root system	RKI	Per cent wilt incidence
	Soil (200cc soil)	Root (5g)			
T_1- application of Neem cake at 200g /m^2 during sowing	462.93	198.50	39.63	2.67	44.27
T_2 -Application of *P. fluroscens* at 50g/m^2 alone during sowing	509.77	200.33	41.67	3.00	27.27
T_3- Application of *T. harzianum* at 50 g. m^2 during sowing	492.27	189.53	39.67	3.00	26.7
T_4- Soil application of *P. lilacinus* alone at 50g/ m^2 during sowing	452.23	182.17	35.10	3.00	29.47
T_5- T_1+T_2	347.37	176.33	33.23	2.33	19.2
T_6 -T_1+T_3	306.23	161.63	29.43	2.33	16.07
T_7 -T_1+T_4	230.43	129.20	25.60	2.00	20.53
T_8 -Carbofuran 3 g a.i.m^2 during sowing	458.40	187.17	66.20	3.00	52.8
T_9 –Carboxil and thiram 3g/l of water	779.13	427.97	155.33	3.33	5.87
T_{10}- Carbendazim(0.1%) and Carbofuran.(3G @ 0.3g a .i/m^2)	651.17	297.70	95.23	2.33	1.37
T_{11}- Cow urine (10 per cent/l)	781.87	426.83	162.57	4.67	49.5
T_{12}- Control	896.40	583.70	180.50	5.00	64.43
S. EM±	7.44	2.41			2.21
CD at 5 %	21.84	7.07			6.55

A. General field view of management of wilt complex in cucumber

B. T_{12} - Control

C. T_{10} - Carbendazim + Carbofuran

D. T_7 – Neem cake + *P. lilacinus*

Plate 13. Management of wilt complex disease in cucumber under field condition

DISCUSSION

V DISCUSSION

Cucumber is prone to attack by wide range of diseases among them,wilt disease complex caused by *F. oxysporum* f. sp. *cucumerinum* (Owen) and root–knot nematode, *M. incognita* are the major are cause wilt complex disease with considerable yield loss. Fewer attempts have been made to study the *Fusarium* and *Meloidogyne* on wilt complex in cucumber including the management of fungal nematode wilt complex.

In the soil ecosystem, plants are constantly exposed to variety of organisms, many of which are common components of the soil biosphere. As they occupy the same environmental niche, such organisms besides influence each other as well. The combinations of nematodes and fungus often results in synergistic interaction where in, the crop loss is greater than the expected from either of the pathogens alone or an additive effect of the two together (Francl and Wheeler, 1993).

Inspite of its destructive nature, not much work with respect to different aspect has been carried out. Hence, the present study was undertaken considering different aspects like cultural, morphological character of *F. oxysporum* f. sp. *cucumerinum*, screening of cucumber varieties against wilt complex resistance under green house condition, evaluation chemicals, botanicals, bio-agent against *F. oxysporum* f. sp. *cucumerinum* and *M. incognita*. Management of wilt complex disease by using different bioagents, soil amendments and chemicals under field condition. The results of the above study have been discussed here under.

5.1 Symptomatology

Wilt complex is caused by the soil borne fungus and nematode, *F. oxysporum* f. sp. *cucumerinum* (Owen) and *M. incognita* showed pathogen attacks plants at any stage of plant growth. *Fusarium* causes vascular discoloration in stem and *Meloidogyne* cause gall on roots. Wilt symptoms develop first on lower or middle leaves final result in vascular discoloration of roots and stems was observed.

Similar symptoms showed by Majdah and Altuwaijri (2015), *Fusarium* infects the roots of cucumber plants. It causes wilting and develops first on lower and middle leaves and then spared to the top of plats. Early infection of plants prevented fruit set while later infection resulted in small abnormal fruits. Cracks appear on diseased vines. Vascular discoloration of the roots and stems was noticed.

5.1.1 Identification of F. oxysporum f. sp. cucumerinum

The cultures were identified based on the morphological characters of mycelia and spores. The fungus has white to pink colored sporulation on potato dextrose agar and produced the septate mycelium. Microconidia are one or two celled and oval to kidney shaped. Macro conidia are three to five celled, sickle shaped, thin walled and

delicate.Chlamydospores are round thick walled spores. The conidia appeared in pinkish slimy drops on the culture plates.

5.1.2 Pathogenicity test

The pathogeniciy was proved the pathogen for isolates (*F. oxysporum* f. sp. *cucumerinum*) on inoculated plants, the visual symptoms were observed.

5.2 To study the cultural and morphological characteristics of the *F. oxysporium* f. sp.*cucumerinum*

Effect of different solid and liquid media on the growth of *F. oxysporum* f. sp. *cucumerinum.* Eight solid media were tested to know the best media for the growth of *F.oxysporum* f. sp. *cucumerinum*. The maximum mean radial growth of *F. oxysporum* f. sp. *cucumerinum* was recorded significantly on potato dextrose agar (90 mm), followed by Richard's agar medium (86.53 mm), V8 juice agar (84.62 mm), host extract agar (76.74 mm). corn meal agar (59.67 mm), Czepek's dox agar (55.80 mm), Sabourauds dextrose agar (45.77 mm), and while, least radial growth was noticed on oat meal agar (52.23 mm).

In different liquid media, maximum mean dry mycelia weight of funguswas recorded in potato dextrose media broth (131.73 mg), which was found significantly superior over other tested broths, followed by richards agar media (312.10 mg), host extract agar media (304.20 mg, corn meal broth (227.20 mg) and least growth were recorded in Sabourauds dextrose agar (166.53 mg).

Similar findings confirmed with Imran Khan *et al.* (2011) who found maximum growth of *F. oxysporum* f. sp. *ciceri* on PDA (85.76 mm) followed by Richard agar (84.62 mm) and Czapek's agar (72.56 mm). Sowmya (1993) studied seven different solid media for the growth on *F. oxysporum* and revealed that maximum radial growth and sporulation was recorded in potato sucrose agar (71.71 mm) followed Richard's agar (65.21 mm) and least radial growth was recorded in host leaf extract agar (59.84 mm).

5.3 Screening of cucumber varieties for disease resistance.

Green long variety has showed the maximum per cent wilt incidence of 52.50 per cent followed by White Long (47.60 %) and Ranebennure Local (46.80 %). Whereas, minimum per cent wilt incidence was recorded in variety Sambar soudhe (24.00 %) followed by Dharwad Green (26.60 %). Similar results agreement with Krishnaveni and Subramanian (2003) that, cucumber cultivar Green long plants were inoculated with 1000 or more nematodes were heavily galled and plant growth was significantly reduced and exhibited symptoms such as stunted growth, yellowing and dropping of leaves. similarly Bharali and Phukan (1996) reported that the cucumber cultivar Poinsette, was moderately resistant to *M. incognita* out of six varieties.

5.4 *In vitro* evaluation of chemicals, botanicals and bio-agents against pathogens

5.4.1 Evaluation of fungicides against F. oxysporum f. sp. cucumerinum

5.4.1.1 Systemic fungicides against F. oxysporum f. sp. cucumerinum

Among five systemic fungicides, maximum per cent inhibition of growth of *F. oxysporum* f. sp. *cucumerinum* was observed in carbendazim (100 %) which was significantly superior to all other fungicides followed by hexaconozole (98.15 %) and propiconozole (98.15 %) which are on par each other followed by difenconazole (84.69 %). The least per cent inhibition of fungus was recorded in thiophanate methyl (75.43 %) at 0.05 per cent concentration.

Similar results observed Pushpa *et al.* (1999) they showed that, carbendazim as most effective fungicide against *F. oxysporum* f. sp. *melonis*. Bardia and Rai (2007) also showed per cent inhibition of mycelial growth of *F. oxysporum* f. sp, *cumini* by carbendazim. These results also agreement with Somu *et al.* (2008) reported that, carbendazim and carboxin were highly fungtoxic and showed 100 per cent inhibition in case of *F. oxysporum* at 100 ppm and 200 ppm concentrations.

5.4.1.2 Evaluation of non systemic and combi fungicides against F. oxysporum f. sp cucumerinum

The use of fungicides is an important method of managing the *Fusarium* wilt disease using fungicides are quick in their mode of action and with the best option available. These fungicides have to be used judiciously according to the need and kind of organism involved. In the present investigation four non systemic combi fungicide were used against *F. oxysporum* f. sp.*cucumerinum* to know their efficacy under laboratory condition.

Among fungicide tested maximum per cent growth inhibition was noticed in carbendazim 12% + mancozeb 63% (100%), tricyclazole 18% + mancozeb 62 % (100 %), copper oxycloride (100 %), thiram 37.5 + carboxin 37.5 (100 %) followed by tebuconazole 50 % + trifloxystrobin 25 % (93.89 %). Significantly least per cent inhibition was recorded in mancozeb (66.11 %).

Similar results showed by Sunita and Katoch (2001) in case of *F. oxysporum* f. sp. *dianthi* who reported that carbendazim 12 % + Mancozeb 63 % which completely inhibited the growth of the fungus. Same results agreement with Barhate (2015) showed Carbendazim 12 % + Mancozeb 63 % (0.125 + 0.05 %) had completely checked the growth of pathogen which inhibited 100 per cent growth of *F. oxysporum* f. sp. *lycopersici* followed by thiram + carbendazim (0.15 + 0.05).

5.4.2 Evaluation of botanicals against F. oxysporum f. sp. cucumerinum

Fungicides in the management of disease also brought new problems along with them and alarming, among them are the pollution of air, water, soil residual

toxicity and development of resistance of pathogen against chemical. Hence, there is need to apply them with their escalating process and harmful effects on non target organisms. Botanicals are eco-friendly renewable, inexhaustible, indigenously available and easily assessable largely non phytotoxic, thus radically biodegradable, relatively cost effective constitute suitable plant protection in the strategy of integrated disease management.

Hence, screening of botanicals for their effective and antifungal activity against the pathogen is essentially required to minimize the use of fungicides and to consider as one of the component in the integrated disease management.

The present investigation was carried out to evaluate the different botanicals against *F. oxysporum* f. sp. *cucumerinum.* In the present study, *in vitro* evaluation of botanicals was carried out with respect to inhibition of mycelial growth at different concentrations (5, 10 and 20 %).

Maximum per cent inhibition of mycelial growth of the fungus (68.35 %) was recorded at 15 per cent concentration in turmeric rhizome extract which was significantly superior over all treatments tested botanicals, followed by garlic (65.19%), eukalyptus (51.00%), whereas, least per cent inhibition was recorded in neem (35.11 %) followed by marigold (42.70 %). Similar results were corresponding with Kishore (2007) who showed clove oil is most effective with per cent inhibition followed by garlic extract. Also same results were corresponding with Khaleel and shubhan (2014), *F. oxysporum* f. sp. *pisi* and showed that, ginger extract (57.7 %) was effective high inhibition. and result are inagreement with Ankita and Dwivedi (2012) who showed garlic, turmeric and black pepper reduced the growth up to 94.63 %, 87.96 % and 77.74 % respectively.

5.4.3 Evaluation of bio-agents

5.4.3.1 Evaluation of bio-agents against F. oxysporum f. sp. cucumerinum

There was significant difference among all the tested bio-agents against *F. oxysporum* f. sp. *cucumerinum.T. viride*-II was significantly recorded maximum inhibition of mycelial growth (72.00 %) followed by *T. viride-I* (64.89 %) isolate, *T. harzianum*-I (64.89%), *T. harzianum*-II (60.96 %), *P. fluroscense* (52.52 %) whereas, least mycelia inhibition was recorded in *B. subtilis* (52.52 %) and *T. viride*- II isolate (72.00%) was found to be superior to over all their bio-agents. Similar results were inagreement with findings of Bardia and Rai (2007), who tested bioagents against *F. oxysporum* f. sp. *cumini* revealed that, maximum inhibition of growth of mycelia was observed with *T. harzianum* followed by *T. viride*. Also showed by Vanilla *et al.* (2013) who showed per cent inhibition of 83 per cent was recorded with *T. viride* and minimum with *T. harzianum* (67.00%). The inhibitory effect of these bioagents against the pathogen was probably due to competation, antibiosis and mycoparasitism (Cook

and Baker, 1983). Antagonistic activity of *Trichoderma* speciesagainst soil borne fungal pathogens were reported by Scher and Baker (1982).

5.4.3.2 Effect of bio-agents on juvenile mortality of M. incognita.

All the bio-agents tested showed maximum larval mortality. Also, there was a positive correlation between incubation period and larval mortality with an increase in incubation period there was increase in juvenile mortality. At 24 hrs, all the bio-agents recorded maximum larval mortality ranging from 24.00 to 46.00 per cent compared to control. At 48 hrs also, all the bioagents recorded highest larval mortality ranging from 48.00 to 66.00 per cent and at 72 hrs ranged from 48.67 to 71.33 per cent. Overall, the different bio-agents tested, *P. lilacinus* was more effective in increasing larval mortality than others.

Similar results showed by Samina and Reddy (2012) at 24 hrs, all the bioagents recorded maximum juvenile mortality ranging from 39.33 to 52.33 per cent compared untreated check. At 48 hrs also, all the bio-agents recorded highest juvenile mortality ranging from 44.66 to 62.00 per cent. At 72 hrs ranged from 49.66 to 68.33 per cent compared to untreated check (7.66%). Anita andSelvaraj (2010) showed that, the mortality of the juveniles increased with increase in the concentration and exposure to antagonist fungal culture filtrates. The mortality percentage was found to increase with time and the concentration of the fungal culture filtrate among the three fungal culture filtrates the highest mortality (95 %) was observed in *P. lilacinus* culture after 72 hrs at 50 per cent concentration. There was minimal mortality in the control.

This antagonistic effect against *M. incognita* juvenile is due to permeability changes of juvenile cuticle which is characterized by its selective permeability and the visual abnormalities probably were due to the effect of fungal toxic metabolic products produced by the bio-agents.

5.5 Management of wilt disease complex in cucumber under field condition

The present study was conducted in randomized block design with three replications during 2016-17 in the farmer field of Budigere village (Shivamogga taluka) on management of disease management of cucumber wilt complex disease using chemicals, bio-agents, and soil amendments are evaluated separately and in combination for the management wilt complex disease discussion as follows.,

5.5.1 Plant parameters

The results of the trial conducted on efficacy of various treatments on plant parameters *viz*., plant fresh and dry shoot weight, root length, fresh and dry root weight and yield of cucumber. Infected by *F. oxysporum* f. sp. *cucumerinum* and *M. incognita* were analyzed.

5.5.1.1 shoot weight

5.5.1.1.1 Fresh shoot weight

The effect of treatments on the fresh shoot weight of cucumber plant was recorded at the time of harvest. The fresh shoot weight of cucumber was recorded at the time of harvest and was varied from 113.86-365.65 g. The maximum fresh shoot weight was recorded neem cake + *P. lilacinus* (365.62 g) followed by neem cake + *T. hargianum* (350.37 g) followed by neem cake +*P. fluoscence* (333.99g). whereas the minimum fresh weight was recorded in untreated check (113.86 g). All the treatments were significantly superior over untreated check. However, treatment which received significantly superior over all other treatments and significantly higher fresh shoot weight.

5.5.1.1.2 Dry shoot weight

The maximum dry shoot weight was recorded in *T*7 (neem cake and *P. lilacinus)* followed by T6 (neem cake + *T. harzianum*) (139.26 g), T5 (neem cake +*P. fluoscence)* (130.47 g), the minimum dry weight was recorded in case of untreated check (38.07 g). However, the treatment which received T7 (neem cakeand *Pacilomyces lilacinus)* was significantly superior over all other treatments. The untreated check recorded least dry shoot weight.

5.5.1.2 Root length and yield

At the time of harvest, all the treatments recorded significantly better root length. The maximum root length was observed T7(neem cakeand *P. lilacinus*) (33.05cm) which were superior over all the treatments. Among the treatments maximum root length (33.05 cm) was observed in T7 (neem cakeand *P. lilacinus)* followed by T6 (neem cake + *T. harzianum*) (32.17 cm), T5 (neem cake +*P. fluoscence)* whereas, least root length observed in control (0.17cm)

The maximum fruit yield per plot was recorded in T7(neem cakeand *P. lilacinus)* (2.56 kg plot) treatment and the least fruit yield per plot was observed in control (0.77 kg). All the treatments were significantly superior over untreated check. However, the treatments which received *T*7(neem cakeand *P. lilacinus)* was recorded significantly higher yield compared to other treatments followed by T6 (neem cake + *T. harzianum*) (2.39 kg per plot).

5.5.1.3 Root weight

5.5.1.3.1 Fresh root weight

The minimum Fresh root weight was recorded in T7 (neem cake and *P. lilacinus)* (8.07 g) treatment and the maximum fresh root weight were observed in control (125.49 g). All the treatments were significantly superior over untreated check. However, the treatments which received T7(neem cake and *P. lilacinus)* was recorded

significantly higher fresh root weight compared to other treatments followed by T6 (10.38 g). The increase in root weight is due more multiplication of *M. incognita* and formation of non- functional roots.

5.5.1.3.2 Dry root weight

The dry root weight was significantly higher in untreated check (44.09 g). The maximum dry root weight was recorded in untreated check. The minimum dry root weight was recorded in T7 (neem cake and *P. lilacinus)* (3.22 g) treatment and the maximum fresh root weight were observed in control (125.49 g). All the treatments were significantly superior over untreated check. However, the treatments which received T7 (neem cake and *P. lilacinus)* was recorded significantly higher dry root weight compared to other treatments followed by T6 (3.63 g).

In general, control plants were recorded significantly lower dry root weight compared to other treatments.

With respect to oilcake (Neem cake) and bio-control (*P. lilacinus*) and their effectiveness in improving the growth of plant, the present results are in conformity with the results obtained by Gowda (1972), Mishra and Gouda (1972), Khan *et al.* (2004), Azim *et al*. (2011) in cucumber and Tulika Singh *et al.* and Thumar *et al.* (2012) in bottle gourd, Konsam *et al.* (2015) reported that oil cake improves plant height and yield as compared to the control. The results were also in conformity with the results obtained by Kshetrimayum and Debanand (2014) who reported neem cake 2 t/ha increase in growth parameters on green gram and the increase in root weight is due more multiplication of *M. incognita* and formation of non- functional roots.

Hassan and Saxena (1974), Chen and Tue (1991) showed that, neem cake 20 g per pot was found effective increasing the plant growth. The effect of bio-control agent in improving plant growth parameters is comparable with the effect of *P lilacinus* against *M. incognita* of ridge gourd. These results are in accordance with the results obtained by(Stephen *et al*., 1996) effect of *T. viride,P. lilacinus* against root-knot / wilt diseases complex of tomato.

Krishnaveni and Subramanian (2005), opined that *T. viride* recorded significantly higher growth parameters and yield with reduced nematode population compared to control in cucumber plants.

The reason for increased plant growth parameters observed in the present study could be attributed to the release of growth promoting substances by bio-agents or by producing toxic metabolites which inhibit nematodes and exclude other deleterious microorganisms (Baker *et al*., 1986). Anti nematode effect of neem cake may be attributed to the phenolic compounds released during its degradation apart from its stimulatory effect on root growth and predaceous fungi and also to small amount of Azadirachtin (Sundraraju and Kiruthika, 2005).

5.5.2 Nematode population in final root and soil

Observation on *M. incognita* population in soil (200 cc) and root (5 g) was recorded at harvest. The final population of *M. incognita* in soil was lowest in T7(neem cake and *P. lilacinus)* (230.43 in soil and 129.20 in root), followed T6 (neem cake + *T. harzianum*) (306.23 in soil and 161.63 in root), followed by T5 (neem cake +*P.fluoscence)* (347.37 in soil and 176.33 in root), whereas maximum population observed in control (893.40 in soil and 583 in root).

The present study revealed that the treatments *viz*., neem cake, Carbofuran, *T. viride* and *P. lilacinus* were effective both individually and in combination in reducing the nematode population and number of galls per root system.

Similar results were agreement with. Akhtar and Mallik (2000), neem cake @ 1-2 t/ha efficiently reduce nematode population. Zarina *et al.* (2006), Yasmin *et al.* (2003) neem seed extract lethal to root-knot nematode infecting Sweet gourd. Krishnaveni and Subramanian, (2005), Kshetrimayum Sumita and Debanand (2014) reported that organic amendments @ 250 g/m^2 reduce nematode population.

5.5.3 Number of galls and root-knot index

The treatments were significantly superior over the untreated check. However the treatments *T*7 (neem cake and *P. lilacinus*) were significantly superior over all treatments. Among the treatments, T7 (neem cake and *P. lilacinus)* recorded 25.60 per cent galls per root system with Root-knot index with 2.00 which was significantly superior over other treatments followed by T6 (29.43 per cent galls per root system. population galling per plant on tomato. with Root-knot index with 2.33) where as maximum gall per root system observed in cow urine (162.57) galls per root system with 4.67 root gall index) followed by control. (181.50 per cent galls per root system with Root-knot index with 5)

Whereas, the minimum fresh weight was recorded in untreated check (113.86 g). All the treatments were significantly superior over untreated check. However, treatment which received significantly superior over all other treatments and significantly higher fresh shoot weight.

The present results are in conformity with the results obtained by Verma and Anwar (1997) who reported neem cake at 250 kg/ha reduces root gall in pointed gourd. Akhtar and Alam (1990) used neem (*Azadirachta indica*) leaves (80 g/pot) and recorded highest reduction in root-knot index Gowda and settee (1973), Mishra (1972), who have reported that application of neem cake significantly reduced the nematode.

5.5.4 Wilt incidence

The plants treated with T9 (carbendazim at 0.1% and carbofuran 3g a.i.m^2) recorded very less wilt incidence of 1.37 per cent compare to maximum wilt incidence

of 64.43 per cent in control treatment. The next best treatment was plant inoculated with T9 (thiram 37.5 + carboxin 37.5 3g/l of water) (5.87%) followed by T6 (neem cake + *T. harzianum*) (16.07%). Similar results were Comparing effectiveness of systemic, non-systemic and various fungicides was assessed *in vitro* and *in vivo* to control *F. oxysporum* causing wilt diseases. Carbendazim and benomyl totally blocked the growing of the check fungus monitored by thiophanate methyl, aureofungin, bitertinol. In field situations carbendazim and benomyl abridged the wilt frequency winning to 80 to 73 % respectively Santoshreddy *et al.,* 2014 showed that, carboxyl thiram manage disease. Also results are comparable with Coskuntuna and Ozer (2008) reported that application of *T. harzianum*, resulted a significant reduction in basal rot incidence on onion sets under pot and field conditions. This formulation was also found superior to prochloraz by enhancing set diameter in both experiments. *T. harzianum* has the ability to stimulate a chemical response in onion. And they also showed that *T. harzianum* treatment of onion seeds induced the accumulation of antifungal compounds absorbing UV light in onion, which may be involved in the control of *F.oxysporum* f. sp.*cepae* during set development under pot and field conditions. Hunt *et al.* (1997) found that these compounds were involved in induced resistance.

Conclusion

The growth and mean dry mycelial weight of *F.oxysporum* f. sp. *cucumerinum* was maximum on potato dextrose in solid and liquid media respectively. None of the varieties shown resistant to disease. Among the chemicals, batonicals and bio-agents tested against pathogen, carbendazim and hexaconozole, turmeric, garlic extract and *T. viride*-II found effective in inhibiting the mycelial growth of the pathogen respectively under *in vitro* condition. *P.lilacinus* showed maximum mortality of juveniles, for the disease management under field condition the combination of neem cake and *P. lilacinus* showed less disease incidence with increased growth parameters of the crop along with less nematode population. Whereas combination of carbofuran and carbendazim showed less per cent wilt incidence.

Future line of work

1. There is a need undertake an intensive survey for the occurrence of *Fusarium* wilt complex.
2. Conduct detailed studies on the influence of various agro ecological factors on the distribution and incidence of disease complex.
3. To study the interaction studies on different pathogen with *M. incognita* and *F. oxysporum* f. sp. *cucumerinum.*

SUMMARY

VI SUMMARY

Investigations on cucumber wilt complex caused by *F. oxysporum* f. sp. *cucumerinum* and *M. incognita* were carried out with reference to cultural and morphological, characteristics of the pathogen. Screening of cucumber varieties for disease resistance. Evaluation of chemicals, botanicals and bio-agents both under *in vitro* and *in vivo* condition. Further management of disease under field condition.

The pathogen was isolated from infected cucumber and pure culture of fungus, *Fusarium oxysporum* f. sp. *cucumerinum* was obtained by hyphal tip method. On the basis of morphological and cultural studies, the pathogen was identified as *F. oxysporum* f. sp. *cucumerinum.* Fungus produced three kinds of spores *viz.*, microconidia, macroconidia and chlamydospores. Among eight solid and liquid media tested maximum radial growth of *F. oxysporum* f. sp. *cucumerinum* was observed on Potato dextrose agar and Potato dextrose broth.

Studies on screening of different varieties of cucumber against wilt disease complex showed that, varieties. Among nine varieties, Sambar Southe and Uttam were showed moderately susceptible reaction to wilt complex and Green long variety showed susceptible reaction to wilt incidence and highly susceptible reaction to root knot nematode.

Among botanicals tested against *F. oxysporum* f. sp. *cucumerinum* maximum per cent inhibition of mycelia growth of the fungus was recorded in turmeric (68.35 %) maximum weight followed by garlic (65.19 %) at 15 per cent concentration. Whereas, least percent inhibition of mycelia growth was observed in marigold leaf extract (9.61 %).

Five bio-control agents tested against *F. oxysporum* f. sp. *cucumerinum,*out of tested *T. viride*-II recorded significantly maximum inhibition of mycelia growth (72.00 %) followed by *T. viride-I* (68.22 %), whereas least mycelial inhibition was recorded in *B. subtilis* (49.67%).

Among five systemic fungicides tested against *F. oxysporum* f. sp. *cucumerinum,*carbendazim (100 %) which was significantly superior over all other fungicides and per cent inhibition at three concentration (0.3, 0.2, 0.1 %) followed by hexaconozole (98.15 %), propiconozole (98.15 %) and whereas, the least per cent inhibition of fungus was recorded in Thiophanate methyl (75.43 %).

Among the non systemic and combi fungicides tested against *F. oxysporum* f. sp. *cucumerinum,* carbendazim + mancozeb which recorded maximum percent inhibition in all concentratins (0.3, 0.2, 0.1%) whereas minimum per cent inhibition observed in mancozeb (51.05 %) and fond to be least effective in inhibition the growth of mycelia.

Effect of bio-agents used against juveniles of *M. incognita* under *in vitro* condition. *P. lilacinus* (71.*33 %)* recorded the higher juvenile mortality at 72 hrs followed by *T. harzianum* (62 %) whereas; least mortality was recorded by *T. harzianum* at 24 hrs incubation.

Management of wilt complex under field condition by using different chemicals, bioagents, and soil amendments, among them, combined application of neem cake and *P. lilacinus* showed superior over control in management of nematode population and recorded maximum root length, less fresh and dry weight of shoot and root, yield and number of nematodes population with less RKI (1.4) along with wilt incidence (1.37 %). Whereas, in case of wilt incidence less wilt recorded in treatment combination of carbendazin and carbofuran (1.37 %) followed by carboxyl and thiram (5.87 %)

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APPENDIX

VIII APPENDIX

LIST OF ABBREVATIONS AND SYMBOLS USED

%	Percentage
µm	Micrometer
C.D.	Critical difference
cm	Centimetre
g	Grams
ha	Hectare
kg	Kilogram
t/ha	Tonnes per hectare
L	Litre
m	Meter
mg	Milligram
ml	Millilitre
mm	Millimetre
No	Number
˚C	Degree celsius
PDI	Per cent disease index
S.Em	Standard error of mean
PDA	Potato Dextrose Agar
h	Hours
viz.	Namely
et al.	And other co workers
Sec	Second (S)