EVALUATION OF NATIVE ISOLATES OF NEMATODE ANTAGONISTIC FUNGI AGAINST *Meloidogyne incognita* (KOFOID AND WHITE) CHITWOOD IN TOMATO.

by

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THESIS

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2020

DECLARATION

I, hereby declare that this thesis entitled "Evaluation of native isolates of nematode antagonistic fungi against *Meloidogyne incognita* (Kofoid and White) Chitwood in tomato" is a bonafied record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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LIST OF ABBREVIATIOS

AMF	-	Arbuscular mycorrhizal fungi
@	-	at the rate of
^{o}C	-	Degree celsius
%	-	Per cent
BOD	-	Biological oxygen demand
CD	-	Critical difference
CFE	-	Cell free extract
CF	-	Colony forming
Cfu	-	Colony forming unit
CWE	-	Cell wall extract
cm	-	Centimetre
DAT	-	Days after transplanting
DNA	-	Deoxyribo Nucleic Acid
et al.	-	And others
Fig.	-	Figure
Ha	-	Hectare
HAE	-	Hours after exposure
HAT	-	Hours after treatment
G	-	Gram
i.e.	-	That is
kg ha	-	Kilogram per hectare
L	-	Litre
m	-	Metre
mg	-	Milligram
No.	-	Number
NS	-	Not Significant
$Plant^{-1}$	-	Per Plant
PDA	-	Potato Dextrose Agar
PDB	-	Potato Dextrose Broth

%	-	Per cent
PPN	-	Plant Parasitic Nematode
POP	-	Package of practices
CBD	-	Completely block design
SE	-	Standard error
t ha^{-1}	-	Tonnes per hectare
VAM	-	Vesicular Arbuscular Mycorrhizae
µ	-	Micro

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Introduction

1. INTRODUCTION

Plant-parasitic nematodes (PPN) are recognized as major pathogens infecting crop plants and cause crop losses throughout the world. Among them *Meloidogyne incognita* is the most damaging species attacking horticultural and field crops, causing an estimated US$100 billion loss globally on an annual basis (Oka *et al*., 2000). Heavy infestations of these nematodes can lead to yield losses of over 30 per cent in highly susceptible vegetable crops (Sikora and Fernandez, 2005). They caused projected yield loss of 12.3 per cent ($157 billion dollars) worldwide and of which $40.3 million is reported from India (Singh *et al*., 2015).

Several management measures were employed to control Root Knot Nematode (RKN) in infested areas. The common practices were the use of chemical nematicides and its impending negative impact on environment. Introduction of antagonists in the soil microenvironment has resulted in an efficient method for biological control of nematodes (Akhman *et al*., 2002) and gaining importance. Of the microorganisms that parasitize or prey on nematodes, fungi hold an important position and some of them have shown great potential as biocontrol agents (Jatala, 1986; Stirling, 1991). Fungi may contribute up to 80 per cent of the total microbial biomass in many soils (Clark and Paul, 1970; Shields *et al.,* 1973). In nature, fungi continuously destroy nematodes in virtually all soils because of their constant association with nematodes in the rhizosphere. The fungal antagonism consists of a great variety of organisms which vary considerably in their biology and taxonomy and play a major role in recycling the carbon, nitrogen and other important elements from the rather substantial biomass of nematodes. Hence attempts have to be initiated for the isolation of native strains of antagonistic fungi that can be developed as an effective bio-agent against RKN.

Natural control of PPN with adversarial organisms is a promising procedure which might be consolidated in Integrated Nematode Management (Stirling, 1991) and picking up significance. Of the microorganisms that parasitize or go after nematodes, organisms hold a significant position and some of them have indicated incredible potential as biocontrol agents (Jatala, 1986; Stirling, 1991). Organisms may contribute up to 80 per cent of the total microbial biomass in many soils (Clark and Paul, 1970; Shields *et al*., 1973). In nature, growths consistently pulverize nematodes in essentially all soils in view

of their steady relationship with nematodes in the rhizosphere. The contagious opposition comprises of a great variety of organisms which shift impressively in their biology and taxonomy and assume a significant job in reusing the carbon, nitrogen and other important elements from the fairly considerable biomass of nematodes. Thus, endeavours must be started for the isolation of native strains of antagonistic fungi that can be created as a successful bio-agent against RKN.

Parasites and microbes are among the most prevailing soil-borne bunches in normal soil environment and some of them have demonstrated extraordinary potential as organic control operators for root-hitch nematodes (Kerry, 2000). The free-living soil growths *Trichoderma spp*. are potential nematode biocontrol operators on numerous nourishments, vegetable and money crops (Dababat and Sikora, 2007; Affokpon *et al*., 2011).

Chaetomium abuense an ascomyceteous fungus was effective in the management of disease complex caused by *M. incognita - Fusarium solani* complex on soybean (Narayana, 2002). *Aspergillus awamori*, an RKN trapping fungi isolated from the rhizosphere of tomato showed 44.9 % control efficacy against *M. incogtina* (Cui *et al.,* 2015).

The nematode antagonistic fungi belonging to genera *viz, Fusarium*, *Acremonium*, *Trichoderma*, *Chaetominum*, and *Purpureocillium lilacinum*, can produce numerous metabolites *in vitro*. When *Acremonium srictum*, *A. implicatum*, *P. lilacinum*, and *Trichoderma harzianum* were grown in liquid media, their culture filtrates were toxic to J_2 of *M. incognita* (Gowswami *et al.,* 2008). *T. harzianum* was able to penetrate nematode eggs and significantly decrease the hatching of *M. javanica* eggs. The tomato plants inoculated with the fungi showed an increase in the activity of resistance related enzymes *viz*, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase (Sahebani and Hadavi, 2008). Narayana *et al.* (2017) reported that *Purpureocillium lilacinum* $1x10^7$ cfu @ 30g/plant was equally effective in increasing the yield of cardamom and reducing the population of *M. javanica* in soil and roots. Varkey *et al.* (2017) reported that a consortium of rizhobacteria and *Piriformospora indica* suppressed the population of *M. incognita* in soil and roots of tomato.

Information pertaining to different indigenous species of antagonistic fungi effective against RKNs in Kerala is meager. Considering the above evidences, the present study was done with the following objectives:

- To isolate indigenous fungal antagonists from vegetable growing areas of Thiruvananthapuram district.
- To evaluate the ovicidal effect of cell free extracts of fungal isolates against *M. incognita* under laboratory conditions.
- To assess the biocontrol potential of the promising fungal isolates against *M. incognita* through pot culture experiment.
- To identify effective fungi selected by morphological, biochemical and molecular characterization.

Review of literature

2. REVIEW OF LITERATURE

Plant parasitic nematodes are usually found in the rhizosphere of the plants and produce typical symptoms on roots *viz*., galls, necrosis, lesions, stubbiness, dwarfness and bunchy root system. Nematode cause stunting, yellowing of foliage and sickly appearance on plants and even symptoms of nutrients deficiencies due to the damage of roots. Several PPN have been reported to be associated with vegetables and other crops. Majority of the phytoparasitic nematodes are found in association with plant roots, resulting in significant losses. Different microbes can be outstandingly used for the management of RKN. Some microorganisms have been reported which have ability to parasitize nematode, some are nematophagous and some produces chemicals with nematicidal properties. The literature related to present investigation "Evaluation of native isolates of nematode antagonistic fungi against *Meloidogyne incognita* (Kofoid and White) Chitwood in tomato" is briefly reviewed here.

2.1 TOMATO

Solanum lycopersicum (L.,) is a commonly cultivated plant belongs to the family Solanaceae. This fruit can be classified under the nightshade family together with the eggplant, peppers and the potatoes. India (18.7 MT) ranks second in the world after China (56.8 MT) in the world largest tomato producing countries in the world according to FAO (2019).

Tomatoes in most cases are normally used in salads and stew to bring out the flavor. Apart from consuming for the purpose of adding flavour they have medicinal benefit such as source of vitamin C, low in dietary nutrients, prevent cancer from colon, breast, stomach, pancreases, and treat high blood pressure.

2.2 ROOT KNOT NEMATODE (*Meloidogyne incognita*)

The first report of RKN in India was given by Barber (1901) on tea in Kerala and it was identified as *Heterodera radicicola*. Lall and Ansari (1960) identified *M. arenaria* on tomato from Bihar. RKN (*Meloidogyne spp*.) are sedentary endoparasites of various crops and most important pests in agriculture. Health, yield and quality are greatly affected by their infestation. PPN are recognized as major pests infecting crop plants and cause crop losses throughout the world. Among them *Meloidogyne incognita* is the most harming

species attacking horticultural and field crops, causing an expected US$100 billion loss globally on an annual basis (Oka *et al*., 2000).

They parasitize on wide range of crops like vegetables, fruits and ornamental crops (Hussey and Janssen 2002). In tropical and subtropical parts of the world, vegetables are worst affected. In India, various crops are infested by different species of RKN . Nematodes are distributed over different geographical conditions of the world. In a study conducted by Krishnappa (1985) it is reported that in India *M. incognita* and *M. javanica* have the amplest host range contaminating more than 232 and 114 genera of plants respectively. Most preferred hosts of RKN are vegetables, pulses, fruit crops, fibre crops, ornamentals, medicinal and aromatic plants and other important cash and plantation crops. Due to short life cycle of six to eight weeks enable them to survive well in the presence of suitable host.

Characteristic symptoms of RKN infestation seen on all parts of plant. Indications of nematode pervasion include hindering, yellowing, wilting, die back, dead and devitalized buds, crinkling and curling of leaves, gall formation, leaf spots and symptoms similar to nutrient deficiency. Susceptible plants are stunted, with yellow leaves and root galls and finally wilted in severe cases (Walters and Barker 1994). The patches of infestation of nematode are of non-uniform growth.

Infection due to nematode reduces the yield due to reduction in ability of plant to extract available soil water and nutrients. Contingent on the seriousness of contamination, there will be gigantic monetary misfortunes to producers (Mitkowski and Abawi, 2003). They are sedentary parasites of vascular tissues of plant roots. Tainted plants will grow enormous large galls all through the root system. Enormous infestation of nematodes results in reduced yield and affects consumer recognition in crops like vegetables.

RKN galls on lettuce are beadlike while on grasses and onions, they are typically little with slight swellings. Feeding activities cause cellular browning or necrosis which interferes with transportation of water and solutes and leakage of stored photosynthetic products. The altered tissues at feeding sites also block the vascular system interfering the upward movement of water and dissolved nutrients by xylem and translocation of photosynthates to other regions of the plant by phloem (Hajera *et al.,* 2009).

Nematode infested plants are unable to ripe and their fruits are of poor size (Dropkin, 1980). It was reported that infestation of nematode was higher on younger plants of tomato as compared to older ones as the older plants being harder and stronger, suffered less. In a study conducted by Khan *et al.* (2004) reported the effect of *M. incognita* initial inoculum densities on rate of nematode multiplication, it was observed that higher multiplication rates were observed with lower initial densities of nematodes. *M. incognita* is pathogenic at all population heights and more damaging at higher densities.

Sasser (1978) reported that RKN have been a hazard for global food production and depending upon nematode species the crop loss was estimated to be 5-43 per cent. Reddy (1985) observed 39.8 per cent decrease in tomato yield due to nematode population at 20 J_2/g soil. In India because PPN invasion the yearly evaluated reduction was assessed to be about Rs. 242.1 billion (Jain *et al*., 2007).

2.3 MANAGEMENT OF ROOT KNOT NEMATODE

The susceptible stages of PPN to manage with biological control are the egg and second-stage juveniles which exist stages outside of the plant in the water film on soil particles whereby the antagonistic microorganisms will come in contact, infect and parasitize the nematodes. If these two phases of the PPN are controlled, the existence pattern of the nematodes will be hindered and bring about decreased populace of the nematode.

Development of control measures of nematodes is a great challenge, because they spend their lives in soil or plant root, delivery of chemicals to the surrounding area of nematode is difficult (Chitwood, 2003). Moreover, due to alarming health and environment factors related to use of chemical nematicides, a resurgence of biocontrol alternatives is much safer.

2.4 FUNGAL BIOCONTROL AGENTS

The fungal antagonists of PPN comprise of a wide series of organisms which incorporates the nematode-catching or predacious fungal growths, nematode endoparasitic organisms, nematode egg parasitic fungi, and fungi which produce metabolites dangerous to nematodes (Mankau, 1980; Yang *et al*., 2007).

2.4.1 EGG PARASITIC FUNGUS

Pochonia chlamydosporia (Goddard), was first announced in 1974 as a parasite of nematode eggs by Wilcox and Tribe in the UK, the organism therefore getting one of the most determined natural biocontrol agents of nematodes (Zare *et al.,* 2001). Research conducted on this nematophagous fungus has been reviewed extensively by researchers (Kerry, 1997; Llorca *et al*., 2008; Kerry and Hirsch, 2011; Lopez *et al*., 2011; Chen and Dickson, 2012).

Kerry (1980) revealed that *Verticillium chlamydosporium* is a facultative parasite of eggs and females of cyst and RKN. Jones *et al.* (1983) reported that *V. chlamydosporium* was capable of preventing egg hatching of *Meloidogyne spp*. and to colonize eggs by hyphal penetration. *P. lilacinum* successfully controlled the nematode *M. incognita* on tomato (Villanueva and Davide 1984) and on banana (Jonathan and Rajendran, 2000). Jatala *et al*. (1985) also reported that *P. lilacinum* caused substantial egg deformation in *M. incognita* and these deformed eggs never matured or hatched Dube and Smart Jr (1987) observed that soil applications of *Paecilomyces lilacinum* (Thom) Samson and *Pasteuria penetrans* (Thorne) Sayre and Starr resulted in higher control levels of *M. incognita* (Kofoid and White) Chitwood population, when compared to control treatments or to the antagonist alone.

The fungal organism *P. chlamydosporia* Zare and Gams (sin. *Verticillium chlamydosporium* Goddard) is considered as one of the most capable biocontrol agents for PPN (Freitas *et al.,* 2009). Siddiqui and Haque (2000) additionally observed comparable outcomes for the decrease in *M. javanica* (Treub) Chitwood when the biocontrol agents *P. chlamydosporia* was used.

The serine protease produced by *P. lilacinum* might play a key role in infiltration of the growth through the egg shell of the nematode (Bonants *et al*., 1995). Pathak and Saikia (1999) observed that inoculation with *P. lilacinum* reduced the damaging effect of *M. incognita* in betel vine.

Siddiqui *et al.* (2000) detailed that *P. lilacinum* decreased *Meloidogyne spp*. infection in tomato. Khan and Goswami (2000) in an experiment on banana demonstrated that the plant growth characters considerably increased with *P. lilacinum* application. Khan and Verma (2004) reported that nematode multiplication was diminished by the utilization

of *P. lilacinum* @ 0.25g to 2.0 g/pot. Khan *et al.* (2012) recorded an enhancement in growth and yield of eggplant with biocontrol agents *P. chlamydosporia, P. lilacinum*, and *T. harzianum* by the suppression of galls formation. Kiewnick and Sikora (2006) reported that in tomato a pre-planting soil treatment with *P. lilacinum* decreased root galling by 66 per cent, number of egg masses by 74 per cent and the last nematode populace in the roots by 71 per cent contrasted with the inoculated control. Monjil *et al*. (2017) found that *P. lilacinum* attacked eggs of *Meloidogyne spp*. and inhibit the nematode incubating from the egg masses and increased plant growth parameters. Narayana *et al.* (2017) observed that *P. lilacinum* $1x10^7$ cfu @ 30g/plant was successful in increasing the yield of cardamom and decreasing the population of *M. javanica* in both soil and roots.

2.4.2. ENDOPHYTIC FUNGUS

Sikora and Schonpeck (1975) in their investigation set up that there is decrease in root infiltration and advancement of *M. incognita* by Vesicular Arbuscular Mycorrhizae (VAM) in tomato. Kellam and Scheck (1980) indicated that gall formation by *M. incognita* in soybean was decreased distinctly in root portions mycorrhized with *G. macrocarpum* recommending an immediate short-range effect and also observed that the yield and root weight were increased in dually inoculated plants and had significantly fewer galls per gram root than inoculating with nematode alone.

John *et al.* (2004) examined the influence of six isolates of AMF *viz*., *G. fasciculata*, *G. etunicatum, G. constrictum, G. mosseae, G. monosporum* and *Acaulospora morroweae* on the root-knot infestation and biomass production in amaranthus. Plants treated with *G. monosporum*, *G. etunicatum* and G. *mosseae* resulted in an increased fresh weight of plants and reduced nematode population in root and soil. Masadeh *et al*. (2004) examined the impacts of the blend of the AMF, *G. intraradices* and the fungus *T. viride* on the control of RKN. *M. hapla*, in green house experiment on the tomato cultivars 'Hildares' and 'Tiptop' and detailed that neither of the beneficial organisms, inoculated separately or together, changed general susceptibility of the cultivars. Use of the beneficial fungi decreased the quantity of galls and egg sacs of Hildares. While in Tiptop, biocontrol of RKN was not accomplished. Prakob *et al*. (2007) examined the impacts of blended AMF, rhizobacteria *P. aeruginosa*, *B. subtilis*, and antagonistic fungus *P. lilacinum* on development and gall development of tomatoes contaminated by RKN *Meloidogyne spp*. under greenhouse

conditions. In a study conducted by Saikia and Aparajita (2008) to find out the comparative efficacy of *G. fasciculatum*, *P. penetrans* and *T. harzianum* with carbofuran against *M. incognita* on brinjal it was observed that among the treatments *G. fascicnlatum* + carbofuran 3G blend was seen as the best in growing plant development characters and yield and diminishing the quantity of galls and egg masses followed by *T. harzianum*+ carbofuran 3G.

There is restricted information about the chemical compositions of the cell wall extract and CF. *P. indica* has also been reported to increase plant stress tolerance towards abiotic stresses like drought, acidity, and heavy metals (Kumari *et al*., 2004) and against biotic stresses such as plant pathogens (Waller *et al*., 2008; Zuccaro *et al*., 2009). In barley, *P. indica* triggers resistance against Fusarium head blight (*Fusarium graminearum*) (Deshmukh and Kogel, 2007) as well as against the leaf pathogen *Blumeria graminis f. sp. hordei* (Waller *et al*., 2005). As per Fukhro *et al*. (2009) it is suggested that *P. indica* may induce systemic resistance in plants. The fungus interacts with fungal and viral pathogens and also improves the growth of tomato plants. *P. indica* colonization, as well as the application of fungal exudates and cell wall extracts, significantly affects the vitality, infectivity, development, and reproduction of the cyst nematode *Heterodera schachtii* in *Arabidopsis thaliana* (Daneshkhah *et al*., 2013).

2.4.3 TOXIN PRODUCING FUNGUS

Khan and Saxena (1997) observed that *M. incognita* damage could be reduced with root dip treatment with culture filtrates of *A. niger*, *P. lilacinum* and *T. viride*. Sankaranarayanan *et al*. (1997) observed that *Trichoderma* and *Gliocladium* as could be used as a fungal biocontrol agent against PPN. Reddy *et al*. (1996) and Rao *et al.* (1998) reported *T. harzianum* as a prospective biocontrol agent against PPN. Reddy *et al.* (I996) and Sankaranarayanan and Sundarababu, (1997) also reported the synergistic impact of fungi *T. viride* along with the organic amendment for increasing the nematode trapping fungus population and plant growth enhancement.

The nematode antagonistic fungi belonging to genera *viz, Fusarium*, *Acremonium*, *Trichoderma*, *Chaetominum*, and *P. lilacinum*, can deliver various metabolites *in vitro*. When *Acremonium srictum*, *A. implicatum*, *P. lilacinum*, and *T. harzianum* were grown in fluid media, their culture filtrates were lethal to J_2 of *M. incognita* (Gowswami *et al.,* 2008).

Nitao *et al*. (1999) reported lower egg hatching rates in potato dextrose broth than in water. *T. harzianum* was able to penetrate nematode eggs and significantly decrease the hatching of *M. javanica* eggs. The tomato plants inoculated with the fungi showed an increase in the activity of resistance related enzymes *viz*, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase (Sahebani and Hadavi, 2008).

Acharya *et al*. (1988) reported good control of RKN in betel vine by field application of *T. viride*. Lal and Rana (2013) evaluated four fungi, *T. harzianum, T. viride, Gliocladium viren* and *A. ochraceous* as soil treatment, in screen house pot experiment against *M. incognita* infecting okra. Among all the fungi, *T. harzianum* was the most effective fungus in reducing nematode growth followed by *T. viride, G. virens* and *A. ochraceous*.

2.4.4 NEMATODE TRAPPING FUNGI

Nematode catching fungi are soil-borne fungi that capture moving phases of nematodes utilizing trapping structures of different shapes and sizes. These fungi are not having specific and could trap all dirt dwelling nematodes. Distinctive fungal species produce at least one sort of various trapping devices. These structures can differ from simple fungal hyphae covered to much more complex structures. They can be adhesive branches, simple loops, two-dimensional, or three-dimensional networks.

Trapping structures may vary even inside a genus, for instance *Nematoctonus robustus* produce adhesive knobs solely on hyphae, *N. leptosporus* only on sprouted conidia, and *N. angustatus* on both hyphae and conidia (Spadaro *et al.,* 2004).

A few detriments like unpredictability in the establishment in the soil, their constrained capturing activity and above all non-specific trap of PPN lessen their potential in natural control. Some *Arthrobotrys* species have been formulated and applied under precise conditions, yet the outcomes were conflicting (Camili *et al.*, 2010).

It was demonstrated that trapping fungi also have the capacity of secreting antimicrobial and nematicidal compounds like linoleic acid (*A. oligospora, A. conoides*) or pleurotin (*N. robustus*, *N. concurrens*). The creation of linoleic acid was absolutely correlated with the quantity of traps formed (Wang *et al.*, 2010).

Aspergillus awamori, an RKN trapping fungus secluded from the rhizosphere of tomato indicated 44.9 per cent control efficacy against *M. incogtina* (Cui *et al.,* 2015*).*

2.4.5 NEMATODE ENDOPARASITIC FUNGI

Few endoparasitic fungi produce zoospores that swim toward the nematode, attach to the cuticle usually around the natural openings, and afterward encyst. The encysted zoospores infiltrate the host body by means of those natural openings and start their vegetative growth. Later the hyphae develop some sporangium containing zoospores (Camili, 2010).

Drechmeria coniospora form huge numbers of conidia in contrast to production of hyphal material. In a single contaminated nematode, *D. coniospora* may create as many as 10000 conidia while the endoparasite *Hirsutella rhossoliensis*, which sporulates singly, produces 100–1000 conidia for each infected nematode. Both fungi develop an adhesive bud on their conidia with which they infect the nematode. *Catenaria anguillulae* infects nematodes with their motile zoospores which encyst on and stick to the nematode (Hertz *et al.*, 2001).

Varkey *et al.* (2017) observed that population of *M. incognita* in tomato could be controlled by a consortium of rizhobacteria and *Piriformospora indica.*

2.5 IDENTIFICATION OF NEMATODE PARASITIC FUNGAL ISOLATES

Fungal and bacterial cultures, nematophagous or antagonist to nematode were identified by various workers based on their morphological, physiological and biochemical characteristics. Rifai (1969) first time depicted the morphology of *Trichoderma*. *Trichoderma* is a septate fungus and produces highly 12 branched conidiophores with a cone shaped or pyramidal outline. He further portrayed that *Trichoderma* species form floccose or tufted colonies of a variety of colors (white, yellow, green), which can be utilized to identify and differentiate about various species of the genus.

Gams and Bissett (1998) conducted their research on morphology of *Trichoderma*. They portrayed that *Trichoderma* species are described by quick growth, mostly bright green conidia and a frequently branched conidiophore structure. They produce a wide cluster of shades from splendid greenish-yellow to rosy in shading, even though some are also colorless. Similarly, conidial pigmentation differs from colorless to various shades of

green and occasionally also grey or brown. Other than pigmentation, species identification within the genus is troublesome due to the narrow range of variation of the simplified morphology in *Trichoderma*.

Nagesh *et al*. (2005) reported that *A. oligospora* the nematode predaceous fungus exhibited septate two-celled ovate-oblong conidiospores, trapping hyphae, and radiating and sparse mycelium. Arevalo *et al*. (2009) identified RKN egg parasitic fungi, *P. chlamydosporia* based on their cultural and morphological characters such as white cottony colony, with a light-yellow center and regular edges. Conidiogenous cell were produced singly on vegetative hyphae or in two whorls on erect conidiophores. On the basis of conidial shape particular variety of fungus can be identified.

Hernandez (2013) characterized three fungal strains isolated from *Quercus spp*. and *Larrea tridentata* on morphological, physiological and molecular basis. Strains were genotyped by utilizing polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), random amplified polymorphic DNA (RAPD), inter-transcript spaces (ITS) and intergenic spaces (IGS) with the object to take out duplications.

Materials and Methods

3. MATERIALS AND METHODS

The study entitled "Evaluation of native isolates of nematode antagonistic fungi against *Meloidogyne incognita* (Kofoid and White) Chitwood in tomato" was undertaken in the Department of Nematology, College of Agriculture, Vellayani during 2017-2019. Details of materials used and methods employed for the work are mentioned below.

3.1. ISOLATION AND IDENTIFICATION OF NATIVE STRAINS OF NEMATODE ANTAGONISTIC FUNGI

3.1.1. Survey

A survey was conducted at six Taluks of Thiruvananthapuram district during 2017-18 for the isolation of indigenous fungi. 20 soil and root samples were took from the rhizosphere of vegetable crops like bhindi, tomato, chilly, cucumber and cowpea grown in each taluk by random sampling. Plants showing different disease symptoms such as yellowing, necrosis, withering, galling etc. were selected for sampling. These plants were collected and brought back to the laboratory for the isolation of egg masses and females of RKN. The soil adhered to roots was also collected carefully in polythene bag and labelled for the isolation of soil fungus. The soil samples (weighing 10g each) were collected using sterile spatula and kept in a clean and dry polythene bag under sterile conditions.

3.1.2. Isolation

3.1.2.1. From Rhizosphere

Fungus from soil samples were isolated by serial dilution technique. One g each of soil samples was added to 100 mL distilled water in a conical flask to get the stock solution and mixed thoroughly with a mechanical shaker. Test tubes were labelled from 10^{-1} to 10^{-8}. One mL of stock solution was transferred to the test tube labelled "10^{-1}" using a pipette and made up to 10 mL. Then the tube was capped and agitated gently until the solution was well mixed. One mL of the solution in the 10^{-1} test tube was transferred to 10^{-2} tube with a new pipette tip. This method was repeated to transfer solution from the 10^{-2} tube to the 10^{-3} tube and then from the 10^{-3} tube to the 10^{-4} tube. Five samples each

was spread plated in a Petri dish containing Rose bengal agar medium from the 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} tubes and were incubated at 30ºC for 48 hrs. Number of colonies on each plate was counted from each soil sample and those fungal colonies showing similar characteristics of *Trichoderma* or *Purpureocillium spp*. were selected for preliminary studies. These isolates were sub-cultured on Potato Dextrose Agar [PDA] media to get pure culture of the fungus and the fungus was streaked onto PDA slants for further analysis and storage.

3.1.2.2. Medium used

1. Potato Dextrose Agar (PDA) medium was used for isolation and maintenance of culture.

200 g of peeled and cut potatoes were washed and boiled in 500 mL of distilled water for 30 minutes. The extract was filtered through cheese cloth. 20g Agar-agar strips, washed in distilled water, and 20g dextrose were added to the extract and total volume was made to 1000 mL by adding distilled water. Medium was then poured into 500 mL conical flasks and plugged. The flasks were sterilized at 121^0C and 1.05 kg/cm^2 pressure for 30 minutes in an autoclave before use.

2. Potato Dextrose Broth (PDB) medium was used during the course of study for isolation and maintenance of culture.

200g of peeled and cut potatoes were washed and boiled in 500 mL of distilled water for 30 minutes. The extract was filtered through cheese cloth. 20g dextrose was added to the extract and total volume was made to 1000 mL by adding distilled water. Medium was then poured into 500 mL conical flasks and plugged. The flasks were sterilized at 121^0 C and 1.05 kg/cm^2 pressure for 30 minutes in an autoclave before use.

3.1.3. Identification of *Meloidogyne* Species

Meloidogyne spp. was identified by observing perineal pattern of adult females as described by Taylor and Netscher (1974) later modified and described by Hartman and Sasser (1985). The single galls containing mature females were collected and washed thoroughly. Adult females were removed from the galls with the help of forceps and needle. 10 - 20 females selected randomly from each sample were kept in 45 per cent lactic acid.

A cut was made at the anterior region and pressed the female nematode body against the glass slide. The inner tissues were cleaned with brush and then the posterior part of perineal pattern was cut with sharp blade and put in a drop of 45 per cent lactic acid and further trimmed in square shape under stereoscopic binocular microscope. Thoroughly cleaned the debris from perineal patterns using brush in lactic acid and transfered the trimmed perineal patterns to a drop of glycerin on a cleaned glass microscopic slide. Perineal pattern was aligned so that the anus oriented towards down and interior surface of the cuticle was placed against the glass. Perineal pattern was pressed gently against the glass and placed a cover glass on the glycerin drop, sealed and labelled for microscopic investigation. Perineal patterns of isolated species were studied with the help of identification keys as dorsal arch, striae, lateral field and tail terminus as given by Eisenback (1985).

3.1.4. Maintenance of Nematode Culture

M. incognita population was obtained from naturally infected tomato plant and was multiplied and maintained on susceptible tomato plant variety, Vellayani Vijay, grown in grow bags filled with sterilized loamy soils. The egg masses from infected plants were extracted and placed on culture plates containing distilled water and kept for 48-72 hrs in dark at 23° C to allow hatching. 5mL of freshly hatched juveniles were inoculated in grow bag. Inoculated plants were maintained throughout the experiment for the assortment of egg masses and second stage juveniles as and when required.

3.1.4.1. Collection of Egg Masses of M. incognita

Inoculated tomato plants were uprooted and roots are thoroughly washed for egg masses. Small spherical honey dew like brown colored egg mass adhered to the root surface were picked up carefully using forceps. These are collected in sterile distilled water and surface sterilized in 0.1 per cent sodium hypochlorite for two minutes and 95 per cent ethanol for one minute, followed by three continuous washings with sterile water (Singh and Siddique, 2010).

3.1.4.2. Preparation of Juvenile (J_2) Suspension of M. incognita

Egg masses were collected in sterile distilled water after surface sterilization and incubated at 28±2°C in BOD for 2 days for juvenile emergence (J_2) using Modified

Baermann's funnel technique (Schindler, 1961). The bottom of petri plates (10 cm diameter) were poured with sterile water and above that a concave wire mesh covered with double layered tissue paper was placed carefully without breaking the tissue paper. The egg masses were then spread uniformly on the tissue paper. The edges of the tissue paper spreading outside the wire mesh were bend back to keep away from the trickling of water drops from the edges as it might transmit nematodes. Petri plates were then completely filled with water and maintained a level 5 mm over the wire mesh. This plate was incubated at room temperature. After 24 to 48 hrs the wire mesh along with filter paper was removed and the extracted nematodes in the petri plate were collected and counted under a stereo zoom microscope using hand tally counter.

3.1.5. Preparation of Cell Free Extract (cfe) of Isolates

Five mm diameter mycelium disc was cut out from the fungus grown in PDA plates for 7 days using a sterile cork borer and used for inoculating 100ml of PDB broth (pH 7) in 250 ml conical flask and incubated at 28±2°C for 10 days at 100 rpm in an incubator shaker. Fungal mycelium was separated by centrifugation at 8000rpm for 10 minutes. CFE was collected aseptically and filter sterilized using Bacteriological Filter. The CFE was further diluted to S, S/2, S/3 and S/4 concentrations with appropriate amount of sterile distilled water.

3.1.5.1. Testing the Efficacy of fungal Isolates in vitro

Preliminary screening of 32 fungal colonies showing characteristics similar to *Trichoderma* (colonies with green, cottony white mycelium) and *Purpureocillium* (colonies powdery or suede-like, gold, green-gold, yellow-brown, lilac or tan) was conducted by preparing CFEs as mentioned in the para 3.1.5. Hundred freshly hatched juveniles of *M. incognita* were suspended in 5 mL of CFE of isolates in sterile vials (Standard concentration). Most effective ten isolates showing more than 50 per cent mortality were selected and their morphological, cultural characteristics and colony forming units were recorded.

3.1.5.2. Estimation of cfu mL^{-1}

After testing the efficacy of fungal isolates, number of colonies of selected isolates

is counted using a plate counter. Plate counter has a light source and a magnifying glass making colonies easier to see. Number of colonies was multiplied with dilution factor to determine the number of colony forming units.

3.1.6. *In vitro* Experiment to Test Larval Mortality

3.1.6.1. Preparation of Concentration

Cell free extracts (CFE) of isolates were prepared as mentioned in 3.1.5. Different concentration of CFEs of each isolates *viz*., S, S/2, S/3 and S/4 were prepared as follows:

S - 100% cell free extract (5 mL cfe)

S/2- 50% cell free extract + 50 % sterile water (2.5 mL cfe+2.5 mL sterile water)

S/3- 33.3% cell free extract + 66.7% sterile water (1.67 mL cfe+3.33 mL sterile water)

S/4- 25% cell free extract + 75% sterile water (1.25 mL cfe+3.75 mL sterile water)

Effect of CFEs of selected isolates on mortality of *M. incognita* was studied in four different concentrations (100, 50, 33.3 and 25%) at 24, 48 and 72 hrs after treatment. Hundred freshly hatched second stage juveniles of *M. incognita* were suspended in 5mL of each concentration of CFEs in sterile vials. All vials were kept in, BOD incubator at a temperature of 30°C. Larval mortality was estimated by counting the number of dead juveniles at 24, 48 and 72 hrs after treatment. Of these isolates, most effective three isolates were screened for ovicidal effects against *M. incognita in vitro*. Sterile water and plain broth were maintained as control.

3.2. SCREENING OF FUNGAL ISOLATES FOR NEMATICIDAL PROPERTY *IN-VITRO*

Four different concentrations of CFE of the isolates (S, S/2, S/3 and S/4) were prepared as mentioned in the para 3.1.6.1 and transferred to sterile vials (culture tubes). Hundred J_2 of *M. incognita* were suspended in each CFE of isolate separately and incubated at 28°C. For control, 100 J_2were placed in vials containing 5 mL each of sterile distilled water and plain broth. Each set was replicated four times. Larval mortality was calculated

by counting the number of juveniles dead 24, 48 and 72 hrs after treatment.

The experiment was carried out in completely randomized block design with six treatments and four replications.

3.3. SCREENING OF FUNGAL ISOLATES FOR EVALUATION OF NEMATODE EGG HATCHING *IN-VITRO*

The CFE of selected isolates in para 3.2.1 were transferred to sterile petri dishes of diameter 55mm. Three surface sterilized egg masses of similar size were picked with sterilized forceps and placed in CFE concentrations of S, S/2, S/3 and S/4. Each isolate was incubated at room temperature and egg hatching was observed. For control, three egg masses were placed in 5mL each of sterile distilled water and plain broth. Number of eggs hatched 1, 2, 3, 4, 5, 6, 7 and 8 days after treatment was recorded.

The experiment was carried out in completely randomized block design with six treatments and four replications

3.4 SCREENING OF FUNGAL ISOLATES FOR NEMATODE EGG PARASITIZATION *IN-VITRO*.

The fungus was inoculated to the centre of a Petri dish containing PDA medium amended with antibiotic streptomycin at 1 ml/L, then incubated at 25 ± 2^0C for 10 days. Each petri dish was then spread uniformly with surface sterilized egg masses of same size were picked with sterilized forceps. There were four replicates for each fungus, and egg free plates were served as control. After seven days, eggs were stained with cotton blue and percent egg parasitism assessed by counting the parasitized and non-parasitized eggs under a stereo microscope.

3.5. SCREENING OF FUNGAL ISOLATES FOR NEMATODE TRAP FORMATION *IN-VITRO*.

One g of soil was sprinkled on each of ten 2 % water agar plates amended with antibiotic streptomycin at 1 mL/L which had been inoculated with nematode to stimulate the growth of the fungi (Wyborn *et al.,* 1969). After seven days, the water agar plates are observed under a stereo microscope for trap formation by nematophagous fungus.

3.6. POT CULTURE STUDIES TO EVALUATE THE EFFECTIVE ISOLATE

A pot culture study was conducted to assess the effect of three promising fungal isolates, *P. indica* (1% w/v) and chemical Cartap hydrochloride (4% G) against RKN. The research was conducted in glass house.

3.6.1. Raising Tomato Seedlings

Tomato seedlings (Vellayani Vijay) were raised in pro trays filled with denematised coir pith and vermicompost.

3.6.2. Preparation of Denematized Potting Mixture

Sieved field soil, sand and farm yard manure in the proportion of 2:1:1 were mixed and packed in a polypropylene cover for autoclaving at 15kPa pressure and 121°C for 20 min. This denematised potting mixture was used in maintaining monoxenic culture of nematodes and pot culture experiment.

3.6.3. Fungal inoculation

Fungal inoculation was done by incorporating the mycelium at the rate of 1% (w/v) into the transplanting medium (sterile vermiculite and perlite in the ratio of 3:1 (v/v) and filled in protray cavities (5 cm dia × 5 cm depth)

3.6.4. Transplanting of Seedlings

Twenty one day old tomato seedlings were transplanted in 8 kg capacity pots with denematised potting mixture prepared as explained in the section 3.3.2. RKN culture was maintained as mentioned in section 3.1.3. After establishment of seedlings, freshly hatched second stage 2000 juveniles of *M. incognita* were inoculated in the root zone of transplanted seedlings. Fungal isolates were applied 48 hrs before nematode inoculation as soil drench. Tomato plants were maintained as per the POP Recommendations of Kerala Agricultural University (KAU, 2016).

The experiment was carried out in completely randomized block design with six treatments and four replications

T1, T2, T3: Promising isolates.

T4 : Cartap hydrochloride 4% G (25kg/ha)

T5 : *Piriformospora. indica* (1% w/v)

T6 : Untreated check

3.6.5. Recording Observations

3.6.5.1. Estimation of Nematode Population in Soil

Soil samples (200cc) were collected from the rhizosphere of tomato plants for the estimation of nematode population at the time of harvest. Nematodes were extracted from the representative soil samples as per Cobb's sieving and decanting technique (Cobb, 1918) and modified Baermann's method (Schindler, 1961). The nematodes thus extracted were counted under a stereo zoom microscope using a hand tally counter.

3.6.5.2. Estimation of Nematode Population in Roots

Plants were uprooted for estimating nematode population in roots by modified Baermann funnel technique and root incubation technique as follows.

3.6.5.3. Root-knot Index

Root-knot indexing was done using the technique of Heald *et al*. (1989).

Number of galls or root	Root knot index
0	0
1-25	1
26-50	2
51-75	3
76-100	4
>100	5

3.6.5.4. Number of Females

Root samples (5 g) of tomato were cut into 2- 3 cm length and stained by differential staining method using acid fuschsin-lactophenol mixture. Lacto phenol was prepared by mixing liquid phenol (500 mL), lactic acid (500 mL), glycerine (100 mL) and distilled water (500 mL). Stock solution of acid- fuchsin was prepared by dissolving 3.5g acid fuchsin in 250 mL of acetic acid and 750 mL of distilled water. Working solution of the stain was prepared by adding one mL of the stock solution of the stain into 100 mL of lacto phenol solution. The stain was boiled in a beaker on a hot plate. The infected roots of each treatment were immersed in the boiling stain for one min. rinsed with tap water and then destained in lacto phenol solution until the maximum contrast between the nematodes and the root tissue was obtained. The processed roots were squeezed between glass slides, teased with a clean needle and observed under a microscope to count the number of females.

3.6.5.5. Number of Egg Masses

Egg mass number in roots was calculated following the method of Southey (1986). Phloxine B solution was prepared for staining egg masses by mixing 0.15g of Phloxine B in 1L of water. Stained egg masses from the root system were counted under a stereo zoom microscope.

3.6.5.6. Biometric Observation and Yield

Biometric observations (plant height, shoot and root weight) were recorded from the plants uprooted at harvest. Yield in terms of fruit weight was recorded.

3.7. IDENTIFICATION OF FUNGI

3.7.1 Molecular Characterization

Species level confirmation was done at molecular level by ITS-1F, ITS-4R and 16S sequencing for fungi and bacteria respectively, using the facilities available at Rajiv Gandhi Centre for Biotechnology (RGCB) for molecular identification, Thiruvananthapuram, Kerala, India.

3.7.1.1 ITS Sequencing

3.7.1.1.1 DNA Isolation using NucleoSpin® Plant II Kit (Macherey-Nagel)

Around 100 mg of the mycelium was homogenized using liquid nitrogen and the powdered mycelia were transferred to a micro centrifuge tube. 400 µL of buffer PL1 was added to powdered tissue and vortexed for one minute. Then 10µL of RNase A solution was added and inverted to mix. The homogenate obtained was incubated at 65°C for 10 minutes later this lysate was transferred to a Nucleospin filter and centrifuged at 11000 x g for two minutes. The flow through liquid was collected and 450 µL of buffer PC was added. The solution was transferred to a Nucleospin Plant II column, centrifuged for one minute and the flow through liquid was discarded. 400 µL buffer PW1 was added to the column, centrifuged at 11000 x g for one minute and flow though liquid was discarded. Then 700 µl PW2 was added centrifuged at 11000 x g and flow through liquid was discarded. Finally, 200 µl of PW2 was added and centrifuge at 11000 x g for two minutes to dry the silica membrane. The column was transferred to a new 1.7 mL tub and 50µl of buffer PE was added and incubated at 65°C for five minutes. The column was centrifuged at 11000 x g for one minute to elude the DNA. The eluted DNA was stored at 4°C.

3.7.2 Agarose Gel Electrophoresis for DNA Quality check

The quality of the DNA isolated was checked using agarose gel electrophoresis. One µl of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH-8.0) was added to 5µl of DNA. The samples were loaded to 0.8% agarose gel prepared in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg/mL ethidium bromide. Electrophoresis was performed with 0.5X TBE as electrophoresis buffer at 75 V until bromophenol dye front has migrated to the bottom of the gel. The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio- Rad).

3.7.3 PCR Analysis

PCR amplification reactions were carried out in a 20 µl reaction volume which had 1X Phire PCR buffer (contains 1.5 mM $MgCl_2$), 0.2mM each dNTPs (dATP, dGTP,

dCTP and dTTP), 1 µl DNA, 0.2 µl Phire Hotstart II DNA polymerase enzyme, 0.1 mg/mL BSA and 3% DMSO, 0.5M Betaine, 5pM of forward and reverse primers.

Primers used

Target	Primer Name	Direction	Sequence (5' → 3')
ITS	ITS-1F	Forward	TCCGTAGGTGAACCTGCGG
	ITS-4R	Reverse	TCCTCCGCTTATTGATATGC

The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems).

PCR amplification profile

ITS

98 °C - 30 sec } 40 cycles

98 °C - 5 sec

60 °C - 10 sec

72 °C - 15 sec

72 °C - 60 sec

4 °C- ∞

3.7.4 Agarose Gel Electrophoresis of PCR Products

The PCR products were checked in 1.2% agarose gels prepared in 0.5X TBE buffer containing 0.5 µg/mL ethidium bromide. One µl of 6X loading dye was mixed with 5 µl of PCR products and electrophoresis was performed at 75V power supply with 0.5X TBE as electrophoresis buffer for about one-two hours, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular

standard used was 2-log DNA ladder (NEB). The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.7.5 ExoSAP-IT Treatment

ExoSAP-IT (GE Healthcare) consists of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal of unwanted primers and dNTPs from a PCR product mixture with no interference in downstream applications. Five micro litres of PCR product were mixed with 2 µl of ExoSAP-IT and incubated at 37ºC for 15 minutes followed by enzyme inactivation at 80ºC for 15 minutes.

3.7.6 Sequencing using Big Dye Terminator v3.1

Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufactures protocol.

The PCR mix consists of following components:

PCR Product(ExoSAP treated)	-	10-20 ng
Primer	-	3.2 pM (either Forward or Reverse)
Sequencing Mix	-	0.28 µL
5x Reaction buffer	-	1.86 µL
Sterile distilled water	-	make up to 10µL

The sequencing PCR temperature profile consisted of a first cycle at 96ºC for two minutes followed by 30cycles at 96ºC for 30 sec, 50ºC for 40 sec and 60ºC for 4 minutes for all the primers.

3.7.7 Post Sequencing PCR Clean up

Master mix I containing 10µL milli Q and 2 µL125mM EDTA was added to each reaction containing 10µL of reaction contents and properly mixed. Then master mix II containing of 2 µL of 3M sodium acetate pH 4.6 and 50 µL of ethanol was added to each reaction and mixed by inverting. The reaction mixture was incubated at room temperature for 30 minutes and centrifuged at 14,000 rpm for 30 minutes. The supernatant was decanted and 100 µL of 70% ethanol was added and centrifuged at 14,000 rpm for 20 minutes. The supernatant was discarded and repeat 70% ethanol wash again supernatant was decanted and cleaned up air dried product obtained was sequenced in ABI 3500 DNA Analyzer (Applied Biosystems).

3.7.8 Sequence Analysis

The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems).

3.8. STATISTICAL ANALYSIS

Data generated from the experiment (3.1 to 3.3) were subjected to analysis of variance (ANOVA) test (Cochran and Cox, 1965). Those variables which did not satisfy the basic assumptions of ANOVA were subjected to angular and square root transformation.

Results

4. RESULTS

The results of the study "Evaluation of native isolates of nematode antagonistic fungi against *Meloidogyne incognita* (Kofoid and White) Chitwood in tomato" are presented below.

4.1. ISOLATION OF INDIGENOUS FUNGI

4.1.1. Thiruvananthapuram District.

A total of 100 samples were gathered from the rhizosphere of vegetable plants like chilli, tomato, brinjal, cowpea and snake gourd (healthy and diseased) grown in Thiruvananthapuram District by random sampling. Ten samples were collected from these vegetable plants showing galling, yellowing, rotting and necrosis. Isolates showing characters similar to *Trichoderma* (colonies varied from light green to dark green) and *Purpureocillium* (colonies which are fast growing, powdery or suede-like, gold, green-gold, yellow-brown, lilac or tan) were selected by preliminary screening of juvenile mortality of *M. incognita*. Cell Free Extracts (CFE) of isolate 10, 12 and 27 exhibited 81.00, 80.00 and 83.00 per cent mortality of *M. incognita* juveniles under *in vitro* conditions (Table.1).

4.1.2. Colony Characters and cfu mL^{-1} of Selected Fungal Isolates.

The colony characters of the selected indigenous fungal isolates in potato dextrose agar (PDA) are indicated in Table 2.

4.2. Effect of Cell Free Extracts of Isolates on *M. incognita* Juveniles

Out of thirty-two fungal isolates, the most effective ten isolates (1, 10, 11, 12, 16, 19, 25, 26, 27 and 32) which showed 66.00 to 94.00 per cent juvenile mortality in preliminary screening were selected for further screening in four different concentrations (100, 50, 33.3 and 25%) at 24, 48 and 72 hrs after treatment *in vitro.* Sterile water and plain broth were maintained as control.

4.2.1. Isolate 1

Analysis of the data on mortality of *M. incognita* juveniles to different

Table 1. Details of indigenous fungal isolates collected from rhizosphere of vegetables in Thiruvananthapuram district.

Crop and Location	Disease symptoms	No. of samples collected	No. of Isolates	Larval mortality shown by the isolates
Bhindi (Parassala)	Galling, Yellowing	10(6S+4R)	3	Isolate 1 (72.00%) S
	Galling, necrosis			Isolate 2 (44.50%) S
	Stunting			Isolate 3(21.00%) R
Tomato (Perumkadavila)	Galling, Yellowing	5(4S+1R)	3	Isolate 4 (23.00%) R
	Galling, necrosis			Isolate 5 (21.00%) S
	Dead plant			Isolate 6(45.00%) S
Salad cucumber (Athiyannoor)	Stunting	10(6S+4R)	3	Isolate 7(22.00%) R
	Severe yellowing			Isolate 8(22.00%) S
	wilting			Isolate 9 (24.00%) S
Brinjal (Nemom)	Root galling	10(6S+4R)	2	Isolate 10(81.00%) S
	Stunting			Isolate 11(79.00%) R
Snake gourd (Pothencode)	Yellowing	10(6S+4R)	3	Isolate 12 (80.00%) S
	Galling			Isolate 13 (23.00%) R
	Wilting Wilting			Isolate 14 (20.00%) S
Yard long bean (Vellanad)	Yellowing stunting,	10(8S+2R)	1	Isolate 15(24.00%) R

Table 1. Details of indigenous fungal isolates collected from rhizosphere of vegetables in Thiruvananthapuram district (cont.)

Crop and Location	Disease symptoms	No. of samples collected	No. of Isolates	Larval mortality shown by the isolates
Tomato (Nedumangad)	Galling, Yellowing	5(3S+2R)	3	Isolate 16(62.00%) S
	Galling, necrosis			Isolate 17(23.00%) R
	Dead plant			Isolate 18(15.00%) S
Salad cucumber (Vamanapuram)	Stunting	10(6S+4R)	3	Isolate 19 (51.00%) S
	Yellowing, stunting			Isolate 20 (23.00%) R
	Withering, stunting			Isolate 21 (19.00%) S
Bhindi (Kilimanoor)	Stunting	10(7S+3R)	3	Isolate 22 (23.00%) R
	Yellowing, stunting			Isolate 23 (21.00%) R
	Withering, stunting			Isolate 24 (33.00%) S
Brinjal (Chirayinkeezhu)	Root galling	10(7S+3R)	2	Isolate 25 (79.00%) S
	Stunting			Isolate 26 (55.00%) R
Tomato (Varkala)	Galling, Yellowing	5(4S+1R)	3	Isolate 27(83.00%) R
	Galling, necrosis			Isolate 28 (22.00%) S
	Dead plant			Isolate 29 (23.00%) S
Tomato (Kazhakkuttom)	Galling, Yellowing	5(3S+2R)	3	Isolate 30 (23.00%) S
	Galling, necrosis			Isolate 31 (25.00%) R
	Dead plant			Isolate 32 (77.00%) S

Table 2. Morphological and cultural characteristics of indigenous fungal isolates

Isolates	Morphological and cultural characteristics
Isolate 1	The colour of the colony reverse was greenish yellow. Conidiophores were mostly short and irregularly branched
Isolate 10	Colonies having concentric rings with green conidial which are denser in centre then towards the margins.
Isolate 11	Colonies are fast growing, powdery or suede-like, gold, green-gold, yellow-brown, lilac or tan.
Isolate 12	The colonial growth showed white to greenish colour and eventually, it became dark green was observed with white spores.
Isolate 16	Colonies are usually fast growing, in shades of green, sometimes white, mostly consisting of a dense felt of conidiophores.
Isolate 19	Colonies are usually fast growing, in shades of green, sometimes white, mostly consisting of a dense felt of conidiophores.
Isolate 25	Colonies are usually slow growing, often compact and moist at first, becoming powdery, suede-like or floccose with age, and may be white, grey, pink, rose or orange in colour.
Isolate 26	Colonies are usually fast growing, pale coloured. The colour of the thallus varies from whitish to yellow, pink, red or purple shades.
Isolate 27	The colonial growth showed characteristic, white to creamy and tinge pink tinge, margins slightly smooth
Isolate 32	The colonial growth showed characteristic velvety to the woolly culture of yellow to green colour (especially at centre), with a white border surrounding the yellow to the greenish surface.

concentrations (100, 50, 33.3 and 25%) of CFE of isolate 1 showed statistically significant superiority over the control at 24, 48 and 72 hrs after exposure (Table 3). CFE of isolate 1 at all concentration could not produce any significant difference with regard to mortality of *M. incognita* juveniles at 24 hrs after exposure and 72 hrs after exposure. Effect of CFE isolate 1 at 50% concentration (28.25 per cent) was found as effective as 100% concentration (17.75 per cent mortality of *M. incognita* juveniles), while 33.3 % and 25 % concentrations were also as effective as 100 % concentration at 48 hrs after treatment.

4.2.2. Isolate 10

All the concentrations (100, 50, 33.3 and 25%) of isolate 10 showed statistically significant superiority over control at 24, 48 and 72 hrs after treatment. Isolate 10 at 100% concentration recorded 17.93 per cent mortality of *M. incognita* juveniles at 24 hrs after treatment and it was found as effective as 50% concentration in which 16.50 per cent mortality of *M. incognita* juveniles was observed, which in turn was also comparable with 33.3 % concentration. Effect of isolate 10 at 33.3 % and 25% concentration was statistically on par at 24 hrs after treatment having a juvenile mortality of 14.50 and 12.13 per cent respectively. At 48 hrs after treatment, isolate 10 at 100% concentration recorded 46.88 per cent mortality of *M. incognita* juveniles and it was significantly superior to lower concentrations. Isolate 10 at 50% concentration exhibited 37.50 per cent mortality of *M. incognita* juveniles and it was significantly superior to lower concentrations having a juvenile mortality of 27.25 per cent at 33.3 % concentration and 21.25 per cent at 25% concentration. At 72 hrs after treatment each higher concentration gave significantly superior percentage of juvenile mortality compared to lower concentrations *i.e.*, 85.05, 68.25, 45.25 and 34.50 at 100%,50%,33.33% and 25 % concentrations respectively (Table 4).

4.2.3. Isolate 11

The data displayed at Table 5 revealed that CFE of isolate 11 at 100, 50, 33.3 and 25% concentrations recorded statistically significant superiority over the control at 24, 48 and 72 hrs after treatment. At 24 and 48 hrs after treatment each higher concentration gave significantly superior juvenile mortality compared to lower concentrations *i.e.*, 26.00, 20.25, 14.00 and 8.25 per cent mortality of *M.incognita* juveniles at 100%,50%,33.33%

Table 3. Effect of cell free extracts of isolate 1 on mortality of *M. incognita in vitro*

Treatments	**Mortality (%)**		
Concentrations	**24 HAE***	**48 HAE**	**72 HAE**
100%	13.25 (21.35) [a]	17.75 (24.92) [ab]	23.00 (28.66) [a]
50%	19.75 (26.39) [a]	28.25 (32.11) [a]	30.50 (33.52) [a]
33.3%	12.50 (20.70) [a]	13.25 (21.35) [b]	18.00 (25.10) [a]
25%	11.50 (19.82) [a]	13.50 (21.56) [b]	20.75 (27.10) [a]
Sterile water	1.00 (5.74) [b]	1.50 (7.03) [c]	1.50 (7.03) [b]
Plain broth	0.50 (4.05) [b]	0.50 (4.05) [c]	1.00 (5.74) [b]
CD (0.05)	**(6.767)**	**(8.768)**	**(9.215)**

Figures in the parenthesis are Angular transformed values *Hours After Exposure

Table 4. Effect of cell free extracts of isolate 10 on mortality of *M. incognita in vitro*

Treatments	Mortality (%)		
Concentrations	**24 HAE***	**48 HAE**	**72 HAE**
100%	17.93 (25.02) [a]	46.88 (43.19) [a]	85.05 (67.60) [a]
50%	16.50 (23.95) [ab]	37.50 (37.72) [b]	68.25 (55.72) [b]
33.3%	14.50 (22.36) [bc]	27.25 (31.45) [c]	45.25 (42.26) [c]
25%	12.13 (20.36) [c]	21.25 (27.34) [c]	34.50 (35.93) [d]
Sterile water	0.5 (2.87) [d]	0.50 (2.87) [d]	0.50 (2.87) [e]
Plain broth	0.25 (1.43) [d]	0.75 (3.47) [d]	1.00 (4.06) [e]
CD (0.05)	**(2.965)**	**(4.561)**	**(5.419)**

Figures in the parenthesis are Angular transformed values *Hours After Exposure

Table 5. Effect of cell free extracts of isolate 11 on mortality of *M. incognita in vitro*

Treatments	Mortality (%)		
Concentrations	**24 HAE***	**48 HAE**	**72 HAE**
100%	26.00 (30.66) a	32.50 (34.76) a	39.75 (39.09) a
50%	20.25 (26.74) b	26.25 (30.82) b	33.25 (32.21) b
33.3%	14.00 (21.97) c	18.00 (25.10) c	27.25 (31.47) b
25%	8.25 (16.69) d	12.50 (20.70) d	15.75 (23.38) c
Sterile water	0.75 (4.97) e	1.50 (7.03) e	2.00 (8.13) d
Plain broth	0.75 (4.97) e	1.25 (6.42) e	1.50 (7.03) d
CD (0.05)	**(3.413)**	**(2.472)**	**(2.288)**

Figures in the parenthesis are Angular transformed values *Hours After Exposure

and 25 % concentrations respectively at 24 hrs after treatment and 32.50,26.25,18.00 and 12.50 per cent mortality of *M. incognita* juveniles at 48 hrs after treatment. At 72 hrs after treatment, 100% concentration recorded 39.75 per cent mortality of *M. incognita* juveniles and it was significantly superior to lower concentrations. Isolate 11 at 50% concentration recorded 33.25 per cent mortality of *M. incognita* juveniles and it was on par with 33.3% concentration having 27.25 per cent mortality of *M.incognita* juveniles. At 25% concentration 15.75 per cent mortality of *M.incognita* juveniles was observed.

4.2.4 Isolate 12

All the concentrations (100, 50, 33.3 and 25%) of isolate 12 showed statistically significant superiority over the control at 24, 48 and 72 hrs after treatment (Table 6). At 24, 48 and 72 hrs after treatment each higher concentration gave significantly superior juvenile mortality compared to lower concentrations *i.e.*, 59.00, 47.25, 32.25 and 17.25 per cent at 24 hrs after treatment for 100%, 50%, 33.33% and 25 % concentrations respectively, while at 48 hrs after treatment the values ranged from 74.75 per cent at 100 %, 52.50 per cent at 50%, 36.00 per cent at 33.3 % and 19.50 per cent at 25% concentration. At 72 hrs after treatment percentage of juvenile mortality recorded were 76.50, 68.25, 45.25 and 34.50 at 100%, 50%, 33.33% and 25 % concentrations respectively.

4.2.5 Isolate 16

Analysis of the data on mortality of *M. incognita* juveniles to different concentrations (100, 50, 33.3 and 25%) of CFE of isolate 16 showed statistically significant superiority over the control at 24, 48 and 72 hrs after exposure (Table 7). At 24 hrs after treatment all concentration showed more or less same mortality of *M. incognita* juveniles. At 48 hrs after treatment 25 % concentration reported more juvenile mortality (71.00 per cent) and it was comparable with 50 % concentration (66.00 per cent) and 33.3 % concentration (64.75 per cent). 100 % concentration was on a par with 50 % and 33.3%. At 72 hrs after treatment 100 % concentration registered the lowest juvenile mortality and the mortality reported by all other concentrations were comparable.

Table 6. Effect of cell free extracts of isolate 12 on mortality of *M. incognita in vitro*

Treatments	**Mortality (%)**		
Concentrations	**24 HAE***	**48 HAE**	**72 HAE**
100%	59.00 (50.18) [a]	74.75 (59.83) [a]	76.50 (61.00) [a]
50%	47.25 (43.42) [b]	52.50 (46.43) [b]	68.25 (55.70) [b]
33.3%	32.25 (34.60) [c]	36.00 (36.86) [c]	45.25 (42.27) [c]
25%	17.25 (24.54) [d]	19.50 (26.21) [d]	34.50 (35.97) [d]
Sterile water	0.75 (4.97) [e]	1.00 (5.74) [e]	1.25 (6.42) [e]
Plain broth	0.25 (2.87) [e]	0.50 (4.05) [e]	1.00 (5.74) [e]
CD (0.05)	**(4.694)**	**(5.843)**	**(3.976)**

Figures in the parenthesis are Angular transformed values *Hours After Exposure

Table 7. Effect of cell free extracts of isolate 16 on mortality of *M. incognita in vitro*

Treatments	**Mortality (%)**		
Concentrations	**24 HAE***	**48 HAE**	**72 HAE**
100%	30.50 (33.52) [a]	48.25 (44.00) [b]	51.50 (45.86) [b]
50%	22.00 (27.97) [a]	66.00 (54.33) [ab]	62.75 (52.39) [a]
33.3%	23.75 (29.17) [a]	64.75 (53.58) [ab]	69.75 (56.63) [a]
25%	21.25 (27.45) [a]	71.00 (57.42) [a]	76.50 (61.00) [a]
Sterile water	0.75 (4.97) [b]	0.75 (4.97) [c]	1.00 (5.74) [c]
Plain broth	0.50 (4.05) [b]	0.50 (4.05) [c]	0.75 (4.97) [c]
CD (0.05)	**(10.708)**	**(10.952)**	**(8.805)**

Figures in the parenthesis are Angular transformed values *Hours After Exposure

4.2.6 Isolate 19

All the concentrations (100, 50, 33.3 and 25%) of isolate 19 showed statistically significant superiority over the control at 24, 48 and 72 hrs after treatment (Table 8). At 24 hrs after treatment isolate 19 at 50 % concentration showed 32.00 per cent mortality of *M.incognita* juveniles and it was comparable with 100 % concentration with 25.75 per cent mortality of *M.incognita* juveniles. The mortality recorded by 100 % concentration was comparable with 33.3 % (20.00 per cent mortality) and 25 % (20.50 per cent mortality). At 48 hrs after treatment, effect of isolate 19 at 50% concentration (35.50 per cent mortality) was found as effective as 100% concentration (30.50 per cent mortality of *M. incognita* juveniles). The mortality exhibited by 100 % concentration was comparable with 25 % (26.75 mortality) and 33.3 % concentration (22.50% mortality). At 72 hrs after treatment isolate 12 at 100% concentration (40.25% mortality) was found as effective as 50% concentration (39.25 per cent) and 25 % concentration (33.50% mortality).

4.2.7. Isolate 25

All the concentrations (100, 50, 33.3 and 25%) of isolate 25 showed statistically significant superiority over the control at 24, 48 and 72 hrs after treatment (Table 9). CFE of isolate 25 at 100% concentration recorded significant superiority over all other concentrations (50, 33.3 and 25%) at 24, 48 and 72 hrs after treatment. Isolate 25 (100% concentration) recorded 23.25 per cent mortality of *M. incognita* juveniles at 24 hrs after treatment. Isolate 25 (50% concentration) exhibited 16.25 per cent mortality of *M. incognita* juveniles at 24 hrs after treatment and it was as effective as 33.3% concentration (12.25 per cent mortality of *M. incognita* juveniles). At 48 and 72 hrs after treatment each higher concentration gave significantly superior juvenile mortality compared to lower concentrations *i.e.*, 32.25, 24.25, 15.50 and 8.00 per cent mortality of *M. incognita* juveniles at 100%, 50%, 33.33% and 25 % concentrations respectively at 48 hrs after treatment, and 37.75, 26.75, 21.25 and 11.25 per cent mortality of *M. incognita* juveniles at 100%, 50%, 33.33% and 25 % concentration respectively at 72 hrs after treatment

4.2.8. Isolate 26

Data presented in Table 10 showed statistically significant variation between

Table 8. Effect of cell free extracts of isolate 19 on mortality of *M. incognita in vitro*

Treatments	Mortality (%)		
Concentrations	**24 HAE***	**48 HAE**	**72 HAE**
100%	25.75 (30.49) [ab]	30.50 (33.52) [ab]	40.25 (39.38) [a]
50%	32.00 (34.45) [a]	35.50 (36.57) [a]	39.25 (38.79) [a]
33.3%	20.00 (26.57) [b]	22.50 (28.32) [b]	26.75 (31.14) [b]
25%	20.50 (26.92) [b]	26.75 (31.54) [ab]	33.50 (35.37) [ab]
Sterile water	0.75 (4.97) [c]	0.75 (4.97) [c]	0.75 (4.97) [c]
Plain broth	0.25 (2.87) [c]	0.25 (2.87) [c]	1.00 (5.74) [c]
CD (0.05)	**(5.800)**	**(5.242)**	**(4.455)**

Figures in the parenthesis are Angular transformed values *Hours After Exposure

Table 9. Effect of cell free extracts of isolate 25 on mortality of *M. incognita in vitro*

Treatments	Mortality (%)		
Concentrations	**24 HAE***	**48 HAE**	**72 HAE**
100%	23.25 (28.83) [a]	32.25 (34.60) [a]	37.75 (37.91) [a]
50%	16.25 (23.77) [b]	24.25 (29.50) [b]	26.75 (31.14) [b]
33.3%	12.25 (20.49) [b]	15.50 (23.18) [c]	21.25 (27.45) [c]
25%	4.50 (12.25) [c]	8.00 (16.43) [d]	11.25 (19.60) [d]
Sterile water	0.50 (4.05) [d]	0.50 (4.05) [e]	0.50 (4.05) [e]
Plain broth	0.50 (4.05) [d]	0.50 (4.05) [e]	0.50 (4.05) [e]
CD (0.05)	**(3.637)**	**(3.525)**	**(3.442)**

Figures in the parenthesis are Angular transformed values *Hours After Exposure

Table 10. Effect of cell free extracts of isolate 26 on mortality of *M. incognita in vitro*

Treatments	Mortality (%)		
Concentrations	**24 HAE***	**48 HAE**	**72 HAE**
100%	26.75 (31.14) [a]	45.25 (42.27) [a]	54.75 (47.73) [a]
50%	24.25 (29.50) [a]	27.00 (31.31) [b]	29.75 (33.05) [b]
33.3%	20.75 (27.10) [a]	24.00 (29.33) [b]	28.75 (32.42) [b]
25%	14.75 (22.59) [b]	17.00 (24.35) [c]	20.00 (26.57) [c]
Sterile water	0.50 (4.05) [c]	0.50 (4.05) [d]	1.00 (5.74) [d]
Plain broth	0.50 (4.05) [c]	1.00 (5.74) [d]	1.50 (7.03) [d]
CD (0.05)	**(4.579)**	**(4.732)**	**(4.192)**

Figures in the parenthesis are Angular transformed values *Hours After Exposure

treatments. Maximum juvenile mortality (26.75 per cent) was observed in 100% concentration of CFE of isolate 26 at 24 hrs after exposure and it was found as effective as 50 % concentration (24.25 per cent mortality) and 33.3% concentration (20.75 per cent mortality of *M. incognita* juveniles). 25 % concentration recorded the lowest juvenile mortality (14.75 per cent mortality). At 48 hrs after treatment CFE of isolate 26 at 100% concentration exhibited maximum juvenile mortality (45.25 per cent) and it was significantly superior to all other concentrations (50, 33.3 and 25%). CFE of isolate 26 at 50 % concentration was 27.00 per cent and it was on a par with 33.3 % concentration with 24.00 per cent juvenile mortality. At 72 hrs after treatment CFE of isolate 26 at 100% concentration exhibited maximum juvenile mortality (54.75 per cent) and it was significantly superior to all other concentrations followed by 50 % concentration with 29.75 per cent mortality, which was on par with 33.3 % concentration with 28.75 per cent mortality. The lowest mortality was observed at 25 % concentration (20.00 per cent mortality) of *M. incognita* juveniles.

4.2.9. Isolate 27

Data presented in Table 11 revealed statistically significant difference in mortality of *M. incognita* juveniles between different concentrations of isolate 27 at different exposure periods. CFE of isolate 27 at 100% concentration exhibited maximum juvenile mortality (46.50 per cent) at 24 hrs after exposure. It was statistically superior to 50% concentration of CFE which recorded 39.50 per cent mortality of *M. incognita* juveniles at 24 hrs after treatment. Effect of isolate 27 CFE at 33.3% concentration was statistically superior to 25% concentration and the recorded mortality of juveniles was 17.00 per cent. CFE of isolate 27 at 100% concentration exhibited maximum juvenile mortality (52.50 per cent) at 48 hrs after exposure. It was statistically superior to 50% concentration of CFE which recorded 42.75 per cent mortality of *M. incognita* juveniles. Effect of isolate 27 CFE at 33.3% concentration (30.75) was statistically superior to 25% concentration with mortality of juveniles (20.00 per cent). CFE of isolate 27 at 100% concentration exhibited maximum juvenile mortality (62.50 per cent) at 72 hrs after exposure. It was statistically superior to 50% concentration of CFE which recorded 47.00 per cent mortality of *M. incognita* juveniles at 72 hrs after treatment. Effect of isolate 27 CFE at 33.3% concentration (32.75 % mortality) was statistically superior to 25% concentration and

Table 11. Effect of cell free extracts of isolate 27 on mortality of *M. incognita in vitro*

Treatments	**Mortality (%)**		
Concentrations	**24 HAE***	**48 HAE**	**72 HAE**
100%	46.50 (42.99) [a]	52.50 (46.43) [a]	62.50 (52.24) [a]
50%	39.50 (38.94) [b]	42.75 (40.83) [b]	47.00 (43.28) [b]
33.3%	27.75 (31.79) [c]	30.75 (33.68) [c]	32.75 (34.91) [c]
25%	17.00 (24.39) [d]	20.00 (26.57) [d]	24.25 (29.50) [d]
Sterile water	0.50 (4.05) [e]	1.25 (6.42) [e]	1.75 (7.60) [e]
Plain broth	0.50 (4.05) [e]	1.00 (5.74) [e]	2.00 (8.13) [e]
CD (0.05)	**(3.824)**	**(3.352)**	**(2.336)**

Figures in the parenthesis are Angular transformed values *Hours After Exposure

mortality of juveniles was 24.25 per cent.

4.2.10. Isolate 32

Data presented in Table 12 revealed statistically significant difference in mortality of *M. incognita* juveniles between different concentrations of isolate 32 at different exposure periods and it showed statistically significant superiority over control at 24, 48 and 72 hrs after treatment. At 24 and 72 hrs after treatment each higher concentration gave significantly superior percentage of juvenile mortality compared to lower concentrations *i.e.* 27.00, 14.50, 10.75 and 7.00 at 100%, 50%, 33.33% and 25 % concentration respectively at 24 hrs after treatment and 45.25, 32.50, 23.25 and 16.50 at 100%, 50%, 33.33% and 25 % concentration respectively at 72 hrs after treatment. At 48 hrs after treatment higher juvenile mortality (38.25 per cent) was recorded by 100 % concentration and it was on par with 50 % concentration (27.00 per cent). The mortality reported by 33.3 % and 25 % concentrations were on par.

4.3. *IN VITRO* SCREENING OF FUNGAL ISOLATES ON EGG HATCHING

CFEs of isolates 10, 12 and 27 at 100, 50, 33.3 and 25% concentration were tested for egg hatch inhibition of *M.incognita*. The results are given in Table 13.

4.3.1 Effect on Egg Hatching

CFEs of isolates 10, 12 and 27 exhibited statistically significant variation in the hatching of eggs from three to eight days after treatment (Table 13). No hatching was observed on first and second day after treatment.

On the third day of exposure, CFE of isolate 10 at 100% concentration exhibited minimum egg hatching (14.00 per cent) and it was statistically on par with isolate 10 at 50% and 33.3% concentration giving 15.00 and 16.25 per cent egg hatching respectively. Isolate 10 (50% concentration and 33.3 % concentrations) were on par with isolate 10 at 25 % concentration (17.25 per cent egg hatching). 50% concentration and 33.3 % concentrations isolate 10 were statistically on par with isolate 12 at 100 % concentration (18.00 per cent egg hatching), 50 % concentration (18.50 per cent egg hatching), 33.3 % concentration (19.13 per cent egg hatching). All concentrations of isolate 12 were

Table 12. Effect of cell free extracts of isolate 32 on mortality of *M. incognita in vitro*

Treatments	**Mortality (%)**		
Concentrations	**24 HAE***	**48 HAE**	**72 HAE**
100%	27.00 (31.31) [a]	38.25 (32.20) [a]	45.25 (42.27) [a]
50%	14.50 (22.38) [b]	27.00 (31.31) [ab]	32.50 (34.76) [b]
33.3%	10.75 (19.14) [c]	18.25 (25.29) [c]	23.25 (28.83) [c]
25%	7.00 (15.34) [d]	15.50 (23.18) [c]	16.50 (23.97) [d]
Sterile water	0.25 (2.87) [e]	0.75 (4.97) [d]	0.75 (4.97) [e]
Plain broth	0.50 (4.05) [e]	0.50 (4.05) [d]	1.00 (5.74) [e]
CD (0.05)	**(3.759)**	**(3.968)**	**(4.155)**

Figures in the parenthesis are Angular transformed values *Hours After Exposure

Table 13. Effect of cell free extracts of fungal isolates on egg hatching of *M. incognita in vitro*

Isolates	Concentration	Percentage of egg hatching (in days after exposure)					
		3D*	4D	5D	6D	7D	8D
Isolate 10	100%	14.00(21.95)^{h}	15.25(22.97)^{i}	16.50(23.95)^{f}	18.50(25.46)^{g}	20.28(18.25)^{h}	23.00(28.63)^{fg}
	50%	15.00(22.76)^{gh}	15.75(23.37)^{i}	16.75(24.15)^{f}	18.75(25.65)^{g}	18.78(17.50)^{h}	19.75(26.37)^{g}
	33.3%	16.25(23.75)^{fgh}	18.00(25.07)^{h}	18.75(25.65)^{ef}	20.50(26.91)^{fg}	21.37(19.75)^{g}	22.00(27.95)^{fg}
	25%	17.25 (24.52)^{fg}	19.00(25.87)^{g}	19.75(26.37)^{de}	21.75(27.79)^{ef}	26.30(22.50)^{e}	27.25(31.44)^{cde}
Isolate 12	100%	18.00(25.07)^{ef}	18.75(25.64)^{g}	20.00(26.55)^{de}	22.00(27.96)^{ef}	23.26(21.00)^{fg}	24.00(29.32)^{ef}
	50%	18.50(25.46)^{ef}	19.75(26.37)^{fg}	20.25(26.72)^{de}	22.2(28.12)^{ef}	27.76(21.75)^{ef}	24.00(29.32)^{ef}
	33.3%	19.13(25.89)^{ef}	20.50(26.89)^{efg}	21.50(27.61)^{d}	23.50(28.98)^{de}	24.29(22.50)^{e}	24.75(29.80)^{def}
	25%	20.50(26.91)^{de}	21.50(27.61)^{ef}	21.75(27.79)^{d}	23.75(29.15)^{de}	23.47(22.75)^{e}	24.50(29.61)^{def}
Isolate 27	100%	20.88(27.17)^{de}	20.75(27.08)^{efg}	21.75(27.78)^{d}	23.75(29.15)^{de}	26.37(25.00)^{d}	27.25(31.45)^{cde}
	50%	22.50(28.27)^{d}	24.00(29.29)^{cd}	26.00(30.62^{c}	28.00(31.91)^{c}	29.93(28.00)^{d}	30.50(33.51)^{c}
	33.3%	23.75(29.14)^{d}	24.75(29.79)^{c}	26.00(30.63)^{c}	28.00(31.92)^{c}	29.44(27.25)^{c}	30.25(33.35)^{c}
	25%	28.23(32.08)^{c}	22.38(28.22)^{de}	23.75(29.15)^{c}	25.75(30.48)^{cd}	27.30(25.25)^{c}	28.25(32.09)^{cd}
Sterile water		76.59(61.54)^{a}	84.03(66.63)^{a}	93.62(73.55)^{a}	100.00(89.88)^{a}	100.00(89.88)^{a}	100.00(89.88)^{a}
Plainbroth		72.31(55.87)^{b}	81.32(57.90)^{b}	89.63(65.70)^{b}	92.05(74.39)^{b}	95.43(76.79)^{b}	98.01(84.21)^{b}
CD (0.05)		(2.274)	(1.479)	(1.905)	(1.753)	(1.404)	(2.708)

statistically on par with isolate 27 at 100 % concentration (20.88 per cent egg hatching). Isolate 27 at 25% concentration, 33.3% concentration and, 50% concentration recorded 28.23, 23.75 and 22.50 per cent egg hatching respectively and the effect of these isolates were statistically on par. Plain broth and sterile water recorded 72.31 and 76.59 per cent hatching of eggs respectively.

Effect of CFEs of isolate 10 at 100% concentration and 50 % concentration was statistically on par at fourth day after treatment. The per cent of egg hatching in these treatments were 15.25 and 15.75. Isolate 10 at 33.3% concentration exhibited 18.00 per cent egg hatching. The per cent of egg hatching of isolate 10 at 25% concentration (19.00) and isolate 12 at 100 % concentration (18.75), 50 % concentration (19.75) and 33.3 % concentration (20.50) and isolate 27 at 100 % concentration (20.75) were statistically on par. The per cent of egg hatching by the CFE of isolate 12 at 33.3 %, 25 % were 20.50 and 21.50 respectively and these were statistically on par with isolate 27 at 100 % and 25% concentration. The per cent of egg hatching of isolate 27 at 50 % and 25 % concentration were on par. Isolate 27 at 33.3 % concentration recorded significantly inferior egg hatching per cent (24.75) except isolate 27 at 50 % concentration (24.00 per cent egg hatching). Plain broth and sterile water recorded 81.32 and 84.03 per cent hatching of eggs respectively.

On the fifth day of exposure, CFEs of isolate 10 at 100% concentration exhibited minimum egg hatching (16.50 per cent) and it was statistically on par with isolate 10 at 50 % concentration giving 16.75 per cent egg hatching and isolate 10 at 33.3 % concentration (18.75 cent egg hatching). Isolate 10 at 33.3 % concentration was also on par with isolate 10 at 25% concentration (19.75 per cent) and isolate 12 at 100 % and 50 % concentration giving egg hatching value of 20.00 per cent and 20.25 per cent respectively. Isolate 10 at 25 % concentration was on par with isolate 12 at all concentrations and isolate 27 at 100 % concentration giving a percentage of egg hatching ranged from 19.75 to 21.75. The lowest number of egg hatching was recorded by isolate 27 at 33.3 % and 50 % concentration (26.00 per cent) and was on par with isolate 27 at 25 % concentration (23.75 per cent). Plain broth and sterile water recorded 89.63 and 93.62 per cent hatching of eggs respectively.

On the sixth day of exposure, CFEs of all the isolates showed statistically significant variation in egg hatching. Isolate 10 at 100% concentration exhibited minimum egg

hatching (18.50%) and it was statistically on par with isolate 10 at 50% concentration giving 18.75 per cent egg hatching and isolate 10 at 33.3% concentration giving 20.50 per cent egg hatching. CFE of isolate 10 (33.3% concentration) was also on par with isolate 10 (25% concentration) recording 21.75 per cent egg hatching which was also statistically on par with isolate 12 at 100% and 50% concentration giving 22.00 and 22.20 per cent egg hatching respectively. Isolate 10 at 25 % concentration was on par with isolate 12 at all concentrations and isolate 27 at 100 % concentration giving a percentage of egg hatching ranging from 21.75 to 23.75. The lowest number of egg hatching was recorded by isolate 27 at 33.3 % and 50 % concentration (28.00 per cent) and these were on par with isolate 27 at 25 % concentration (25.75 per cent). Plain broth and sterile water recorded 92.05 and 100.00 per cent hatching of eggs respectively.

On the seventh day of exposure, CFE of isolate 10 at 50 % and 100% concentration recorded higher per cent of egg hatching *viz.* 24.78 and 25.28 per cent respectively. Isolate 10 at 33 % concentration recorded 26.37 per cent hatching of eggs and was on par with isolate 12 at 100% concentration (27.26 per cent) which in turn was also on par with isolate 12 at 50% concentration (27.76 per cent). Isolate 12 at 50% concentration was also statistically on par with its 25 % (28.47 per cent) and 33.3 % concentration (28.29 per cent) and isolate 10 at 25 % concentration (28.29 per cent). Effect of CFE of isolate 27 at 100% concentration and 25% concentration was statistically on par in which the per cent egg hatching were 26.37 and 28.30 respectively. Isolate 27 (50% concentration) recorded maximum per cent egg hatching (31.44) and it was on par with isolate 27 at 33.3 % concentration (31.93). Plain broth and sterile water recorded 95.43 and 100.00 per cent hatching of eggs respectively.

On the eighth day of exposure, CFE of isolate 10 at 50% concentration recorded 19.75 per cent hatching of eggs and it was statistically on par with isolate 10 at 33.3.% concentration (22.00 per cent) and isolate 10 at 100% concentration (23.00 per cent). Isolate 10 at 33.3% concentration was statistically comparable with isolate 10 at 100% concentration and all concentration of isolate 12 *i.*e. 24.00 per cent hatching of eggs (100 % and 50 % concentrations), 24.50 per cent hatching of eggs, (25 % concentration), and 24.75 per cent hatching of eggs (33.3 % concentration). All concentrations of isolate 12 was statistically on par with isolate 10 at 25 % concentration and isolate 27 at 100%

concentration (27.25 per cent). Isolate 27 at 50% concentration (30.50 per cent), 33.3% concentration (30.25 per cent), 25 % concentration (28.25 per cent) and 100 % concentration (27.25 per cent) were statistically on par and was also comparable with egg hatching per cent of isolate 10 at 100 % concentration (27.25 per cent). Plain broth and sterile water recorded 98.01 and 100.00 per cent hatching of eggs respectively.

4.4 SCREENING OF FUNGAL ISOLATES FOR NEMATODE EGG PARASITIZATION *IN-VITRO*

From the eggs no parasitized was observed even after inoculation with fungus.

4.5 SCREENING OF FUNGAL ISOLATES FOR NEMATODE TRAP FORMATION *IN VITRO*

The fungus was unable to grow in the artificial medium because of the obligate nature of the fungus.

4.6. POT CULTURE STUDIES TO EVALUATE THE EFFECTIVE ISOLATE

The effect of potential isolates selected from *in vitro* experiments (Isolate 10, 12 and 27) were compared against root-knot nematode in tomato plants under pot culture. The results in terms of nematode population, biometric characters and yield are presented in Table 14, 15 and 16.

4.6.1. Nematode Population (Table 14)

4.6.1.1. Nematode Population in Soil (200cc)

The data on the population build-up of nematodes in tomato rhizosphere at the time of harvest revealed that all the treatments significantly reduced the population of *M. incognita* in soil. Among non-chemical methods, the lowest mean nematode population was recorded in plants drenched with isolate 10(1% w/v) (83.00). Next best treatments in the order of effectiveness were soil drenching with isolate 12 (1% w/v) (147.00), soil drenching with isolate 27(1% w/v) (147.50), Cartap hydrochloride 4% G (25kg/ha) with RKN inoculation (110.25). Lowest mean number of nematodes was recorded in chemical treatment was *P.indica* (1% w/v) with RKN inoculation (105.00). All these treatments

Table 14. Effect of different treatments on population of *M. incognita* in tomato under pot culture condition RKN inoculation

Treatments	Population of nematodes		No. of galls (5g root)	Gall Index	No. of females (5g root)	No. of egg masses (5 g root)
	Soil (200cc)	Root (5g)				
Isolate 10 (1% w/v)	83(9.16)[d]	44.25(6.73)[e]	18(4.36)[d]	2	22.5(4.85)[c]	34.25(5.93)[e]
Isolate 12 (1% w/v)	160(12.66)[b]	94.75(9.78)[b]	23.25(4.92)[b]	2	34.25(5.93)[b]	45.75(6.83)[c]
Isolate 27 (1% w/v)	147.5 (12.18)[b]	61.25(7.88)[c]	26(5.19)[b]	2	33(5.83)[b]	54.25(7.43)[b]
Cartap hydrochloride 4% G	98.25(9.96)[c]	54.00(7.42)[cd]	27(5.29)[b]	1	19.75(4.55)[c]	36.25(6.10)[d]
Piriformospora indica (1% w/v)	99.75(10.31)[c]	52.50(7.31)[d]	21.75(4.77)[c]	2	21.5(4.74)[c]	40.75(6.46)[cd]
Control	372.25(19.31)[a]	183.75(13.59)[a]	87.75(9.42)[a]	4	64.5(8.09)[a]	146(12.12)[a]
CD (0.05)	(2.985)	(0.534)	(0.378)	-	(0.346)	(0.408)
SE± m	(0.996)	(0.178)	(0.126)		(.115)	(.136)

Table 15. Effect of different treatments on biometric characters and yield of tomato under pot culture condition with RKN inoculation

Treatments	Plant height (cm)	Fresh shoot weight(g)	Fresh root weight(g)	Yield(g)
Isolate 10 (1% w/v)	81.23 [a]	176.38 [a]	80.25 [a]	346.75 [a]
Isolate 12 (1% w/v)	72.32 [cd]	165.22 [a]	63.00 [cd]	310.60 [bc]
Isolate 27 (1% w/v)	70.25 [ed]	154.75 [a]	66.25 [bc]	303.00 [c]
Cartap hydrochloride 4% G	77.35 [ab]	167.20 [a]	68.50 [b]	331.50 [ab]
Piriformospora indica (1% w/v)	76.40 [bc]	152.80 [a]	59.00 [d]	313.63 [bc]
Control	57.50 [e]	80.00 [b]	48.50 [e]	59.50 [d]
CD (0.05)	4.314	39.127	4.228	25.083
SE± m	1.441	13.068	1.412	8.388

Table 16. Effect of different treatments on biometric characters and yield of tomato under pot culture condition without RKN inoculation

Treatments	Plant height (cm)	Fresh shoot weight(g)	Fresh root weight(g)	Yield(g)
Isolate 10 (1% w/v)	86.05 [a]	204.88 [a]	81.00 [a]	361.13 [a]
Isolate 12 (1% w/v)	75.34 [cd]	165.22 [bc]	65.50 [b]	324.50 [bc]
Isolate 27 (1% w/v)	71.50 [d]	154.75 [cd]	61.25 [c]	311.50 [c]
Cartap hydrochloride 4% G	80.25 [b]	167.20 [b]	66.00 [b]	339.38 [ab]
Piriformospora indica (1% w/v)	78.32 [cb]	152.80 [d]	62.50 [bc]	320.30 [c]
Control	59.50 [e]	109.25 [e]	48.50 [d]	88.75 [d]
CD (0.05)	4.241	32.789	10.779	22.785
SE± m	1.417	10.951	3.600	7.610

showed statistically significant superiority over control (372.25) in reducing the nematode population in soil.

4.6.2. Biometric Characters

The results revealed that different treatments significantly influenced the biometric characters and yield.

4.6.2.1. Plant Height (cm) (Table 15 and 16)

The perusal of the data revealed that isolate 10(1% w/v) without RKN inoculation, recorded the highest plant height of 86.05 cm followed by Cartap hydrochloride 4G @ 1kg a.i ha^{-1}(80.25 cm) which in turn was on par with *P. indica* (1% w/v) with a height of (78.32 cm).The plant height of *P. indica* (1% w/v) was on par with isolate 12 (1% w/v). The lowest plant height was recorded by control (59.50 cm) and it was significantly inferior to all treatments (Plate 1).

Among the different treatments with RKN inoculation, isolate 10 (1% w/v) recorded maximum height of 81.23 cm and was significantly superior to all other treatments except Cartap hydrochloride 4G @ 1kg a.i ha^{-1} which recorded a height of 77.35 cm and it was on par with *P. indica* (1% w/v) with a height of (76.40 cm). The height recorded by *P. indica* (1% w/v) was on par with isolate 12 (1% w/v) with a height of 72.32 cm. All treatments were significantly superior to control (57.50 cm).

4.6.2.2. Fresh Shoot Weight (g) (Table 15 and 16)

Among the different treatments without RKN inoculation, isolate 10(1% w/v) recorded the highest fresh shoot weight (204.88 g) and it was significantly superior to all other treatments, followed by Cartap hydrochloride 4G @ 1kg a.i ha^{-1}(167.20 g) which was on par with isolate 12 (1% w/v) with a weight of 165.22 g. Isolate 12 was on par with isolate 27 (154.75g). The lowest fresh shoot weight of 109.25 was reported by control. There was no significant variation with respect fresh shoot weight among different treatments with RKN inoculation but were significantly superior to control (80.00 g)

4.6.2.3. Fresh Root Weight (g) (Table 15 and 16)

Among the different treatments without RKN inoculation, isolate 10(1% w/v)

Isolate 10

Isolate 12

Isolate 27

Cartap hydrochloride 4% G

Piriformospora. indica

Untreated Control

Plate 1. Effect of different treatments on plant height in tomato

Isolate 10

Isolate 12

Isolate 27

Cartap hydrochloride 4% G

Piriformospora. indica

Untreated Control

Plate 2. Effect of different treatments on fresh root weight in tomato

recorded maximum fresh root weight (81.00g), followed by Cartap hydrochloride 4G @ 1kg a.i ha^{-1}(66.00 g) which was on par with isolate 12 (1% w/v) and *P. indica* (1% w/v) with a fresh root weight of 65.50 g and 62.50 g respectively. All treatments were significantly superior to control (48.50 g).

Among the different treatments with RKN inoculation, isolate 10 (1% w/v) recorded the highest fresh root weight (80.25g) and it was significantly superior to all other treatments, followed by Cartap hydrochloride 4G @ 1kg ai ha^{-1}(68.50 g) which was on par with isolate 27 (1% w/v) with a fresh root weight of (66.25 g). Isolate 27 (1% w/v) was on par with isolate 12 (1% w/v) with a fresh root weight of 63.00 g. All treatments were significantly superior to control (48.50 g) (Plate 2).

4.6.2.4. Yield (g) (Table 15 and 16)

Among the different treatments without RKN inoculation, isolate 10(1% w/v) recorded the highest fruit yield (361.13 g/plant) and it was on par with Cartap hydrochloride 4G @ 1kg a.i ha^{-1}(339.38 g/plant). Cartap hydrochloride 4G @ 1kg a.i ha^{-1}was on par with isolate 12 (1% w/v) with a yield of (324.50 g/plant). The yield obtained from isolate 12, 27 and *P. indica* (1% w/v) were on par. All treatments were significantly superior to control (88.75 g/plant).

Among the different treatments with RKN inoculation, isolate 10(1% w/v) recorded the highest fruit yield (346.75g/plant) and it was on par with Cartap hydrochloride 4G @ 1kg a.i ha^{-1}(331.50 g). Cartap hydrochloride 4G @ 1kg a.i ha^{-1}was on par with *P. indica* (1% w/v) and isolate 12 (1% w/v). All treatments were significantly superior to control (59.50 g/plant).

4.7. IDENTIFICATION OF FUNGUS

4.7.1 Morphological and Cultural Characterization

Colonies of isolate 10 have concentric rings with green conidial which are denser in centre then towards the margins (Plate 3a).Colonial growth of isolate 12 showed white to greenish color and eventually, it became dark green to brown was observed (Plate 3b). Isolate 27 colony exhibited pale yellow to white hyphae, turning to black / dark brown

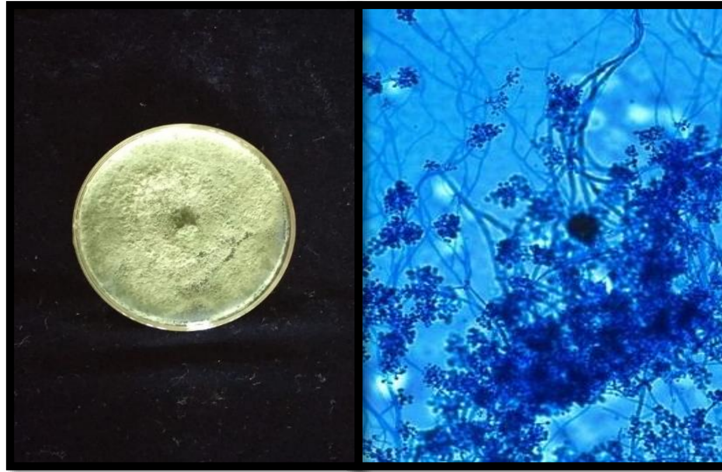

Plate 3a. Morphological and cultural characters of isolate 10

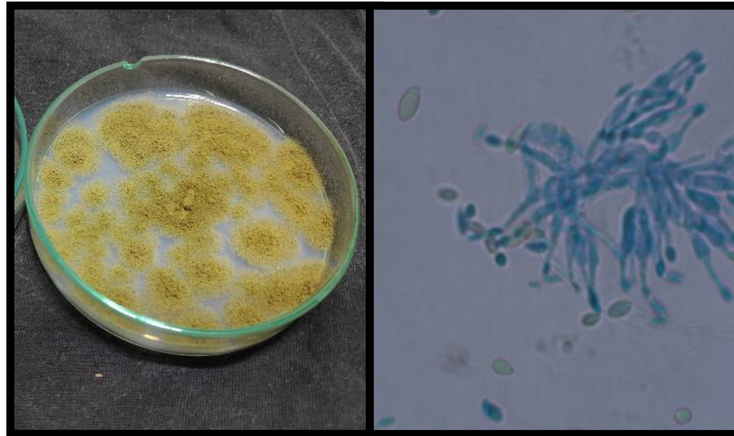

Plate 3b. Morphological and cultural characters of isolate 12

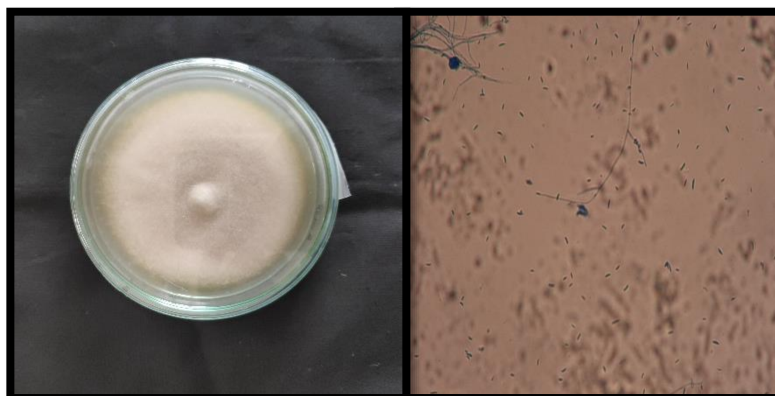

Plate 3c. Morphological and cultural characters of isolate 27

originating from the centre of the culture. (Plate 3c)

4.7.2 Molecular characterization of Fungus

Internal transcribed regions of DNA of ITS regions were amplified by ITS1(5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') regions. Blast search of amplified DNA in NCBI data revealed the identity of isolate 10, 12 and 27 as *Trichoderma viride, Metarhizium anisopliae* and *Fusarium verticillioides* respectively (plate 4, 5a, 5b and 5c). Analysis of nucleotide sequence of the selected fungus is given in table 17.

Table 17. Analysis of nucleotide sequence

Isolates	Sequence	Accession no.
Isolate 10 (*Trichoderma viride* strain T51)	CTTCCCTTCTGCAGCAAGCACCGATGCCAACCGCAAGAACTTTGCCAAGACGGCCATCACCTTCATGAAGGACTGGG GTTTTGATGGTATTGACGTCGACTGGGAGTACCCTGCCGATGACACTCAGGCCACCAACATGGTTCTTCTGCTCAAG GAGATCCGATCTCAACTAGATGCCTATGCTGCGCAATACGCTCCAGGCTACCACTTCCTTCTCTCCATCGCTGCCCCC GCTGGACCAGAGCACTACTCTGCCCTGCACATGGCCGACCTTGGTCAAGTTCTCGACTATGTCAACCTTATGGCCTAT GATTATGCTGGTTCTTGGAGCAGCTACTCTGGACACGATGCCAACTTGTTTGCCAACCCGTCCAACCCCAACTCTTCA CCATACAACACCGATCAGGCTATCAAGGCTTATATCAACGGAGGCGTTCCCGCAAGCAAGATCGTTCTTGGCATGCC TATCTATGGACGATCTTTCGAGAGCACTAATGGCATTGGCCAAACCTACAATGGAATTGGATCTGGAAGCTGGGAGA ACGGTATCTGGGACTACAAGGTTCTTCCCAGGGCCGGCGCTACAGTCCAGTACGACTCTGTCGCACAGGCGTACTAC AGCTATGATTCTAGCAGCAAGGAGCTCATCTCTTTCGATACCCCTGACATGGTCAGCAAAAAGGTCTCTTACCTCAA GAATCTCGGCCTGGGAGGCAGTATGTTCTGGGAGGCTTCTGCTGACAAGACTGGCTCCGACTCCTTGATCGGAACAA GCC	HM17924 2.1
Isolate 12 (*Metarhizium anisopliae* strain STHC-2 18S)	TCGGTTCTAGCGAGCCCAACCTCCCACCCGTGTTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATGGCCGC CGGGGGCTCTCAGCCCCGGGCCCGCGCCCGCCGGAAACACCACGAACTCTGTCTGATCTAGTGAAGTCTGAGTTGAT TGTATCGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGAT AACTAGTGTGAATTGCAGAATTCCGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGC ATGCCTGTCCGAGCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTGGGTCGTCGTCCCCTCTCCGGGGGGGACG GGCCCCAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCC GGCGCTTGCCGAACGCAAATCAATCTTTTCCA	JQ425479.1
Isolate 27 (*Fusarium verticillioides* isolate 2174	GGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGTGAACATACCAATTGTTGCCTCGGCGGATCAGCCCGCT CCCGGTAAAACGGGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTGAGTAAAACCATAAAT AAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTG AATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTC GAGCGTCATTTCAACCCTCAAGCCCAGCTTGGTGTTGGGACTCGCGAGTCAAATCGCGTTCCCCAAATTGATTGGCG GTCACGTCGAGCTTCCATAGCGTAGTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCAA CTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCA	MK32527 5.

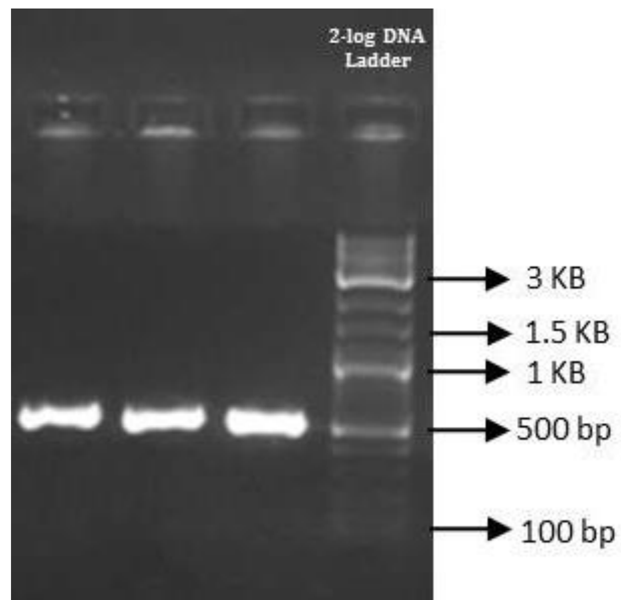

Plate 4. Gel profile of the PCR product of 16sRNS

Trichoderma viride strain T51 endochitinase 42 (ech42) gene, partial cds

Sequence ID: HM179242.1 Length: 776 Number of Matches: 1

Range 1: 117 to 676 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1035 bits(560)	0.0	560/560(100%)	0/560(0%)	Plus/Plus

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Query  1    ATGACACTCAGGCCACCAACATGGTTCTTCTGCTCAAGGAGATCCGATCTCAACTAGATG  60
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Sbjct  117  ATGACACTCAGGCCACCAACATGGTTCTTCTGCTCAAGGAGATCCGATCTCAACTAGATG  176

Query  61   CCTATGCTGCGCAATACGCTCCAGGCTACCACTTCCTTCTCTCCATCGCTGCCCCCGCTG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  177  CCTATGCTGCGCAATACGCTCCAGGCTACCACTTCCTTCTCTCCATCGCTGCCCCCGCTG  236

Query  121  GACCAGAGCACTACTCTGCCCTGCACATGGCCGACCTTGGTCAAGTTCTCGACTATGTCA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  237  GACCAGAGCACTACTCTGCCCTGCACATGGCCGACCTTGGTCAAGTTCTCGACTATGTCA  296

Query  181  ACCTTATGGCCTATGATTATGCTGGTTCTTGGAGCAGCTACTCTGGACACGATGCCAACT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  297  ACCTTATGGCCTATGATTATGCTGGTTCTTGGAGCAGCTACTCTGGACACGATGCCAACT  356

Query  241  TGTTTGCCAACCCGTCCAACCCCAACTCTTCACCATACAACACCGATCAGGCTATCAAGG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  357  TGTTTGCCAACCCGTCCAACCCCAACTCTTCACCATACAACACCGATCAGGCTATCAAGG  416

Query  301  CTTATATCAACGGAGGCGTTCCCGCAAGCAAGATCGTTCTTGGCATGCCTATCTATGGAC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  417  CTTATATCAACGGAGGCGTTCCCGCAAGCAAGATCGTTCTTGGCATGCCTATCTATGGAC  476

Query  361  GATCTTTCGAGAGCACTAATGGCATTGGCCAAACCTACAATGGAATTGGATCTGGAAGCT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  477  GATCTTTCGAGAGCACTAATGGCATTGGCCAAACCTACAATGGAATTGGATCTGGAAGCT  536

Query  421  GGGAGAACGGTATCTGGGACTACAAGGTTCTTCCCAGGGCCGGCGCTACAGTCCAGTACG  480
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Sbjct  537  GGGAGAACGGTATCTGGGACTACAAGGTTCTTCCCAGGGCCGGCGCTACAGTCCAGTACG  596

Query  481  ACTCTGTCGCACAGGCGTACTACAGCTATGATTCTAGCAGCAAGGAGCTCATCTCTTTCG  540
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Sbjct  597  ACTCTGTCGCACAGGCGTACTACAGCTATGATTCTAGCAGCAAGGAGCTCATCTCTTTCG  656

Query  541  ATACCCCTGACATGGTCAGC  560
            ||||||||||||||||||||
Sbjct  657  ATACCCCTGACATGGTCAGC  676
```

Plate 5a. Nucleotide sequencing of Isolate 10

Metarhizium anisopliae strain STHC-2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Sequence ID: JQ425479.1 Length: 495 Number of Matches: 1

Range 1: 18 to 313 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
512 bits(277)	3e-141	290/296(98%)	1/296(0%)	Plus/Plus

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Sbjct  18   AACCTCCCACCCGTGTTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATGGCCG  77

Query  61   ACGGGGGCTCTC-GCCCAGGACCCGCGCCCGCCGGAAACAACACGAACTCTGTCTGATCT  119
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Sbjct  78   CCGGGGGCTCTCAGCCCCGGGCCCGCGCCCGCCGGAAACACCACGAACTCTGTCTGATCT  137

Query  120  AGTGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGG  179
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Sbjct  138  AGTGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGG  197

Query  180  TTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTCCG  239
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Sbjct  198  TTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTCCG  257

Query  240  TGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGC  295
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258  TGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGC  313
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Plate 5b. Nucleotide sequencing of Isolate 12

Fusarium verticillioides isolate 2174 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: MK325275.1 Length: 517 Number of Matches: 1

Range 1: 6 to 470 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
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Query  61   ACTACGCTATGGAAGCTCGACGTGACCGCCAATCAATTTGGGGAACGCGATTTGACTCGC  120
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Sbjct  410  ACTACGCTATGGAAGCTCGACGTGACCGCCAATCAATTTGGGGAACGCGATTTGACTCGC  351

Query  121  GAGTCCCAACACCAAGCTGGGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCGCC  180
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Sbjct  350  GAGTCCCAACACCAAGCTGGGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCGCC  291

Query  181  AGAATACTGGCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  290  AGAATACTGGCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATT  231

Query  241  CACATTACTTATCGCATTTTGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  230  CACATTACTTATCGCATTTTGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTG  171

Query  301  TTGAAAGTTTTGATTTATTTATGGTTTTACTCAGAAGTTACATATAGAAACAGAGTTTAG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Query  361  GGGTCCTCTGGCGGGCCGTCCCGTTTTACCGGGAGCGGGCTGATCCGCCGAGGCAACAAT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  110  GGGTCCTCTGGCGGGCCGTCCCGTTTTACCGGGAGCGGGCTGATCCGCCGAGGCAACAAT  51

Query  421  TGGTATGTTCACAGGGGTTTGGGAGTTGTAAACTCGGTAATGATC  465
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Plate 5c. Nucleotide sequencing of Isolate 27

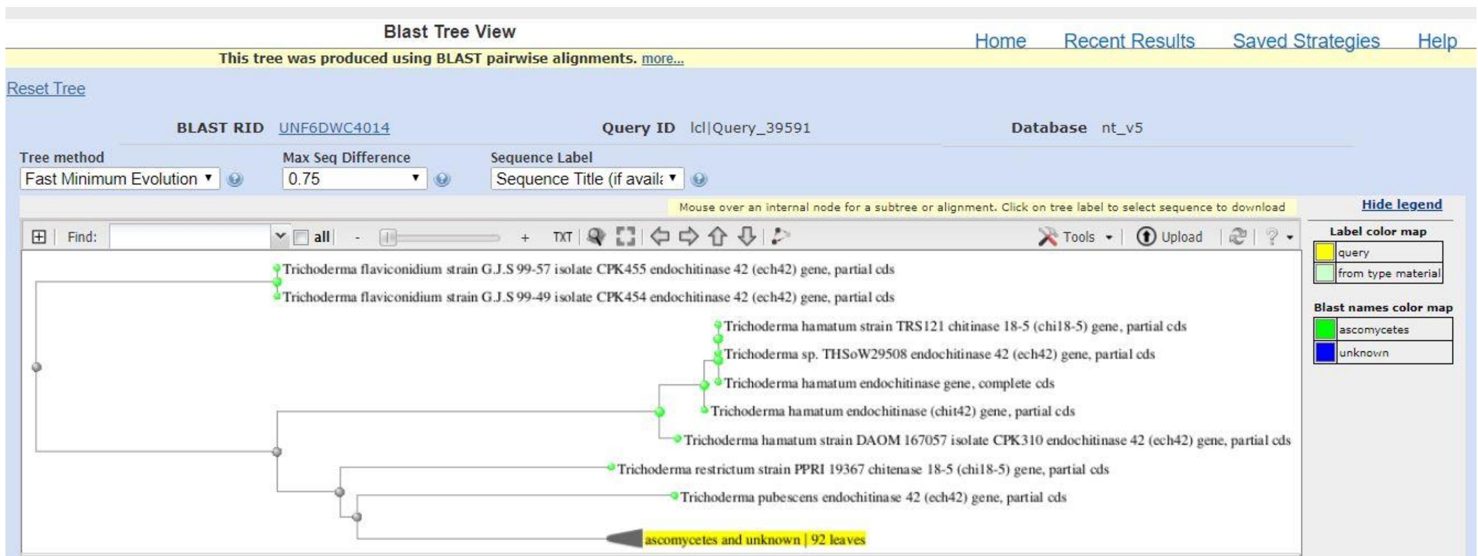

Plate 6a.Phylogenetic tree of Isolate 10

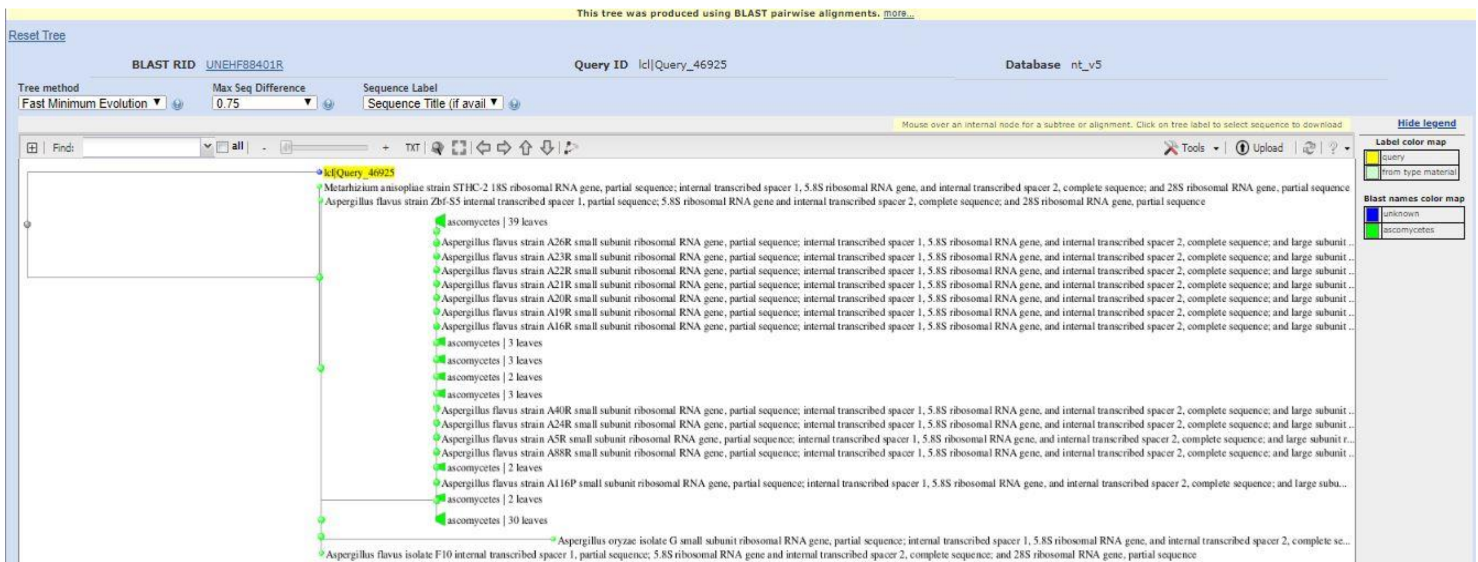

Plate 6b. Phylogenetic tree of Isolate 12

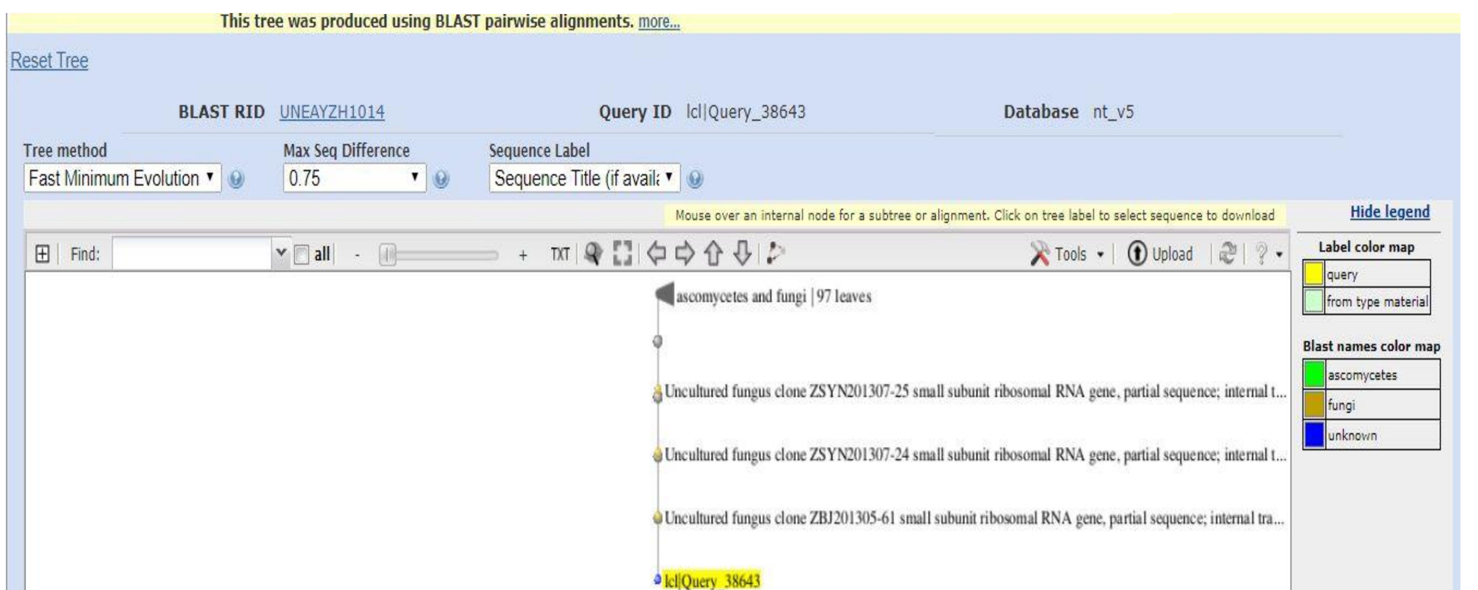

Plate 6c. Phylogenetic tree of Isolate 27

Discussion

5. DISCUSSION

The explanations and understandings conferred after analysis of the important results obtained are discussed in this chapter.

5.1 ISOLATION OF INDIGENOUS FUNGI

A survey was conducted in vegetable growing areas of Thiruvananthapuram district for isolation of indigenous fungi possessing nematicidal property. From the rhizosphere of vegetable crops like bhindi, tomato, chilli, cucumber and cowpea grown in each taluk, a total of sixty-six soil and thirty-four samples of root were collected by random sampling. Plants showing different disease symptoms such as yellowing, necrosis, withering, galling etc. were selected for sampling.

Cell free extracts (CFE) of thirty two fungal colonies showing characters similar to *Trichoderma (*colonies varied from light green to dark green) and *Purpureocillium* (colonies which are fast growing, powdery or suede-like, gold, green-gold, yellow-brown, lilac or tan) were selected for preliminary screening of nematicidal activity against *M. incognita* juveniles, as these two fungal genera possess biocontrol potential against RKN (Khan *et al.*, 2012). Most effective ten isolates which recorded more than fifty per cent juvenile mortality were selected for further screening in four different concentrations *viz.,* 100, 50, 33.3 and 25% at 24, 48 and 72 hr after treatment. Morphological and cultural characteristics and colony forming unit mL^{-1} of ten isolates were studied.

Results of the bio efficacy studies revealed that among the ten isolates, CFE of three isolates (Isolate 10, 12 and 27) at lowest concentration of 25% showed 12.13 to 34.50 per cent mortality of *M. incognita* juveniles at 72hr after exposure (Fig.1). CFE of isolate 10, 12 and 27 at 100 % concentration recorded 85.05, 76.50, and 62.50 per cent mortality of *M. incognita* juveniles respectively at 72hr after treatment (Fig.2).

CFE of these three isolates at 100% concentration exhibited higher mortality of *M. incognita* juveniles (46.88 to 74.75 per cent) compared to other isolates at 48hr after treatment also. Isolate 10 was obtained from samples collected from the rhizosphere of tomato plants grown in Nedumangad area of Thiruvananthapuram district. Isolate 12 and 27 were obtained from soil samples collected from tomato plants grown in Kazhakkuttom

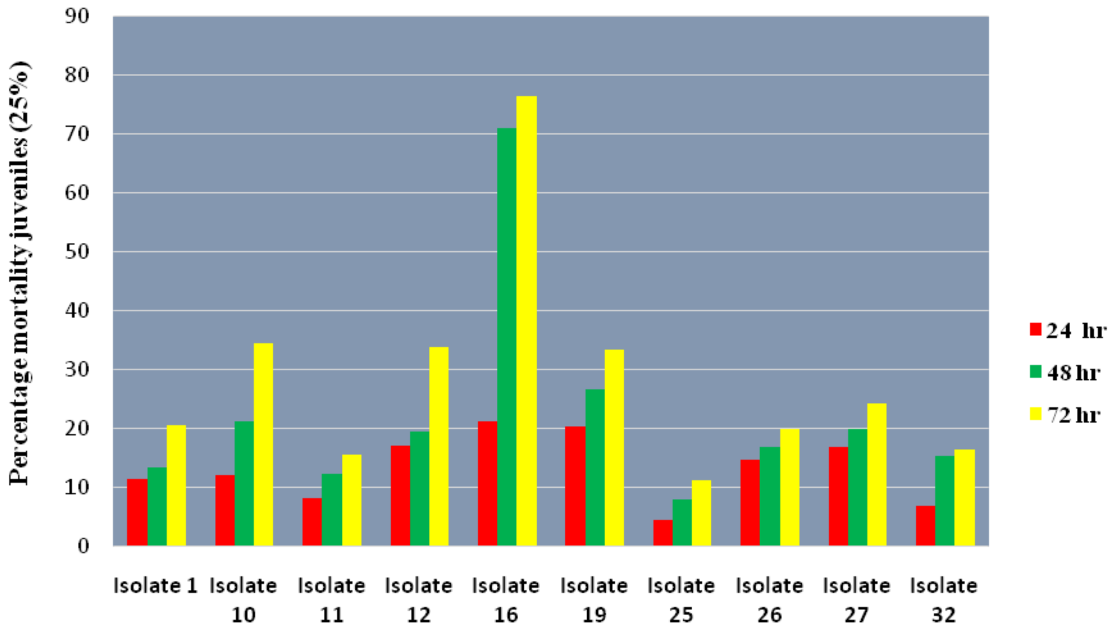

Fig 1. Effect of CEC of fungal isolates on morality of *M. incognita* j_2 at different exposure *in vitro*

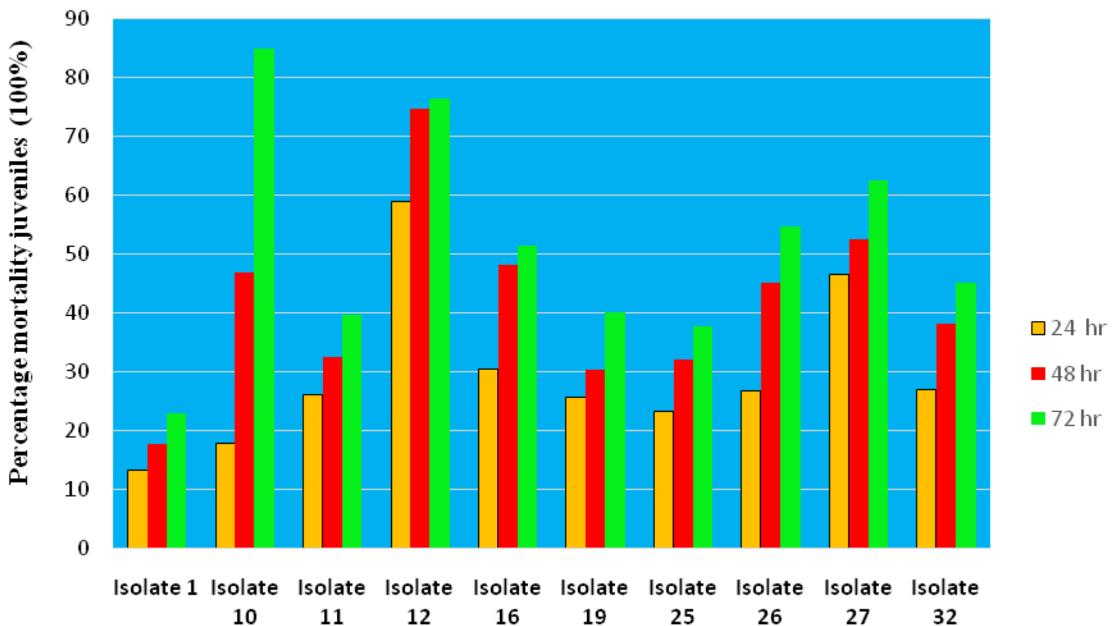

Fig 2. Effect of fungal isolates on morality of *M. Incognita* j_2 at different of exposure *in vitro*

area and Varkala area of Thiruvananthapuram district. CFEs of these three isolates were screened for ovicidal property on *M. incognita in vitro.*

5.2 SCREENING OF FUNGAL ISOLATES ON MORTALITY OF JUVENILES

CFE extracts of isolate 10, 12 and 27 at different concentrations (100, 50, 33.3 and 25%) showed significantly superior mortality of juveniles of *M. incognita* at 24, 48 and 72 hrs after treatment. At 100 % concentration, CFE extracts of isolate 10 recorded 85.05 % mortality, isolate 12 recorded 76.50 % mortality and isolate 27 recorded 62.50 % mortality and these were significantly higher than all other concentrations at 72 hrs after treatment. While at the concentration of 25%, isolate 10 recorded 12.13%, isolate 12 recorded 17.25 %, and isolate 27 recorded 17.00 % mortality and these were significantly inferior to all other concentrations.

The mortality of *M. incognita* juveniles increased with concentration and time of exposure. Higher mortality (74.75 to 46.88 per cent) was observed in higher concentration of isolates (100%) while it was lesser (21.00 to 19.50 per cent) in lower concentration (25%) at 48hr after exposure (Fig.4). Similar trend was observed in 24 and 72 hrs after exposure also. CFE of isolates at 100% concentration recorded 17.93 to 59.00 per cent mortality of juveniles at 24 hr after exposure. This observation clearly highlights the presence of nematicidal compounds in the CFE of indigenous fungal isolates. In this study, the fungal colonies selected for screening showed colony characters similar to *Trichoderma* and *Purpureocillium.* Acharya *et al*. (1988) reported that field application of *T. viride* resulted in good control of RKN. Sankaranarayanan *et al*., (1997) have reported that *Trichoderma* and *Gliocladium* could be used as a fungal biocontrol agent against many plant pathogens and PPN. Reddy *et al.,* (I996) and Sankaranarayanan and Sundarababu (1997) observed increased population of nematode trapping fungus which led to increased plant growth when the fungi *T. viride* was applied along with the organic amendment. Khan and Saxena (1997) also reported reduced *M. incognita* damage in plants when filtrates of *A. niger*, *P. lilacinum* and *T. viride* were used. The free-living soil fungi *Trichoderma* spp. are potential nematode bio-control agents on many food, vegetable and cash crops (Dababat and Sikora, 2007; Affokpon *et al*., 2011). Lal and Rana (2013) found that *T. harzianum* was the most effective fungus in reducing nematode growth followed by *T. viride, Gliocladium virens* and *Aspergillus ochraceous*.

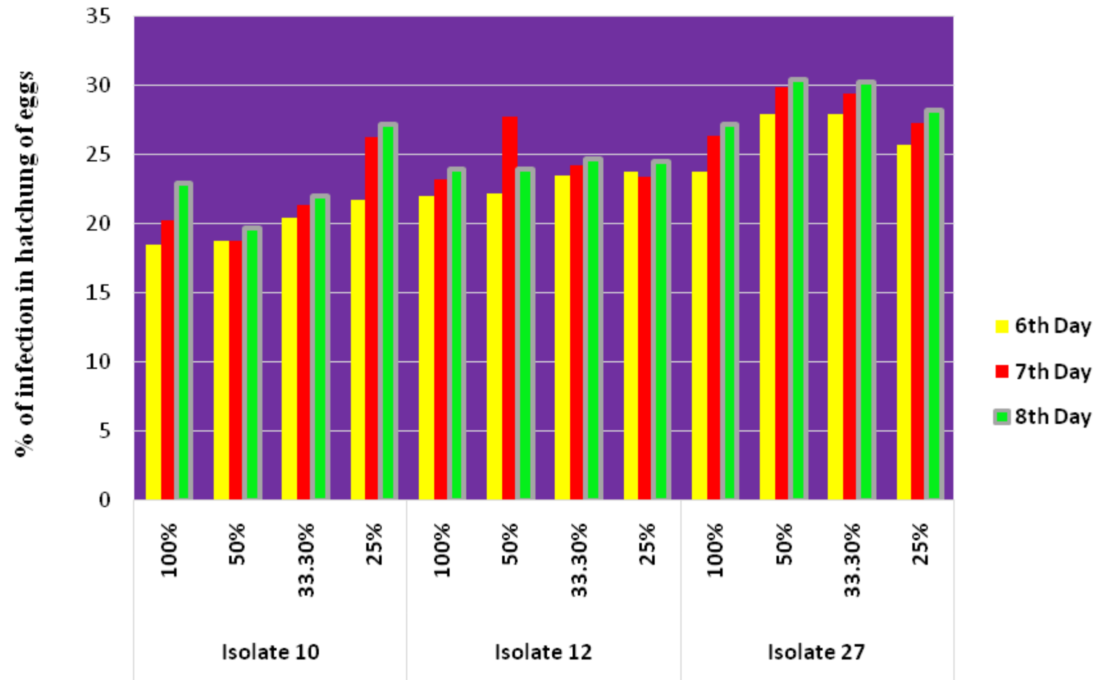

Fig .3. Effect of CFE of 10, 12 and 27 on egg hatching *in vitro*

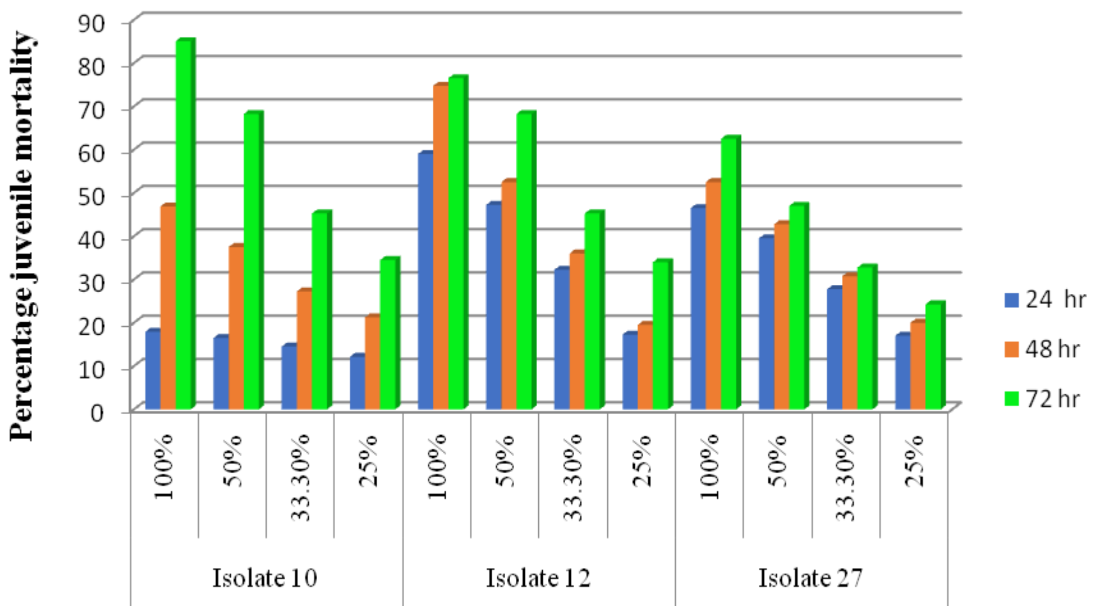

Fig .4. Effect of CFE of 10, 12 and 27 on Mortality of *M. incognita* J2 in vitro

CFE of isolates 10, 12 and 27 reported significantly higher inhibition of egg hatching of *M. incognita* at concentrations of 100, 50, 33.3 and 25%, three to eight days after exposure in comparison with sterile water and plain broth. CFE of isolate 10 at 100% concentration recorded minimum hatching of *M. incognita* eggs (14.00 to 23.00 per cent) at three to eight days after treatment while at lower concentrations (33.3 and 25%) 16.25 to 17.25 per cent egg hatching was observed at three days after treatment to 22.00 and 27.25 per cent egg hatching at eight days after treatment. CFE of isolate 10 at 100% concentration recorded significantly lower hatching of *M. incognita* eggs compared to 25% concentration on 4^{th}, 5^{th}, 6^{th}, 7^{th} and 8^{th} day after treatment (Fig.3).

As the concentration of CFE increased the mortality also increased. Among different concentrations of CFE of isolates 10, 12 and 27, highest egg hatching was observed in lowest concentration (25%) which ranged from 17.25 to 28.25 per cent from three to eight days after treatment while at highest concentration (100%) it was 14.00 to 27.25 per cent. On third day after exposure CFE of these three isolates exhibited 14.00 to 28.23 per cent hatching of eggs while it was 19.75 to 30.50 per cent in 8^{th} day after exposure. Egg hatching percent at highest concentration (100%) ranged from 23.00 to 27.25 on 8^{th} day after exposure while at lowest concentration of 25%, it ranged from 27.25 to 28.25 against 100 in control (sterile water). This finding clearly highlights the inhibitory effect of CFE of the isolated indigenous fungus on *M. incognita* eggs. Jones *et al.,* (1983) reported that *V. chlamydosporium* was capable of preventing egg hatching of *M. arenaria* and to colonize eggs by hyphal penetration. Jatala *et al*., (1985) also reported that *P. lilacinum* caused substantial egg deformation in *M. incognita* and these deformed eggs never matured or hatched.

Fungal penetration of eggs might be enabled by the high production of extracellular cuticle degrading protease (Yang *et al*., 2007). Monjil *et al*. (2017) also found that fungus attacked eggs of *Meloidogyne spp*. and inhibit the nematode hatching from the egg masses and were more susceptible to penetration and killing by fungus.

5.3 POT CULTURE STUDIES TO EVALUATE THE EFFECTIVE ISOLATE

All treatments could significantly reduce the population of nematodes over control both in soil and roots at harvest. Fungal inoculation of mycelium at the rate of 1% (w/v)

recorded the lowest nematode population (83.00) in soil giving 77.71 per cent reduction over the control and (44.25) in root giving 73.19 per cent reduction over the control. Application of Cartap hydrochloride 4% G with RKN inoculation and soil drenching with *Piriformospora indica* (1% w/v) with RKN inoculation gave 98.25 to 99.75 *M. incognita* juveniles in soil respectively (Fig.5), while in roots soil drenching with *P. indica* (1% w/v) with RKN inoculation recorded 52.50 and it was comparable with the application of Cartap hydrochloride 4% G with RKN inoculation (54.00). The efficacies of fungal formulations in managing nematodes have been reported earlier by several scientists. Villanueva and Davide (1984) reported that *P. lilacinum* successfully controlled nematode *M. incognita* in tomato. Dube and Smart Jr (1987) observed that soil applications of *Paecilomyces lilacinus* (Thom) Samson and *Pasteuria penetrans* (Thorne) Sayre and Starr resulted in higher control levels of *M. incognita* (Kofoid and White) Chitwood population, when compared to control treatments or to the antagonist alone. Reduced nematode population with *P. lilacinum* application was reported earlier by Pathak and Saikia (1999), Jonathan and Rajendran (2000), Khan and Verma (2004) and Narayana *et al.,* (2017). Siddiqui and Haque (2000) also observed reduction in *M. javanica* (Treub) Chitwood when the biological control agent *P. chlamydosporia* was used. In this study also the soil drenching of CFEs of isolate 10, 12 and 27 reduced the population of *M. incognita* in soil.

Fungal inoculation of mycelium @ of 1% (w/v) resulted in least number of galls (18.00 in 5 g of roots) giving 79.49 per cent reduction over the control (Fig.5). Next best treatment was application of *P. indica* (1% w/v) with RKN inoculation giving 21.75 and isolate 12 with a gall number of 23.25. Kiewnick and Sikora (2006) also observed that a preplanting soil treatment of *P. lilacinum* strain 251 (PL251), reduced root galling by 66 per cent in tomato. Khan *et al.,* (2012) observed suppression of galls by the biocontrol agents like *P. chlamydosporia, P. lilacinum*, and *T. harzianum*.

The lowest number of females (22.50) in 5g root was recorded with fungal inoculation of mycelium @ 1% (w/v) and it was on par with *P. indica* (1% w/v) with RKN inoculation (21.50) and also chemical treatment with cartap hydrochloride @ 1kg a.i ha^{-1} (19.75). Regarding number of eggs masses, the lowest egg masses (34.25) in 5g root was recorded with fungal inoculation of mycelium @ 1% (w/v) and it was followed by the application of *P. indica* (1% w/v) with RKN inoculation (40.75) and also chemical

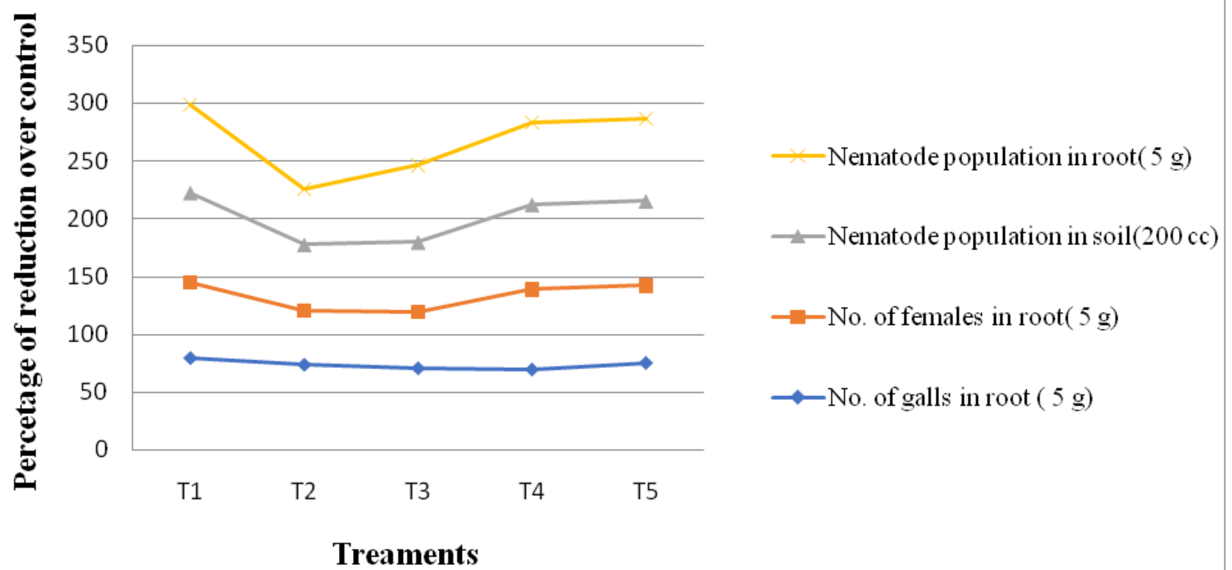

Fig .5. Effect of different treatments on population of *M. incognita* in tomato (pot culture condition)

treatment with cartap hydrochloride @ 1kg a.i ha^{-1} (36.25). This is in line with findings of Jonathan and Rajendran (2000). Kiewnick and Sikora (2006) also observed reduction in root galling, egg mass and final nematode population when treated with *P. lilacinum.*

Results of this study clearly highlight the efficacy of the fungal isolates in improving the plant growth parameters. Isolate 10 and chemical treatment with cartap hydrochloride when inoculated with RKN recorded maximum plant height and fresh root weight (Fig.6) while without RKN inoculation isolate 10 also recorded the highest plant height and fresh root weight followed by chemical treatment with Cartap hydrochloride. Regarding the yield, isolate 10 recorded the highest yield (346.75g/plant) with RKN inoculation and (346.75g/plant) without RKN inoculation (361.13 g/plant) and these were comparable with chemical treatment with Cartap hydrochloride @ 1kg a.i ha^{-1} with (331.50 g/plant) and (339.38g/plant) without RKN inoculation respectively. John *et al.,* (2004) reported that amaranths plants treated with *G. monosporum*, *G. etunicatum* and G. *mosseae* resulted in a significant increase in fresh weight of plants and reduction in nematode population in root and soil. Similar findings of RKN management and increased growth parameters in tomato with *P. lilacinum* were reported by Ahmed and Monjil (2019).

Results of the present study highlights the efficacy of indigenous isolates *viz.*, isolate 10 and 12 and 27 obtained from the rhizosphere of tomato in suppressing the nematode population and increasing the growth parameters and yield of tomato. Hence these three selected isolates were identified by molecular characterization.

5.4. IDENTIFICATION OF FUNGUS

Morphological and cultural characteristics of isolate 10, 12 and 27 were studied. Colonies of isolate 10 having concentric rings with green conidial which are denser in centre then towards the margins. Colonies of isolate 12 showed white to greenish colour and eventually, it became dark green with white spores. Colonies of isolate 27 the colonial growth showed characteristic, white to creamy and tinge pink tinge, margins slightly smooth.

Molecular characterization of isolate 10, 12 and 27 was done by amplifying the internal transcribed region of isolates using TCCGTAGGTGAACCTGCGG as forward

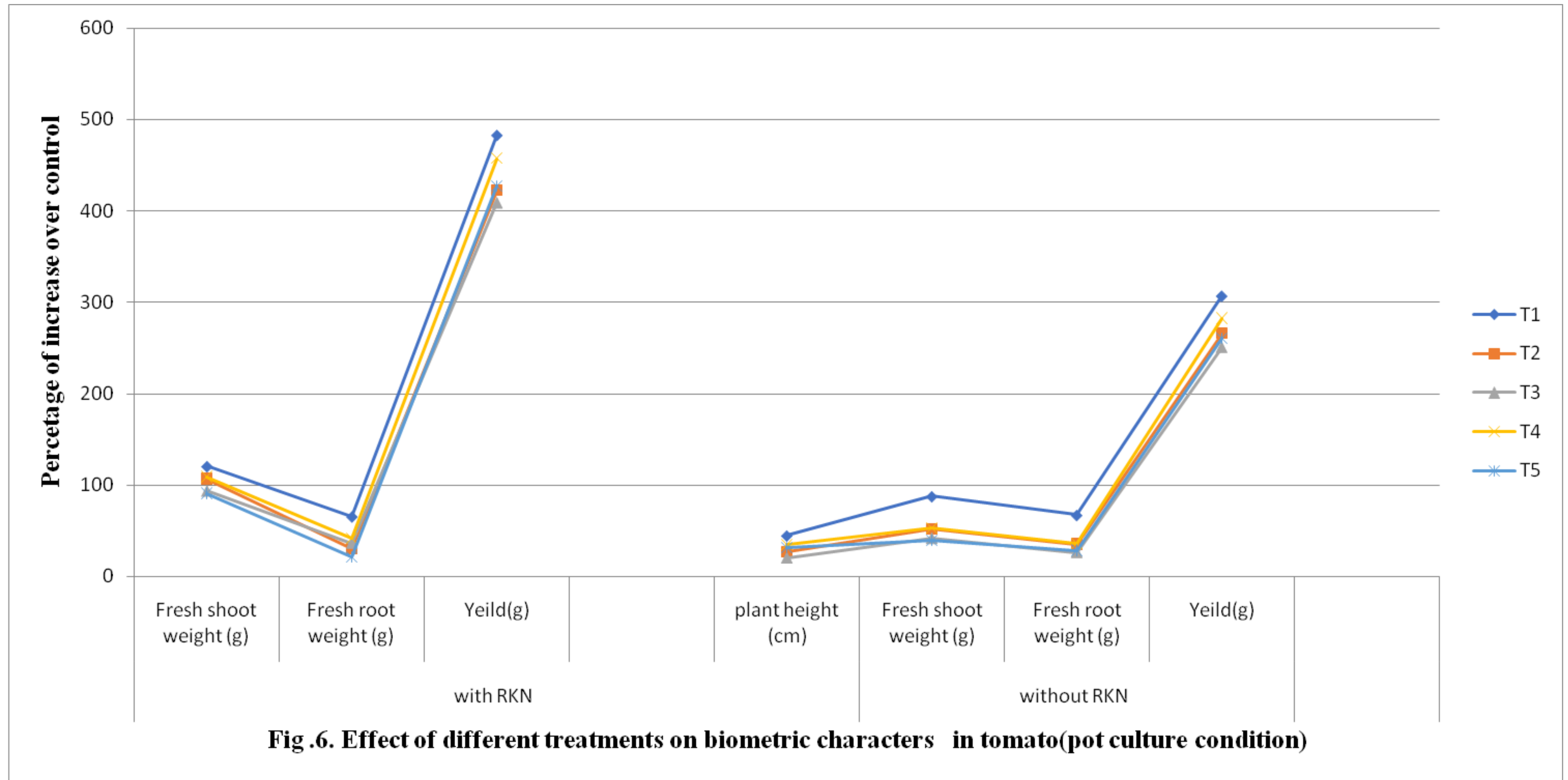

Fig .6. Effect of different treatments on biometric characters in tomato(pot culture condition)

primer and TCCTCCGCTTATTGATATGC as reverse primer. The blast analysis against NCBI revealed the identity of isolate 10, 12 and 27 as *Trichoderma viride, Metarhizium anisopliae* and *Fusarium verticillioides* respectively. Phylogenetic tree is given in plate 6 of the selected isolates.

The result of the present study highlighted the biocontrol of *Trichoderma viride, Metarhizium anisopliae* and *Fusarium verticillioides* against *M. incognita.* Based on the result of present investigation, it can be concluded that isolate10, 12 and 27 can suppress *M. incognita* population and increase the growth and yield of tomato plants. Soil drenching of indigenous fungal isolates can be recommended to manage *M. incognita* in tomato without any detrimental effect on environment.

SUMMARY

6. SUMMARY

The summary of the results is furnished below. Laboratory studies were conducted to evaluate the effect of CFE of isolates 10, 12 and 27 in four different concentrations (100, 50, 33.3 and 25 %) in inhibiting the egg hatching of *M. incognita.* Results of the study revealed that CFE of isolates 10, 12 and 27 showed statistically significant superiority in inhibiting the egg hatching of *M. incognita* at concentrations of 100, 50, 33.3 and 25%, three to eight days after exposure in comparison with sterile water and plain broth. CFE of isolate 10 at 100% concentration recorded minimum hatching of *M. incognita* eggs (14.00 to 23.00 per cent) at three to eight days after treatment while at lower concentrations (33.3 and 25%) 16.25 to 17.25 per cent egg hatching was observed at three days after treatment to 22.00 and 27.25 per cent egg hatching at eight days after treatment. CFE of isolate 10 at 100% concentration and 50% concentration recorded significantly lower hatching of *M. incognita* eggs compared to 25% concentration on 4^{th}, 5^{th} ,6^{th} ,7^{th} and 8^{th} days after treatment.

Pot culture studies revealed that all the treatments were significantly superior to control in reducing the nematode population in soil and roots at harvest. Fungal inoculation of mycelium at the rate of 1% (w/v) recorded lowest nematode population (83.00) in soil (44.25) in root. Fungal inoculation of mycelium at the rate of 1% (w/v) recorded the lowest number of galls (18.00) in roots. The lowest number of females (22.50) in 5g root was recorded with fungal inoculation of mycelium at the rate of 1% (w/v) and it was on par with *P. indica* (1% w/v) with RKN inoculation (21.50) and also chemical treatment with Cartap hydrochloride @ 1kg a.i ha^{-1} (19.75).

The lowest egg masses (34.25) in 5g root was recorded with fungal inoculation of mycelium at the rate of 1% (w/v) and it was followed by the application of *P. indica* (1% w/v) with RKN inoculation (40.75) and also chemical treatment with Cartap hydrochloride @ 1kg a.i ha^{-1} (36.25).

With RKN inoculation fungal inoculation of mycelium at the rate of 1% (w/v) (81.23 cm) and chemical treatment with Cartap hydrochloride @ 1kg a.i ha^{-1} (77.35 cm) recorded taller plants. Soil drenching of isolate 10 and 12 and 27 at the rate of 1% (w/v) and chemical Cartap hydrochloride 4% G 2000 J_2 per plant with RKN inoculation and *P. indica* (1%

w/v) 2000 J_2 per plant with RKN inoculation recorded higher fresh shoot weight compared to control. Isolate 10 (1% w/v) 2000 J_2 per plant with RKN inoculation recorded the highest fresh root weight (80.25 g/plant) and it was 39.56 % more compared to control, followed by Cartap hydrochloride 4%G 2000 J_2 per plant with RKN (68.50 g/plant).

With and without RKN inoculation fungal inoculation of isolate 10 recorded taller plants, highest fresh shoot weight and fresh root weight (81.00 g/plant) and higher yield plant^{-1}.

Results of this study clearly highlight the efficacy of the fungal isolates in improving the plant growth parameters. Regarding the yield, fungal inoculation of mycelium at the rate of 1% (w/v) recorded the highest yield plant $^{-1}$ with RKN inoculation (346.75g) and without RKN inoculation (361.13 g/plant) and these were comparable with chemical treatment with Cartap hydrochloride @ 1kg a.i ha^{-1} with (331.50 g/plant) and (339.38 g/plant) without RKN inoculation respectively.

Results of the present study highlights the efficacy of indigenous isolates *viz.*, isolate 10 and 12 and 27 obtained from the rhizosphere of tomato in suppressing the nematode population and increasing the growth parameters and yield of tomato. Hence these three selected isolates were identified by molecular characterization.

Results of the *in vitro* screening studies revealed that CFE of isolate 10 (100% concentration) was effective in inhibiting the egg hatching at three to eight days after treatment (14.00 to 23.00 per cent).

Isolate 10 at 100 % concentration was effective in increasing the mortality of *M. incognita* juveniles at 24, 48 and 72 hrs after treatment (17.93 to 85.05 per cent).

The results revealed that the soil drenching of isolate 10, with RKN inoculation was effective in reducing the nematode population in soil (83.88 per cent) and root (75. 91 per cent) and it as significantly superior to isolate 12 and isolate 27.

The lowest number of nematodes were reported by isolate 10 (76.43). Lowest number of galls were reported by the soil drenching of isolate 10 (79.48 per cent). Efficacy of isolate 10 was found to be statistically on par with Cartap hydrochloride and *P. indica*

in reducing the number of females and it also recorded the lowest number of egg masses.

Isolate 10 was significantly superior to all other treatments in improving the growth parameters like plant height (86.05 cm), fresh shoot weight (204.88 g/plant), fresh root weight (81.00) compared to control plant height (59.50 cm), fresh shoot weight (109.25 g/plant), fresh root weight (48.50 g/plant) respectively. Significantly superior yield was also recorded by isolate 10 both with and without RKN inoculation.

Morphological, Cultural and Molecular characterization of the fungal isolates were done for identification of isolates. Internal transcribed regions of DNA of ITS regions were amplified by ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') regions. Blast search of amplified DNA in NCBI data revealed the identity of isolate 10, 12 and 27 as *Trichoderma viride and Metarhizium anisopliae* and *Fusarium verticillioides* respectively.

Results revealed that these three isolates suppressed population of *M. incognita* and increased growth and yield in tomato plants. Soil drenching of this indigenous fungal isolates 1% (w/v) can be recommended to manage *M. incognita* in tomato without any detrimental effect on environment.

References

REFERENCES

Acharya, A., Padhi, N. N., Swain, P. K., and Dash, S. C. 1988. Occurrence of nematodes on betel vine in Orissa. *Indian J. Nematology* 18(2): 363.

Affokpon, A., Coyne, D. L., Htay, C. C., Agbede, R. D., Lawouin, L., and Coosemans, J. 2011. Biocontrol potential of native *Trichoderma* isolates against root-knot nematodes in West African vegetable production systems. *Soil Biol. Biochem.* 43: 600–608.

Ahmad, S.F. and Khan, T.A. 2004. Management of root-knot nematode, *Meloidogyne incognita*, by integration of *Paecilomyces lilacinus* with organic materials in Chilli. *Arch. Phytopathol. Plant Prot.* 37(1): 35-40.

Ahmed, S. and Monjil, M. S., 2019. Effect of *Paecilomyces lilacinus* on tomato plants and the management of root knot nematodes. *J. Bangladesh Agric. Univ.* 17(1): 9-13.

Akhman, J., Johansson., T., Olsson, M., Punt, P.J., Van Den Hondel, C.A.M.J.J., and Tunlid, A. 2002. Improving the pathogenicity of a nematode trapping fungus by genetic engineering of a subtilisin with nematotoxic Activity. *Appl. Environ. Microbiol.* 68: 3408–3415.

Arevalo, J., Díaz, H.L., Martins, I., Souza, J. F., Castro, J. M. C., Carneiro, R.M.D., and Tigano, M.S. 2009. Cultural and morphological characterization *of Pochonia chlamydosporia* and *Lecanicillium psalliotae* isolated from *Meloidogyne mayaguensis* eggs in Brazil. *Trop. Plant Pathol.* 34(3): 158-163.

Barber, C.A. 1901. *Root knot nematode infesting tea in South India*. Bulletin, Department of Land Records of Agriculture (45). Madras Agriculture Branch, 2p.

Camili, E.C., Benato, E.A., Pascholati, S.F., and Cia, P. 2010. Acetic acid vaporization for postharvest control of *Botrytis cinerea* in 'Italia' grapes. *Rev. Bras. Frutic*. 32 (2): 436-443.

Chen, S. and Dickson, D.W. 2012. Biological control of plant-parasitic nematodes. In: López, R.H.M., and Mendoza, N.M. (eds), Practical plant nematology. Guadalajara, México: Colegio de Postgraduados and Mundi-Prensa*, BibliotecaBásica de Agricultura.* pp. 761–811.

Chitwood, D.J. 2003. Research on plant-parasitic nematode biology conducted by the United States Department of Agriculture–Agricultural Research Service. *Pest Manag. Sci.* 59(6-7): 748-753.

Clark, F.E. and Paul, E.A. 1970. The microflora of grassland. *Advances in Agronomy* Vol. 22: pp. 375-435.

Cobb, N.A., 1918. *Estimating the nema population of soil, with special reference to the sugar-beet and root-gall nemas, Heterodera schachtii Schmidt and Heterodera radicicola (Greef) Müller: and with a description of Tylencholaimus aequalis n. sp* Agricultural Technology Circular 1, Bureau of Plant Industry. *US Department of Agriculture, Batesville* US Government Printing Office, 48p.

Cochran, W. and Cox, G. 1965. *Experimental design*. pp: 131-157.

Cui. R., Chengming, F., and Xiaotang S. 2015. Isolation and characterisation of *Aspergillus awamori* BS05, a root-knot-nematode-trapping fungus. *Biocontrol sci. Tech.*25(11): 1233–1240.

Dababat, A.A. and Sikora, R.A. 2007. Use of *Trichoderma harzianum* and *Trichoderma viride* for the biological control of *Meloidogyne incognita* on tomato. *Jordan J. Agric. Sci.* 3(3): 297-309.

Daneshkhah, R., Cabello, S., Rozanska, E., Sobczak, M., Grundler, F.M.W., Wieczorek, K., and Hofmann, J. 2013. *Piriformospora indica* antagonizes cyst nematode infection and development in Arabidopsis roots. *J. exp. botany* 64(12): 3763-3774.

Deshmukh, S.D. and Kogel, K.H. 2007. *Piriformospora indica* protects barley from root rot caused by *Fusarium graminearum*. *J. Plant Di. Prot.* 114(6): 263-268.

Dropkin, V.H. 1980. *Introduction to Plant Nematology.* John Wiley and Sons, New York, USA, 293.p.

Dube, B. and Smart Jr, G.C. 1987. Biological control of *Meloidogyne incognita* by *Paecilomyces lilacinus* and *Pasteuria penetrans*. *J. Nematology*. 19(2): 222.

Eisenback, J.D. 1985. Detailed morphology and anatomy of second-stage juveniles, males, and females of the genus *Meloidogyne* (root-knot nematodes). *An advanced treatise on Meloidogyne*, 1: 47-77.

Fakhro, A., Linares, A.D.R., Bargen, V.S., Bandte, M., Buttner, C., Grosch, R., Schwarz, D., and Franken, P. 2009. Impact of *Piriformospora indica* on tomato growth and on interaction with fungal and viral pathogens. *Mycorrhiza* 20: 191–200.

FAOSTAT 2019. http://www.fao.org/faostat/en/#data/QC. Accessed on 2019-3-27

Freitas, L.G., Giaretta, D.R., Ferraz, S., Zooca, R.J.F., and Podestá, G.S. 2009 Controlebiológico de nematoides: Estudo de casos. pp. 41–82 In: L. Zambolim and M. C. Picanço, eds. Controlebiológico de pragas e doenças: Exemplospráticos. Viçosa, Brazil: UFV/DFP.

Gams, W. and Bissett, J. 1998. Morphology and identification of Trichoderma. In: Harman G.E. and Kubicek, C.P.(eds.), *Trichoderma* and *Gliocladium.* Basic biology, taxonomy and genetics, Taylor and Francis, London, pp. 3-34.

Goswami, J., Pandey, R. K., Tiwari, J. P., and Goswami, B. K. 2008. Management of root-knot nematode on tomato through application of fungal antagonists *Acremonium strictum* and *Trichoderma harzianum*. *J. Environ. Sci. Health* 43: 237-240.

Hajera, H., Feroza, N., and Shahina, F. 2009. Effect of yam and nematode interaction on some biochemical parameters of sunflower. *Pakistan J. Nematology* 27: 193-201.

Hartman, K.M. and Sasser, J.N. 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal-pattern morphology. In: Barker,K.R., Carter,C.C., and Sasser, J.N. (eds), *Meloidogyne,* an Advanced Treatise, North arolina State.Univ,Releigh, pp.69-77.

Heald, C.M., Bruton, B.D., and Davis, R.M. 1989. Influence of *Glomus intraradices* and soil phosphorus on *Meloidogyne incognita* infecting *Cucumis melo*. *J. Nematology* 21(1): 69.

Hernandez, E.T.C., Herrera, R. R., Gonzalez, A.C.N., Victoriano, L.F., Valdes, R.M.H., and Reyes, C.F. 2013. Characterization of three novel pigment-producing *Penicillium* strains isolated from the Mexican semi-desert. *African J. Biotechnol.* 12(22).

Hertz, N.B., Jansson, H.B., and Tunlid, A. 2001. Nematophagous fungi. [Online]. Available: https://onlinelibrary.wiley.com/doi/abs/10.1038/npg.els.0000374.

Hussey, R.S. and Janssen G.J.W. 2002. Root-knot nematodes: *Meloidogyne* species. In: Starr, J.L., Cook, R., and Bridge, J. (eds), Plant Resistance to Parasitic Nematodes. Wallingford, UK, pp. 43–70.

Jain, R.K., Mathur, K.N., and Singh, R.V. 2007. Estimation of losses due to plant parasitic nematodes on different crops in India. *Indian J. Nematology*, 37(2): 219-221.

Jatala, P. 1986. Biological control of plant-parasitic nematodes. *Annu. Rev. phytopathol.* 24(1): 453-489.

Jatala, P., Franco, J., Gonzalez, A., and Ohara, C.M. 1985. Hatching stimulation and inhibition of *Globodera pallida* eggs by the enzymatic and exopathic toxic compounds of some biocontrol fungi. *J. Nematology* 17(4): 501-501.

John, A., Sivaprasad, P., and Bai, H. 2004. Influence of VAM on the biomass production and root-knot nematode infestation in amaranthus. *Indian J. Nematology.* 34(1): 99-102.

Jonathan, E.I. and Rajendran, G. 2000. Assessment of avoidable yield loss in banana due to root-knot nematode *Meloidogyne incognita*. *Indian J. Nematology*. 30(2): 162-164.

Jones, M.G., White, J.F., and Kabana, R.R. 1983. Phytonematode pathology: Ultrastructural studies. I. Parasitism of *Meloidogyne arenaria* eggs by *Verticillium chlamydosporium*. *Nematropica*. 13(2): 245-260.

KAU (Kerala Agriculture University) 2016. Package of Practices Recommendations: *Crops* (15th Ed.). Kerala Agricultural University, Thrissur, 392p.

Kellam, M.K. and Schenck, N.C. 1980. Interaction between a vesicular-arbuscular mycorrhizal fungus and root-knot nematode on soybean. *Phytopathol*. 70(4): 293-296.

Kerry, B. 1980. Biocontrol: fungal parasites of female cyst nematodes. *J. Nematology* 12(4): 253.

Kerry, B. 1997. Biological control of nematodes: prospects and opportunities. Plant Nematode Problems and their Control in the Near East Region, Rome pp.79-92.

Kerry, B.R. and Bourne, J.M. 2002. *A Manual for research on Verticillium chlamydosporium: a potential biological control agent for root-knot nematodes*. Gent, Belgium.

Kerry, B.R. and Hirsch, P.R. 2011. Ecology of *Pochonia chlamydosporia* in the rhizosphere at the population, whole organisms and molecular scales. In: Davies, K. and Spiegel, Y. (eds), Biological control of plant-parasitic nematodes: Building coherence between microbial ecology and molecular mechanisms. Progress in biological control, Dordrecht, The Netherlands. pp: 71–182.

Khan, M.N. and Verma, A.C. 2004. Biological control of *Meloidogyne incognita* in pointed gourd (*Trichosanthes dioica* Roxb.). *Ann. Plant Prot. Sci.* 12(1): 115-117.

Khan, M.R. and Goswami, B.K. 2000. Effect of culture filtrates of *Paecilomyces lilacinus* isolates on hatching of *Meloidogyne incognita* eggs. *Annu. Plant Prot. Sci.* 8(1): 62-65.

Khan, M.R., Solanki, R.D., Bohra, B., and Vyas, B.N. 2012. Evaluation of Achook (Azadirachtin 1500 ppm) against root knot nematode (*Meloidogyne incognita*) infecting okra. *South Asian J. Exp. Biol.* 2(4): 149-156.

Khan, T.A. and Saxena, S.K. 1997. Effect of root-dip treatment with fungal filtrates on root penetration, development and reproduction of *Meloidogyne javanica* on tomato. *Int. J. Nematology*, *7*: 85-88.

Kiewnick, S. and Sikora, R.A. 2006. Biological control of the root-knot nematode *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. *Biol. control*. 38(2): 179-187.

Krishnappa, K. 1985. Nematology in developing countries, India-IMP region VIII, In: Sasser, J.N. and Carter, C.C. (eds), *An Advanced Treatise on Meloidogyne -Biology and Control* North Carolina State University Graphics, Raleigh, U.S.A. pp. 379-398.

Kumari, R., Giang, P. H., Sachdev, M., Garg, A.P., and Varma, A. 2004. Symbiotic fungi for eco-friendly environment: a perspective.

Lal, B. and Rana, B.P. 2013. Evaluation of fungi as seed and soil treatment against root knot nematode, *Meloidogyne incognita* in okra. *Agricul. Sci. Digest* 33(3): 226-229

Lall, B. and Ansari, M.N.A. 1960. Field studies on the root-knot nematodes (*Meloidogyne spp*.) Nematoda Heteroderidae. *Sci. Culture*, 26: 279-81.

Llorca, L.L.V., Jansson, H.B., Salinas, J., and Guerrero, P.J. 2008. Effect of chitosan on hyphal growth and spore germination of plant pathogenic and biocontrol fungi. *J. Appl. Microbiol.*104(2): 541-553.

Lopez, M.R.H., Clark, I.M., Atkins S.D., Hirsch P.R., and Kerry B.R. 2011. Exploring competitiveness and variation in the nematophagous fungus *Pochonia chlamydosporia* var. *chlamydosporia* and its significance for biological control. Bulletin OILB/SROP. 63: 37-40.

Mankau, R. 1980. Biocontrol fungi as nematode control agents. *J. Nematololy* 12: 244–252.

Masadeh, B., Von Alten, H., Grunewaldt-Stoecker, G., and Sikora, R.A., 2004. Biocontrol of root-knot nematodes using the arbuscular mycorrhizal fungus *Glomus intraradices* and the antagonist *Trichoderma viride* in two tomato cultivars differing in their suitability as hosts for the nematodes. *J. Plant Dis. Prot.* 322-333.

Mitkowski, A. and Abawi, G. 2003. Plant disease lessons: Root-knot nematode pathogen: *Meloidogyne* species. *The Am. Phytopatholo. Soc.*

Monjil, M.S., Ahmed, S., and Mymensingh, B. 2017. *Paecilomyces lilacinus* on egg hatching and larval population of *Meloidogyne* sp. *Bangladesh J. Plant Pathol.* 33: 71-78.

Nagesh, M., Hussaini, S.S., Chidanandaswamy, B.S., and Biswas, S.R. 2005. Isolation, *in vitro* characterization and predaceous activity of an indian isolate of the fungus, *Arthrobotrys oligospora* on the root-knot nematode, *Meloidogyne*

incognita. *Nematologia Mediterranea* 33(2).

Narayana, R., Sheela, M.S., and Sunu, T. 2017. Management of root-knot nematode, *Meloidogyne javanica* infecting cardamom. *Indian J. Nematology.*47 (1): 60-64.

Nitao, J. K., Meyer, S.L., and Chitwood, D.J. 1999. *In-vitro* assays of *Meloidogyne incognita* and *Heterodera glycines* for detection of nematode-antagonistic fungal compounds. *J. Nematology* 31(2): 172.

Oka, Y., Koltai, H., Eyal, B.M., Mor, M., Sharon, E., Chet, I., and Spiegel, Y. 2000. New strategies for the control of plant-parasitic nematodes. *Pest Mgmt. Sci.* 56: 983–988.

Pathak, J. J. and Saikia, B. 1999. Biological control of root knot nematode of betel vine. *J. Inter. Academicia* 3:117-120.

Prakob, W., Kanthasab, V., Supina, V., Chaimeungchern, N., and Kidtayo, T. 2007. Use of arbuscular mycorrhizal fungi, antagonistic fungus and rhizobacteria *P. aeruginosa* and *B. subtillis* in controlling tomato root-knot nematodes. *J. Agric*. 23*:* 403-406.

Rao, M.S., Reddy, P.P., and Nagesh, M. 1998. Evaluation of plant-based formulations of *Trichoderma harzianum* for the management of *Meloidogyne incognita* on eggplant. *Nematologia Mediterranea 26* (1): 59-62.

Reddy, D.D.R. 1985. Analysis of crop losses in tomato due to *Meloidogyne incognita*. *Indian J. nematology* 15(1): 55-59.

Reddy, P.P., Rao, M.S., and Nagesh, M. 1996. Management of citrus nematode, *Tylenchulus semipenetrans,* by integration of *Trichoderma harzianum* with oil cakes. *Nematologia Mediterranea* 24(2): 265-267.

Rifai, M.A. 1969. Sarawakus Lloyd, a genus of the pyrenomycete family Hypocreaceae. *Reinwardtia* 7(5): 561-578.

Sahebani, N. and Hadavi, N. 2008. Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum. Soil Biol. Biochem.* 40: 2016–2020.

Sakia, J. and Aparajitha. 2008. Comparative efficacy of fungal and bacterial bioagents with nematicides against *Meloidogyne incognita* on brinjal. *Indian J. Nematology* 38(2): 165-167.

Sankaranarayanan, C. and Sundarababu, R. 1997. Effect of oil cakes and nematicides on the growth of blackgram (*Vigna mungo*) inoculated with VAM fungus (*Glomus*

fasciculutum) and root-knot nematode (*Meloidogyne incognita*). *Indian J. Nematology* 27(1):128-130.

Sankaranarayanan, C., Hussaini, S. S., Sreeramakumar, S., and Prasad, R. D. 1997. Nematicidal effect of fungal filtrates against root-knot nematodes. *J. Biol. Control*. 11: 37-41.

Sasser, J.N. 1978. *Biology, identification and control of root-knot nematodes (Meloidogyne species)*. Department of Plant Pathology, North Carolina State University and the United States Agency for International Development.

Schindler, A.F. 1961. A simple substitute for a Baermann funnel. *Plant Dis. Rep.* 45: 747-748.

Shields, J.A., Paul, E.A., Lowe, W.E., and Parkinson, D. 1973. Turnover of microbial tissue in soil under field conditions. *Soil Biol. Biochem.* 5: 753-764.

Siddiqui, I.A. and Haque, E.S. 2000. Use of *Pseudomonas aeruginosa* for the control of root rot-root knot disease complex in tomato. *Nematologia Mediterranea*. 28(2): 189-192.

Siddiqui, I.A., Qureshi, S.A., Sultana, V., Haque, E.S., and Ghaffar, A. 2000. Biological control of root rot-root knot disease complex of tomato. *Plant and soil* 227(1-2): 163-169.

Sikora, R.A. and Fernandez, E. 2005. Nematode parasites of vegetables. *Plant parasitic nematodes in subtropical and trop. agric.* 2: 319-392.

Sikora, R.A. and Schonbeck, F. 1975, Effect of vesicular-arbuscular mycorrhizae (Endogone mosseae) on the population dynamics of the root-knot nematodes *Meloidogyne incognita* and *Meloidogyne hapla*. In: *Proceedings VIII international congress on plant protection* pp. 158-166.

Singh, P. and Siddiqui, Z.A. 2010. Biocontrol of root-knot nematode *Meloidogyne incognita* by the isolates of *Pseudomonas* on tomato. *Arch. Phytopathol. Plant Prot*. 43(14): 1423-1434.

Singh, S., Singh, B., and Singh, A.P. 2015. Nematodes: A threat to sustainability of agriculture. *Procedia Environ. Sci.* 29: 215-216.

Southey, J.F. 1986. Laboratory methods for work with plant and soil nematodes. Ministry of Agriculture Fisheries and Food, London, 202p.

Spadaro, D., Garibaldi, A., and Gullino, M.L. 2004. Control of *Penicillium expansum* and *Botrytis cinerea* on apple combining a biocontrol agent with hot water dipping and

acibenzolar-S-methyl, baking soda, or ethanol application. *Post harvest Biol. and Technol. 33*(2): 141-151.

Stirling, G.R. 1991. Biological Control of Plant Parasitic Nematodes. Progress, Problems and Prospects. CAB International, Wallingford, UK.

Taylor, A.L. and Sasser, J.N. 1978. Biology, identification and control of root-knot nematodes. *North Carolina State University Graphics*, 111.

Taylor, D.P. and Netscher, C. 1974. An improved technique for preparing perineal patterns of *Meloidogyne spp*. *Nematologica* 20(2): 268-269.

Varkey, S., Anith, K.N., Narayana, R., and Aswini, S. 2017. A consortium of rhizobacteria and fungal endophyte suppress the root-knot nematode parasite in tomato. *Rhizosphere* 5: 38-42.

Villanueva, L.M. and Davide, R.G. 1984. Evaluation of several isolates of soil fungi for biological control of root-knot nematodes. *Philippine Agriculturist* 67(4): 361-371.

Waller, F., Mukherjee, K., Deshmukh, S.D., Achatz, B., Sharma, M., Schafer, P., and Kogel, K. H. 2008. Systemic and local modulation of plant responses by *Piriformospora indica* and related Sebacinales species. *J. plant physiol.* 165(1): 60-70.

Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., Fischer, M., Heier, T., Hückelhoven, R., Neumann, C., Von Wettstein, D., and Franken, P. 2005. The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proceedings of the Natl. Acad. Sci.* 102(38):13386-13391.

Walters, S.A. and Barker, K.R. 1994. Efficacy of *Paecilomyces lilacinus* in suppressing *Rotylenchulus reniformis* on tomato. *J. Nematology* 26(4S): 600.

Wang, J., Lee, C., Replogle, A., Joshi, S., Korkin, D., Hussey, R., Baum, T.J., Davis, E.L., Wang, X., and Mitchum, M.G. 2010. Dual roles for the variable domain in protein trafficking and host-specific recognition of *Heterodera glycines* CLE effector proteins. *New Phytol*. 187:1003- 1017.

Wyborn, C.H.E., Priest, D., and Duddington, C.L. 1969. Selective technique for the determination of nematophagous fungi in soils. *Soil Biol. Biochem.* 1(1): 101-102.

Yang, Y., Yang, E., An, Z., and Liu, X. 2007. Evolution of nematode-trapping cells of predatory fungi of the Orbiliaceae based on evidence from rRNA-encoding DNA and multiprotein sequences. *Proc. Natl. Acad. Sci.* 104(20): 8379-8384.

Zuccaro, A., Basiewicz, M., Zurawska, M., Biedenkopf, D., and Kogel, K.H. 2009. Karyotype analysis, genome organization, and stable genetic transformation of the root colonizing fungus *Piriformospora indica*. *Fungal Genet. and Bio.* 46(8): 543-550.

EVALUATION OF NATIVE ISOLATES OF NEMATODE ANTAGONISTIC FUNGI AGAINST *Meloidogyne incognita* (KOFOID AND WHITE) CHITWOOD IN TOMATO.

by

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ABSTRACT

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ABSTRACT

An investigation entitled "Evaluation of native isolates of nematode antagonistic fungi against *Meloidogyne incognita* (Kofoid and White) Chitwood in tomato" was carried out at Department of Nematology, College of Agriculture, Vellayani during 2017-2019. The objective was to get native isolates of nematode antagonistic fungi and evaluate its bio-control potential against *Meloidogyne incognita* and growth promotion.

A survey was conducted in six Taluks of Thiruvananthapuram districts during 2017-18 for isolation of indigenous fungi. Twenty soil and root samples were collected from the rhizosphere of vegetable crops like bhindi, tomato, chilly, cucumber and cowpea grown in each taluk by random sampling. Preliminary screening of 32 fungal colonies showing characteristics similar to *Trichoderma* (colonies with green, cottony white mycelium) and *Purpureocillium* (colonies powdery or suede-like, gold, green-gold, yellow-brown, lilac or tan) were selected and brought to pure culture by sub culturing technique. Thirty-two fungal isolates were subjected to preliminary screening under invitro conditions for testing its efficacy to bring about J_2 mortality at standard concentration (100%). Among them ten isolates showed more than 50.00 per cent mortality of *M. incognita* juveniles were selected for further studies.

Morphological and cultural characteristics of ten isolates were studied. Bio efficacy study of ten isolates against J_2 mortality of *M. incognita* revealed that three isolates at lowest concentration (25%) showed 24.25, 34.00 and 34.50 per cent mortality of *M. incognita* juveniles 72 hrs after exposure. Isolate 10, 12, and 27 showed 85.05, 76.50 and 62.50 per cent mortality of *M. incognita* juveniles at 100 per cent concentration, 72 hr after treatment.

CFEs of these three isolates were screened for ovicidal effects against *M. incognita in vitro*. Sterile water and plain broth were maintained as control. Results of the *in vitro* screening studies revealed that CFE of isolate 10 (100% concentration) was effective in inhibiting the egg hatching at three to eight days after treatment (14.00 to 23.00 per cent).

Isolate 10 at 100% concentration was effective in increasing the mortality of *M. incognita* juveniles at 24, 48 and 72 hr after treatment (17.93 to 85.05 per cent).

Based on ovicidal properties of CFE, three isolates were selected for pot culture experiment to find out the efficacy in comparison with Cartap hydrochloride and *P. indica.* The results revealed that soil drenching of isolate 10 1%(w/v) with RKN inoculation was effective in reducing the nematode population in soil (83.88 per cent) and root (75.91 per cent) and it was significantly superior to isolate 12 and isolate 27.

The lowest number of nematodes were reported by isolate 10 (76.43). Lowest number of galls were reported by the soil drenching of isolate 10 (79.48 per cent). Efficacy of isolate 10 was found to be statistically on par with Cartap hydrochloride and *P. indica* in reducing the number of females and it also recorded the lowest number of egg masses. Isolate 10 was significantly superior to all other treatments in improving the growth parameters like plant height (86.05), fresh shoot weight (204.88), fresh root weight (81.00) compared to control plant height (59.50), fresh shoot weight (109.25), fresh root weight (48.50) respectively. Significantly superior yield was also recorded by Isolate 10 both with and without RKN inoculation.

Morphological, Cultural and Molecular characterization of the fungal isolates were done for identification of isolates. Internal transcribed regions of DNA of ITS regions were amplified by ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') regions. Blast search of amplified DNA in NCBI data revealed the identity of isolate 10, 12 and 27 as *Trichoderma viride* and *Metarhizium anisopliae* and *Fusarium verticillioides* respectively.

Results revealed that these three isolates suppressed population of *M. incognita* and increased growth and yield in tomato plants. Soil drenching of this indigenous fungal isolates 1% (w/v) can be recommended to manage *M. incognita* in tomato without any detrimental effect on environment.