Int.J.Curr.Microbiol.App.Sci (2019) *8*(2): 2176-2188

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 02 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.802.252

Biological Control of *Meloidogyne incognita* by *Trichoderma harzianum*

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Keywords

M. incognita, Eggmass, Juvenile, *Trichoderma harzianum*, Okra

Article Info

Accepted:
18 January 2019
Available Online:
10 February 2019

A B S T R A C T

Trichoderma harzianum parasitism on *Meloidogyne incognita* eggs and juveniles was examined *in-vitro* under Assam condition. *M. incognita* egg masses, their derived eggs and second-stage juveniles (J_2) were parasitized by *T. harzianum*. The conidia of the *T. harzianum* were found inside of the eggs and attached to the J_2s with the gelatinous matrix. The eggs were penetrated and parasitized by the hyphae of *T. harzianum*, while eggs containing juveniles were also parasitised by *T. harzianum*. Further, isolate *T. harzainum* was used for to know the bio-efficacy against *M. incognita* infected on okra under pot condition. For this *T. harzianum* was applied either as a seed treatment and/or soil application or both. Carbosulfan as a seed treatment and carbofuran as soil application was applied as chemical checks both either singly or in combination. The results showed that either *T. harzianum* or the chemicals (Carbofuran and Carbosulfan) when applied together as a seed treatment and soil application, improved plant growth parameters of okra and reduced the nematode multiplication as compared to when they were applied either as a seed treatment or soil application. Application of chemicals either as a seed treatment or soil application emerged as the most effective treatment as compared to the *T. harzianum*. However, in respect of *T. harzianum* when applied together as a seed treatment and soil application showed significantly better results in an improving the plant growth parameters and reduction in the nematode multiplication as compared to the treatments with carbosulfan as a seed treatment and carbofuran as soil application alone.

Introduction

Root-knot nematodes *Meloidogyne* spp. is one of the major pathogens of vegetable crops in Assam (Anon., 2011) and it caused five per cent of global crop loss (Hussey and Janssen, 2002). These microscopic species may not cause appreciable crop loss or symptom development as other pests and pathogens do and regarded as the hidden enemy of the farmers. *Meloidogyne* spp exhibit obligate type of relationship with host and produced the giant cell as feeding cell and it act as a metabolic sink which diverts the nutrient towards them (Davis *et al.*, 2004) as a result they produced galls on the roots. However, root-knot nematode laid their eggs in a gelatinous matrix and collectively known as egg mass. Such egg masses are exposed to the rhizosphere. Further, these egg masses are heavily colonized by microorganisms which are present in the rhizosphere and become an important factor in finding the nematode antagonists (Kok *et al.*, 2001). Kok *et al.*,

2001 reported that bacteria, fungi, protozoa, mites, etc. are feed on the egg mass of root-knot nematode but the utilization of fungi are unique natural enemies for the management of plant parasitic nematodes (Mark *et al.*, 2010). The fungi which feed on the nematodes are called as nematophagous fungi. Such fungi are obligate parasites of nematodes and some are opportunistic fungi which are mostly saprophytic in nature but when nematode will come in contact with them suddenly they trigger their nematocidal activity (Jansson and Nordbring-Hertz, 1988) like predation, parasitism etc. They can be categorized into four major groups: nematode-trapping fungi, endoparasitic fungi, egg-parasitic fungi, and toxin-producing fungi (Zhang and Hyde, 2014).

The activity of egg-parasitic fungi is essential because they mostly prefer the adults, eggs, and juveniles so it helps in the reducing the nematode inoculums while in the nematode-trapping fungi the juveniles of nematode some time escape from the traps and such trapping fungi either showed a poor competitive saprophytes or are susceptible to antagonism from other soil fungi (Mankau, 1962). Lysek (1963) for the first time observed invasion and destruction of nematode eggs by *Fusarium* spp. and *Cephalosporium* spp. and later so many egg parasitic fungi like *Acremonium bacilosporum, Helicoon farinosum, Mortierella nana, Paecilomyces lilacinus, Verticiluum chlamydosporium* and *V. bulbillosum* (Lysek, 1966), *P lilacinus* (Pau *et al.,* 2012), *T. atroviride* and *T. asperellum* (Sharon *et al.*, 2007), *P. chlamydosporia*, *P. lilacinus* and *A. strictum, F. oxysporium*, *T. harzianum*, *T. viride*, *F. chlamydosporium*, *C. oxysporum* and *C. aubense* (Singh and Mathur, 2010) and *A. implicatum* (Yao *et al.*, 2015) were reported from *Meloidogyne* spp. However, *Trichoderma* spp. are more rhizospheric competent than other fungi and showed nematicidal activity like (i) production of mycotoxins that immobilized J_2, (ii) direct antagonism on the pathogen like nematode (Shoresh *et al.*, 2010; Hermosa *et al.*, 2012; Brotman *et al.*, 2013) and pathogenic fungi by the action of antibiosis, competition, enzymatic hydrolysis, parasitism and systemic induced resistance (Chet *et al.*, 1997; Harman *et al.*, 2004) and (ii) It showed root colonization and directly influence the growth of the plants, either reduced abiotic stresses or increase the nutrient uptake (Harman *et al.*, 2004). The use of native biocontrol agents for the controlling of exotic plants appears to be beneficial because they are easy to apply and showed less environmental risk (Cofrancesco, 2000). Hence the present study was undertaking to determine the biocontrol activity of *T. harzianum* against *M. incognita* with the following two objectives (i) mycoparasitism of *T. harzianum* on *M. incognita* eggs and (ii) bio-efficacy of *T. harzianum* against *M. incognita* on okra under pot condition.

Materials and Methods

Collection of sample

Trichoderma harzianum isolated from the egg masses of *M incognita* and identified from the Department of Plant Pathology, AAU, Jorhat, Assam.

Collection of egg masses

Egg masses were collected from the galled root from each sample. Root pieces with galls were mixed thoroughly, washed in running tap water for 5 minute to get rid of soil and placed under a stereomicroscope. Egg masses were handpicked from the galled roots with help of a sterilized forceps. The egg masses thus collected were kept in sterilized cavity block containing 2 ml sterile distilled water.

Surface sterilization of egg masses

The collected egg masses were surface sterilized in 0.4 percent sodium hypochlorite (NaOCl) for two minutes (Singh and Mathur, 2010). Egg masses were washed thoroughly with sterile distilled water until the traces of NaOCl was removed and placed in cavity block for further use.

Preparation of media

The ingredients used for preparation of potato dextrose agar (PDA) are peeled potato (200 gm), dextrose (20 gm), agar-agar (20 gm) and distilled water (1000 ml). Peeled potatoes were boiled in 500 ml water. Potato extract was separated by using double layer muslin cloth and measured amount of dextrose was added to the extract. In another flask, remaining 500 ml distilled water was taken, required amount of agar-agar was added and molted by boiling. The molten agar- agar was strained through double layer muslin cloth and mixed with potato dextrose extract solution. The volume was made upto 1000 ml by adding distilled water. P^H was measured and maintained at 7.0 by NaOH. The medium was poured into culture tubes and conical flask plugged by non-absorbent cotton and then sterilized in autoclave at 121^0C for 20 minutes**.**

Mycoparasitism of *T. harzianum* on *M. incognita* eggs

Culture of fungal specie were inoculated to the center of a petriplate containing PDA medium amended with streptomycin as antibiotic @ 1 ml/L at full growth, 4 egg masses were placed on the petriplate and incubated at 25± 2^oC for 7 days. After 7 days of incubation, the portion of the fungal growth containing egg masses were collected on Hawkshely counting dish and stained with lactophenol cotton blue. The eggs were observed under compound microscope (60× objective lens) for the presences of morphological structures (hyphae, conidiophores, conidia, chlamydospores) of *T. harzianum* were noted during microscopic observations.

Bio-efficacy of *T. harzainum* against *M. incognita* on okra under pot conditions

Experimental site

The experiment was conducted in the net house of the Department of Nematology, AAU Jorhat during 2015-2016.

Mass culture of *T. harzianum* for soil application

For mass culture of isolated *T. harzianum*, 1kg vermicompost was put in to polypropylene bags plugged with nonabsorbent cotton and autoclaved at 121 ^{o}C for 30 minutes. Each bag containing the sterilized medium was inoculated with *T. harzianum* under aseptic conditions and was incubated at 25 ± 2 ^{o}C for 15 days. After 15 days of incubation the materials were mixed thoroughly and *cfu* was counted, maintained at 1×10^7 cfu/gm and used for application in pots (@ 5gm/kg soil).

Seed treatment with *T. harzianum*

Spore suspension of isolated *T. harzianum* was prepared from 15 day old culture grown in PDA slants. The spores were suspended in sterile distilled water and the concentration was adjusted to $1x10^7$ spores/ml with the help of a haemocytometer. Carboxy methyl cellulose (CMC) was used as an adhesive for treating okra seeds with *T. harzianum* spore suspension. For preparing 2% (w/v) adhesive solution, 200 mg of adhesive was added to 10 ml of antagonist suspension. Now required amount of seeds was taken in a petri plate and

the antagonist suspension with the adhesive was added drop by drop on the seeds stirring continuously. Addition of spore suspension was stopped when all the seeds got smeared with the spore suspension. After treating, the seeds were dried in shade for 6 hours and used for sowing.

Seed treatment with chemicals

Seeds were treated with Carbosulfan 25STD @ 3% and gum arabic was used as sticker. The weighed quantity of seed was mixed properly to form uniform coating over the seeds. Treated seeds were dried in shade and were sown in pots.

Soil application of chemical

Carbofuran @ 1 kg *a.i*/ha were applied and mixed thoroughly with the soil before sowing of the seed in pot.

Source of seeds

Seeds of okra cv. 'Parvani Kranti highly susceptible to *M. incognita* was obtained from Assam Seed Corporation ltd., Jorhat Branch, Assam.

Sterilization of seeds

Seeds were washed with clean tap water and were surface sterilized with 0.1 per cent mercuric chloride solution for 1-2 minutes and then washed with sterile water. The wet seeds were then dried in the air.

Collection and sterilization of soil

Required amount of soil was collected from upland near the nematode culture house, Department of Nematology, Assam Agricultural University, Jorhat. The soil was mixed thoroughly after removing unwanted materials like stones and roots. Then the soil was mixed homogenously with finely dried cow dung and sand in the ratio of 2:1:1, respectively. The soil mixture was put in a gunny bag and sterilized in an autoclave at 121^0C for half an hour.

Filling up of pots

Earthen pots with 1 kg capacity were selected, cleaned and sterilized in sunshine for conducting the experiment on biochemical analysis. Few broken pieces of bricks were placed at the bottom of the pots before filling up with sterilized soil mixture. Proper labeling of each pot was done.

Extraction of *M. incognita* juveniles (J_2) from eggs

For extraction of juveniles (J_2), the sterilized eggs collected as described above were placed on a double layer facial tissue paper supported on a course aluminum wire mesh. This was placed over a 10 cm diameter petriplate filled with required quantity of water at 24-26 ^{o}C in BOD incubator for hatching. Several such assemblies were maintained. The juveniles collected from these were mixed together at the time of inoculation in *in-vitro* studies. The counting of juveniles in the suspension was made by using Hawkshley counting dish. Five aliquots of 1 ml suspension were counted and their average number was multiplied with total volume of suspension prepared.

Inoculation of root knot nematode *M. incognita* juveniles (J_2)

Freshly hatched second stages of juveniles (J_2) of *M. incognita* were inoculated @1 J_2/cc of soil.

Treatment details

T_1- Control, T_2- Seed treatment with *T. harzainum* @ $1x10^7$ cfu/ml, T_3- Soil

application of *T. harzainum* @ $1x10^7$ cfu/gm at 5g/kg of soil, T_4- Seed treatment with *T. harzainum* @ $1x10^7$ cfu/ml + Soil application of *T. harzainum* @ $1x10^7$ cfu/gm at 5g/kg of soil, T_5- Seed treatment with Carbosulfan 25STD @ 3%, T_6- Soil application of Carbofuran @ 1kg *a.i*/ha, T_7- Seed treatment with Carbosulfan 25STD @ 3% + Soil application of Carbofuran @ 1kg *a.i*/ha. Further each treatment is replicated four times in completely randomized design.

Observations

Shoot length (cm)

The main shoot was measured in centimeter from the ground level up to tip of the longest leaf after 60 days of sowing.

Root length (cm)

The main root length was measured in centimeter from the ground level up to tip of the longest root after 60 days of sowing.

Fresh shoot and root weight (gm)

The fresh shoot and root weight per plant was measured in gram after 60 days of sowing. These plants were weighed on the weigh balance at Nematology laboratory.

Dry shoot and root weight (gm)

For recording dry weights, shoots and roots were separately cut into small pieces and kept in an oven running constantly at 60ºC at Nematology laboratory. The materials were weighed at every 24 hrs interval until a constant weight was obtained.

Number of galls and egg masses per root system

The number of galls and egg masses per root system was measured after 60 days of sowing.

Final nematode population

For recording the final nematode population in soil, 200 cc of soil was collected from each pot separately and processed by modified Cobb's sieving and decanting technique (Christie and Perry, 1951).

Statistical analysis

The data were analyzed by using WASP - Web Agri Stat Package 2.0 version software. Duncan's Multiple Range Test (DMRT) was conducted to determine the significance of treatments.

Results and Discussion

Mycoparasitsm by isolate *T. harzianum* on *M. incognita* eggs

Root knot nematode laid their eggs in a gelatinous matrix (gm) which is secreted by the six rectal glands of the adult female which covers the eggs (*i.e.,* egg mass) and exposed to the rhizospher by rupturing the roots. The chemical composition of the gelatinous matrix contains fucose and N-acetyl-glucosamine as carbohydrates which protect the eggs from the adverse environmental condition (Sharon and Spiegel, 1993). However, gm acts as a food source for the fungi and when come in contact with it suddenly they trigger the production of lytic enzymes like chitinase, protease and collagenase (Mortan *et al.,* 2004 and Sharon *et al.,* 2007). Such enzymes in combination, destroyed the lipid layer, hydrolyzed the chitin and altered the vitelline layer that causes the physiological and morphological changes in the eggs (Tikhonov *et al.,* 2002 and Khan *et al.,* 2004). Such fungi are able to feed on the inner content of the eggs and proliferated inside of the eggs, when the egg content is finished they produced the resting spores inside or outside of the eggs. In the present study, the egg masses of *M. incognita*

were directly exposed to the pure culture of *T. harzianum* isolated from the eggmass of *M. incognita* and studied the parasitism of *T. harzianum* on eggs after 7 days of incubation. However, under microscope it observed that *T. harzianum* grow on the egg mass surface and the hyphae of *T. harzianum* were observed to be tightly attached to the egg surface and penetrated inside of the egg shell (Fig. 1a) as a result they completely fed on the internal contents of the eggs (Fig. 1a) and they formed conidia inside of the eggs. Further, the complete proliferation of *T. harzianum* was observed inside the eggs (Fig. 1b). However, the *T. harzianum* not only prefer the immature eggs but also parasitized to the egg containing juveniles as a result the complete proliferation of hyphae of *T. harzianum* is seen to parasitized the juvenile which emerged from the egg (Fig. 2c). However, it is observed that, the isolate showed a complete morphological alteration of juvenile inside of the egg (Fig. 2d) and inhibited the mature eggs to hatch. Similar type of observations were reported by Saifullah and Thomas, 1996 who reported that *T. harzianum* was able to grow on the egg surface and penetrated the egg shell. Szabo' *et al.,* (2012) also showed that *Trichoderma* sp. formed the appresorium like structure and penetrated inside of the eggs and developed into a trophic hyphae inside the eggs of *C. elegans*. Sharon *et al.,* (2007) observed that conidia and hyphae of *Trichoderma* species were tightly attached to the surfaces of egg and further, they recorded that germinating hyphae of *Trichoderma* species directly penetrated to the egg masses and not only parasitised to the eggs but also parasitized to the J_2s within eggs and thus confirm the result of the present study. In the present investigation it reveals that the fungi, *T. harzianum* directly parasitized to J_2 which emerged from the eggs and proliferate inside the J_2 of *M. incognita* (Fig. 2). However, similar type of observation also reported by Sharon *et al.,* (2007) who suggested that gelatinous matrix of egg mass contains fucose (carbohydrate) which attached to the surface coat of J_2s during hatching and it can change the binding properties of conidia that contain fucose-binding domains so that gelatinous matrix -J_2s are efficiently attached and parasitized by the fungus.

Bio-efficacy of *T. harzainum* against *M. incognita* on okra under pot conditions

The data on plant growth parameters (Table 1, Fig. 3, 5 and 6) *viz*., plant height, shoot weight (fresh and dry), root weight (fresh and dry) reveal that all the treatments significantly improved the plant height from that of control. The maximum plant height, shoot weight (fresh and dry), root weight (fresh and dry) were recorded in the treatment T_7 *i.e* seed treatment with Carbosulfan 25STD @ 3% + soil application of Carbofuran @ 1kg *a.i*/ha followed by T_4 *i.e* seed treatment with *T. harzainum* @ $1x10^7$ cfu/ml + soil application of *T. harzainum* @ $1x10^7$ cfu/gm at 5g/kg of soil. Among the treatments with bioagents, the treatment T_4 was found significantly superior to rest of the treatments. The results showed that *T. harzianum* when applied together as a seed treatment and soil application significantly improved the plant growth parameters as compared to when it was applied either as a seed treatment or soil application. The growth promotion in the treatments receiving *Trichoderma* spp. are because of it is more rhizospheric competent and have their direct influence on either plant's growth or induction of plant defensive activity against pathogens (Shoresh *et al.*, 2010, Hermosa *et al.*, 2012, Brotman *et al*.,, 2013). Naserinasab *et al.,* (2012) observed that application of *Trichoderma* spp found to improve the plant growth parameters through enzymatic activities in the treated *Lycopersicon* spp. which ultimately reduced the biotic potentiality of plant-parasitic

nematode, *M. incognita*. Similarly, Annapurna *et al.*, (2018) reported that soil application of *T. harzianum* induce defence-related enzymatic activity like peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and total phenol content in tomato against *M. incognita* and as a result improved the plant growth parameters like shoot height, shoot weight, root length, root weight after 15, 30 and 45 days after inoculation and reduced the nematode multiplication on the tomato and in the soil as compared to the untreated control after 30 and 45 days after inoculation. However, the same type modes of action might be posses by the isolated *T. harzianum* against *M. incognita* in the present investigation.

Table.1 Effect of *T. harzianum* on growth parameters of okra infected by *M. incognita*

Treatments	Plant height (cm)	fresh shoot weight (gm)	Dry shoot weight (gm)	fresh root weight (gm)	Dry root weight (gm)
T_1	48.75^{e}	37.56^{f}	4.27^{g}	4.28^{e}	1.42^{g}
T_2	52.90^{d}	44.25^{d}	6.46^{e}	7.63^{d}	2.23^{f}
T_3	51.25^{d}	41.00^{e}	4.76^{f}	7.33^{d}	2.56^{e}
T_4	57.30^{b}	51.99^{b}	7.89$_{b}$	7.89^{b}	3.31^{b}
T_5	54.35^{c}	46.50^{d}	7.63^{c}	6.46^{c}	2.60^{d}
T_6	53.02^{c}	49.00^{c}	7.33^{d}	6.26^{c}	2.85^{c}
T_7	60.50^{a}	55.80^{a}	8.04^{a}	8.04^{a}	3.60^{a}
S. Ed ±	1.15	1.31	0.05	0.22	0.05
p≤ 0.05	2.38	2.74	0.12	0.46	0.11

Mean with different letters in the column are significantly different from each other based on Turky HDS test

T_1- Control, **T_2-** Seed treatment with *T. harzainum* @ 1x10^{7} cfu/ml, **T_3-** Soil application of *T. harzainum* @ 1x10^{7} cfu/gm at 5g/kg of soil, **T_4-** T2 + T3, **T_5-** Seed treatment with Carbosulfan 25STD @ 3%, **T_6-** Soil application Carbofuran @ 1kg *a.i*/ha, **T_7-** T5 + T6

Table.2 Effect of *T. harzianum* on nematode multiplication on okra infected by *M. incognita*

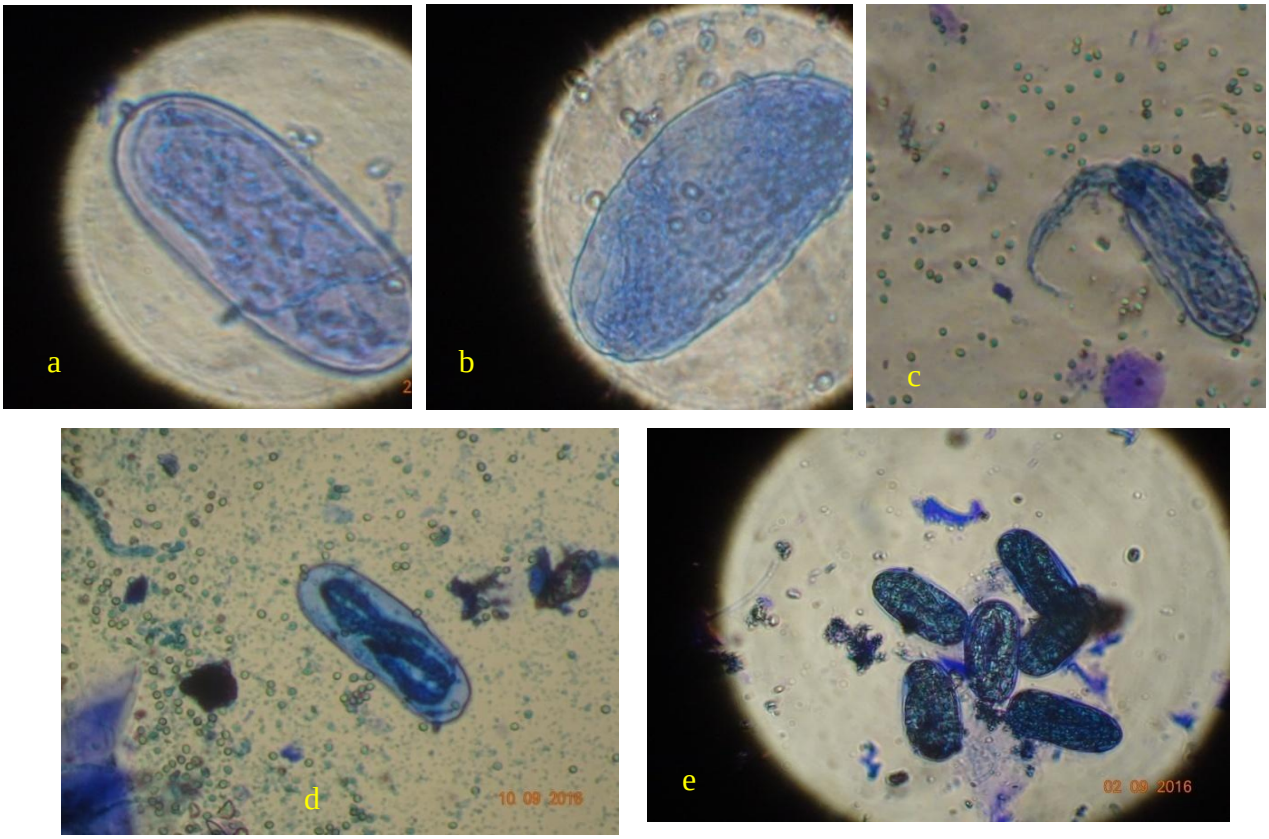
Treatments	Galls/root system	Eggmasses/root system	Eggs/eggmass	FNP
T_1	213.25 (14.61)^{a}	154.50 (12.43)^{a}	275.00 (16.57)^{a}	1514.00 (38.90)^{a}
T_2	108.50 (10.44)^{c}	93.75 (9.71)^{b}	156.00 (12.47)^{b}	783.67 (27.98)^{b}
T_3	127.50 (11.31)^{b}	98.25 (9.94)^{b}	162.75 (12.76)^{b}	866.75 (29.39)^{b}
T_4	97.00 (9.87)^{d}	84.00 (9.19)^{c}	113.75 (10.67)^{e}	533.15 (23.07)^{d}
T_5	85.00 (9.27)^{f}	51.50 (7.21)^{e}	129.00 (11.36)^{d}	600.00 (24.43)^{c}
T_6	91.00 (9.56)^{e}	56.50 (7.55)^{d}	138.75 (11.78)^{c}	650.00 (25.47)^{c}
T_7	56.25 (7.54)^{g}	35.75 (6.02)^{f}	106.50 (10.32)^{f}	400.00 (19.97)^{e}
S. Ed ±	0.13	0.15	0.14	0.52
P≤ 0.05	0.27	0.25	0.29	1.08

Figure in parenthesis are square root transform value before analysis.

Mean with different letters in the column are significantly different from each other based on Turky HDS test

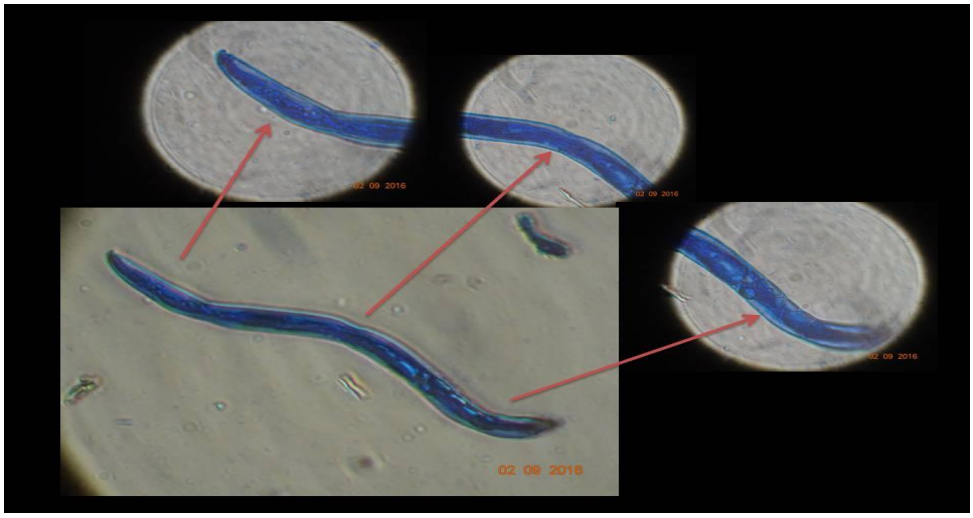
T_1- Control, **T_2-** Seed treatment with *T. harzainum* @ 1x10^{7} cfu/ml, **T_3-** Soil application of *T. harzainum* @ 1x10^{7} cfu/gm at 5g/kg of soil, **T_4-** T2 + T3, **T_5-** Seed treatment with Carbosulfan 25STD @ 3%, **T_6-** Soil application Carbofuran @ 1kg a.i/ha, **T_7-** T5 + T6

Fig.1 Meloidogyne *incognita* eggs/juvenile parasitised by *Trichoderma harzianum*



a. Penetration of the egg shell and degradation of egg embryo b. Extensive network of hyphae inside the egg c. Parasitised to J_2 emerging from the egg (arrow pointing at J_2) d. Morphological alteration of juvenile inside the egg e. Unparasitised mature eggs

Fig.2 *T. harzianum* parasitised to *M. incognita* J_2



(Arrow indicate extensive network of hyphae inside the J_2)

Fig.3 General view of pot experiment

Fig.4 Root galls in different treatments *(Th- T. harzianum)*

Fig.5 Effect of different treatments on plant height of okra infected by *M. incognita*

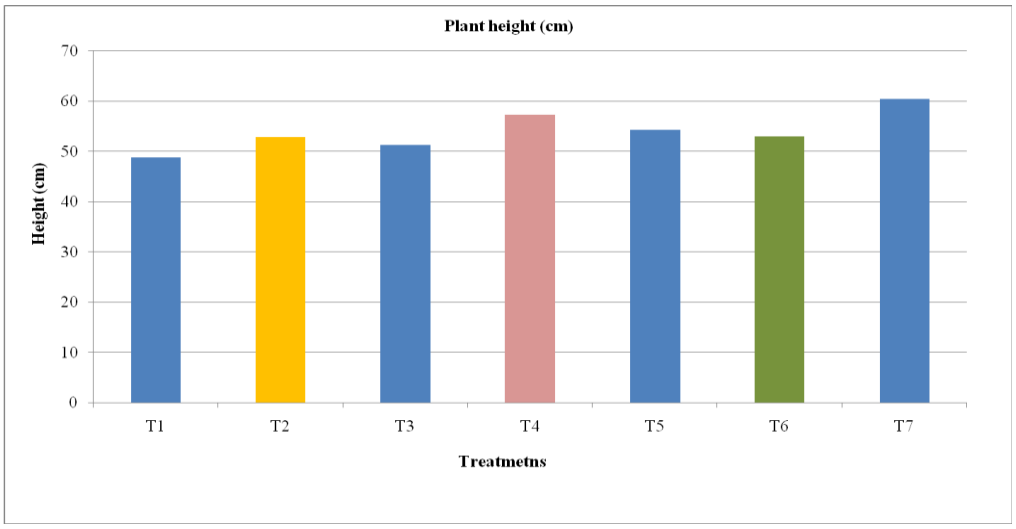


Fig.6 Effect of different treatments on growth parameters of okra infected by *M. incognita*

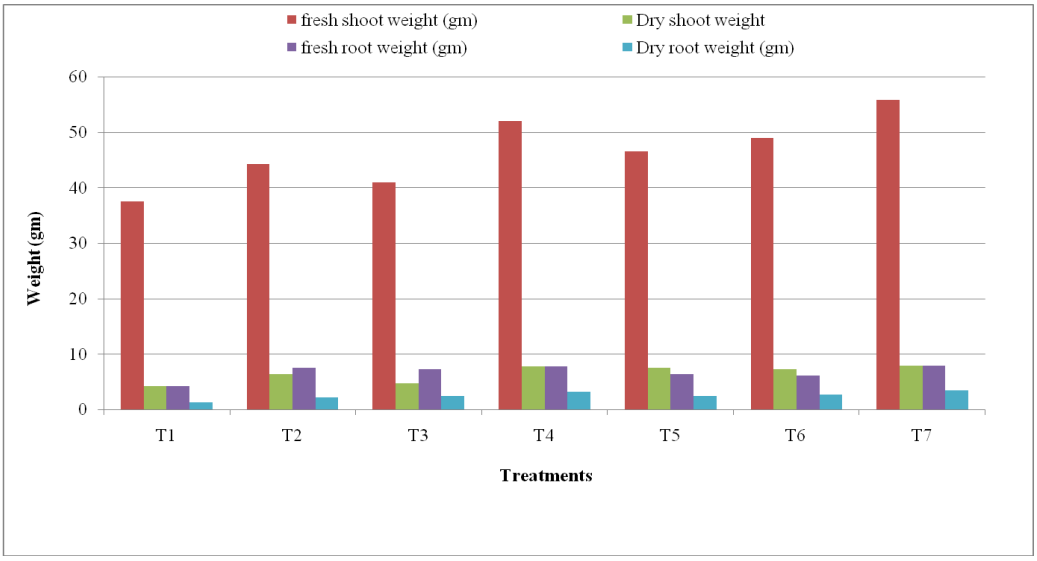
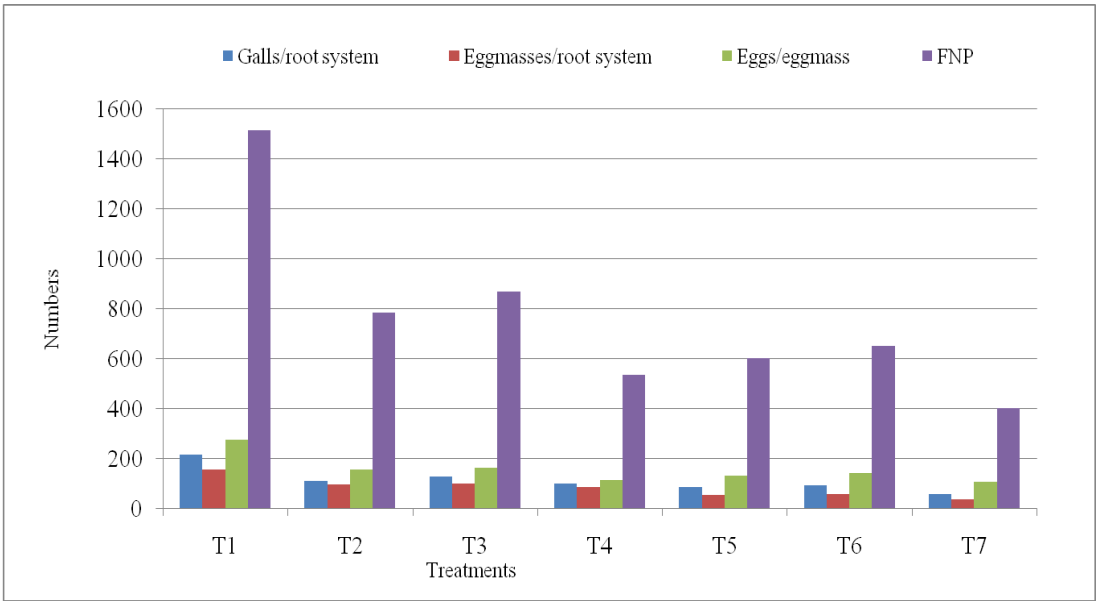


Fig.7 Effect of different treatments on nematode multiplication on okra infected by *M. incognita*



Data on the number of galls per root system, egg masses per root system, eggs per egg mass and final nematode population in the soil (Table 2, Fig. 7) recorded in all the treatments significantly differed from that of control. The treatments T_7 *i.e.* seed treatment with Carbosulfan 25STD @ 3% + soil application of Carbofuran @ 1kg *a.i*/ha was found to be best in reducing the nematode multiplication followed by T4 *i.e.* seed treatment with *T. harzainum* @ $1x10^7$ cfu/ml + soil application of *T. harzainum* @ $1x10^7$ cfu/gm at 5g/kg of soil. The results indicated that chemicals and bioagent when applied as a seed treatments and soil application were found to be significantly superior to those

when they were applied either as a seed treatment or soil application in reducing galls in roots. Sundaram and Hangaraj (2001) also reported a reduction of the population of *M. incognita*, when *T. harzianum* were applied as a seed treatment. The fungal bioagent *T. harzianum* showed their bioefficacy against *M. incognita* in respect of reducing their reproduction rate as compared to the untreated control (Khan and Haque, 2011 T). Similarly, Lal and Rana (2013) who recorded the lowest number of galls, egg masses and final nematode population of *M. incognita* in okra plants treated with *T. harzainum* as a seed treatment and/or soil application thus confirmed the results of the present investigation.

The possible mechanism involved in *Trichoderma* antagonist against root-knot nematode had been studied extensively by Sharon *et al.,* (2001). They reported two mechanisms (i) *Trichoderma* produced metabolites with an antinematode activity that immobilized J_2 thus reduced root penetration and (ii) direct parasitism by the antagonist. The decrease in nematode root galls and nematode population by *Trichoderma* spp. has been reported by several workers (Tripathi *et al.,* 2003, Kalita *et al.,* 2012, Annapurna *et al.,* 2018). The results of the present study also recorded that isolated *T. harzainum* parasitized to the egg and juvenile of *M. incognita*, as a result, reduced the nematode population when applied as a soil application and seed treatment on okra infected by *M. incognita*. Thus, the use of biocontrol agent' *i.e. T. harzainum* may be an effective alternative to the chemicals when applied together as a seed treatment and soil application showed significantly better results in an improving the plant growth parameters and reduction in the nematode multiplication as compared to the treatments with carbosulfan as a seed treatment and carbofuran as soil application alone for the management of *M. incognita* infected on okra under Assam condition..

Acknowledgement

The author is greatly thankful to Department of Plant Pathology, Assam Agricultural University, Jorhat, Assam for the identification of *T. harzainum*.

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How to cite this article:

Kurulkar Uday, Bhabesh Bhagawati and Pranjal Pratim Neog. 2019. Biological Control of *Meloidogyne incognita* by *Trichoderma harzianum*. *Int.J.Curr.Microbiol.App.Sci.* 8(02): 2176-2188. doi: https://doi.org/10.20546/ijcmas.2019.802.252