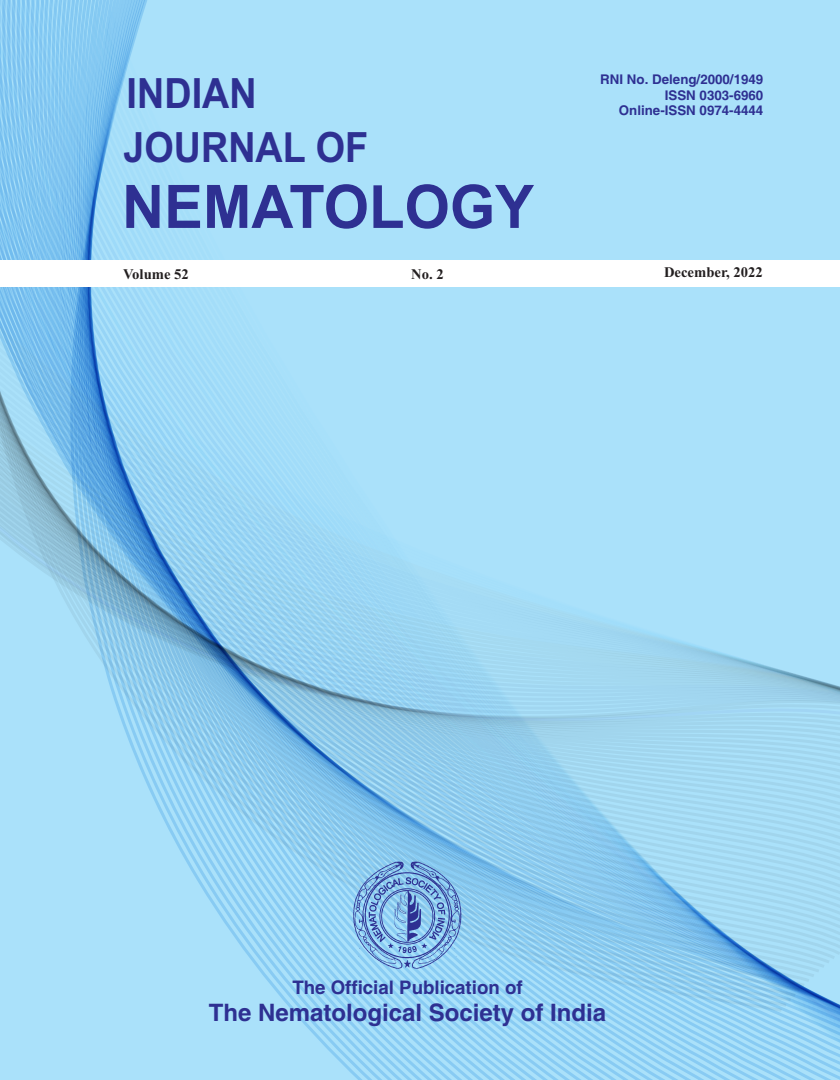

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Biological Control of Rice Root-Knot Nematode, *Meloidogyne graminicola* in Transplanted Rice

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ABSTRACT: The current study is designed for the management of rice root-knot nematode, *Meloidogyne graminicola* in rice (*Oryza sativa* L.) under laboratory, nursery and field conditions. Under laboratory conditions, all the substances *viz.,* phytotherapeutic substances, rhizobacteria and chemicals inhibited the juvenile mortality of *M. graminicola* at all dilution levels at each interval of exposure periods. Among the rhizobacterial strains and plant extracts, the maximum juvenile mortality was observed in *Azotobacter chroococcum* (HT 54) which is statistically at par with aqueous extracts of *Azadirachta indica* and only 65.2 and 61.8% juvenile mortality was observed in this case, respectively. However, *A. chroococcum, A. indica* and carbosulfan gave maximum mortality at 1:5 and 1:10 dilutions irrespective of period of exposure. Under nursery conditions, treatments of neem cake @ 50g/pot (5 kg soil capacity) in combination with *Pseudomonas fluorescens* @ 50 g/pot significantly reduced the nematode reproduction and multiplication and improved the plant growth parameters of rice (var. PB 1121). In main field experiment, where treated nursery treatments were integrated with main field treatments, significantly highest and maximum yield (51.25 q/ha) was obtained in combination of treated nursery (neem cake @ 50g/pot+ *P. fluorescens* @ 50 g/pot) and two deep summer ploughing during the month of June also significantly reduced the nematode reproduction and multiplication. So, combination of treated nursery with neem cake and *P. fluorescens* along with deep summer ploughing in the main field before transplanting has reduced the nematode population and enhanced rice yield.

Keywords: Deep summer ploughing, *Meloidogyne graminicola*, Neem cake, Rice, *Pseudomonas fluorescens*

Rice (*Oryza sativa* L.) belongs to the family Poaceae and affected by number of biotic and abiotic factors. Among biotic factors, plant parasitic nematodes (PPNs) constitute an important component (Sharma *et al.,* 2002) which causes 16-20% yield loss in lowland rainfed rice, 16-32% under irrigated and 11-73% in flooded conditions in India (Pankaj *et al.,* 2015). However, rice root-knot nematode, *Meloidogyne graminicola* has become a most important destructive nematode and serious problem in major rice producing countries of the world (Jain *et al.,* 2012). This nematode is also a major problem in the nurseries and main field (rice) but recently has been found to be widespread in the deep-water and irrigated rice in India (Rusinque *et al.,* 2021). The diagnostic symptoms of *M. graminicola* affected rice show less vigor, stuned growth, yellowing, late maturity of the crop, production of poorly filled kernels and chlorophyll content of infected leaves was also reduced. The galls incited by *M. graminicola* on rice are terminal, horse shoe shaped or typical hook-shaped. The increasing demand of the agricultural produce can be attained by optimizing the productivity potential and minimizing the yield loss caused by PPNs. Hence, the search of alternate to non-chemical strategy, eco-friendly approaches are highly desirable for nematode management in rice. Among the parasitic organisms, management of PPNs is more difficult due to their inhabitation and mode of parasitism (Gillet *et al.,* 2017).

Mostly, PPNs attack underground part of the plants and cause serious yield loss in the crops. So, to avoid these unnecessary losses, better management practices must be adopted. Out of different eco-friendly approaches, plant growth promoting rhizobacteria (PGPR) may act as an efficient nematode bio-control as well as plant growth promoting agents. PGPR like *Psuedomonas* spp., *Gluconacetobacter diazotrophicus* and *Azotobacter chroococcum* are having protection potential in modern agricultural system. PGPR are capable of improving the plant growth in many plants and they also act as biological control agents against various soil borne pathogens including root infecting nematodes. Biological control using PGPR is a promising tool and safe alternative approach for managing PPNs (Lee and Kim, 2016; Priyank *et al.,* 2018). Bacterial application produces additional positive effects on growth stimulation, increased yields and suppresses PPNs population (Varvara and Nicola, 2021). Therefore, PGPR plays significant role in the development of sustainable agriculture system and one of the major components in integrated nematode management (Abd-Elgawad and Kabeil, 2012). The use of plant extracts and their products are gaining attention due to their availability, cost effectiveness, proven nature of specificity, low phytotoxicity and minimum residual toxicity in the ecosystem (Maji *et al.,* 2005). Many of the plant extracts containing phytochemicals *viz.,* alkaloids, tannins, saponins, flavonoids, diterpenes, glucosinolates, acetylenes and thinlys which are found effective nematicidal (Bansal and Bajaj, 2003). Since, the initial infection starts from the nursery, so, the management of nematode in rice nursery shall prove much more effective strategy in preventing the spread of *M. graminicola from infected areas to uninfected areas by infected seedlings.* In the view of increasing importance of *M. graminicola* in the rice based cropping system sequence in the changing agricultural scenario; an investigation was undertaken to develop a biological control of *Meloidogyne graminicola* in transplanted rice.

MATERIALS AND METHODS

The present studies were conducted in laboratory and screen house in the Department of Nematology, CCS HAU, Hisar and field experiments at village Premnagar, Hansi, Hisar (Haryana) India.

Propagation of pure culture of *M. graminicola*: Pure cultures were raised in screen house in earthen pots filled with steam sterilized sandy loam soil at 15 lb pressure/sq inch. Healthy seedlings of rice (PB 1121) were transplanted in the pots and second-stage juveniles (J2) of *M. graminicola* were inoculated. The cultures were allowed to multiply for 2-3 generations and were further sub-cultured periodically. The root-knot nematode species was first identified before propagation of pure culture. For the confirmation of purity, 5-10 matured females were extracted from these roots by teasing them under a stereoscopic binocular microscope with the help of needle and forceps for preparation of perineal pattern for identification and confirmation of species of *M. graminicola* (Mulk, 1976; Eisenback, 1985). Unlike other species of *Meloidogyne*, *M. graminicola* perineal patterns are located on raised portions (protuberances).

***In vitro* Preparation of aqueous plant extracts:** Healthy leaves, delicate twigs and stems of 2-3 years old plant of *Azadirachta indica, Phyllanthus niruri, Achyranthus aspera, Eucalyptus globulus* and *Xanthium strumarium* were washed with sterile distilled water. The aqueous extract of each plant was prepared separately by grinding 2 g of plant material in 5 ml of distilled water. To obtain a clear and transparent extract free of all plant debris, the water extracts were filtered through a four ply muslin cloth and then Whatman's filter paper. The aqueous extracts thus prepared were kept in a covered flask in refrigerator and this concentrated form were taken as stock solution for evaluating their nematicidal effects larval mortality under *in vitro* conditions.

Preparation of culture filtrates (CFs): Five strains of rhizobacteria viz., *Azotobacter chroococcum* (HT 54, HT 57, Mac 27), *Gluconacetobacter diazotrophicus* (33-47) and *Pseudomonas* spp. (P36) were procured from Department of Microbiology, College of Basic Sciences and Humanities, CCS HAU, Hisar, Haryana, India. The culture thus obtained was centrifuged at 6000 rpm for 10 minutes. All the filtrates were maintained on their respective medium slant i.e. I-GI (lacto-glucose infusion) for *Gluconacetobacter*, Jensen's for *Azotobacter* and King's B for *Pseudomonas* spp. Single colony of each strain was inoculated and grown in respective medium in a rotary shaker at 150 rpm for 48h at room temperature. The culture thus obtained was centrifuged at 6000 rpm for 10 min. The supernatant were injected through a 0.2 mm filter to remove the bacterial cell and the filtrates were collected (1×10^8 cfu/ml).

Procedure: Nematicidal activity of plants (*Azadirachta indica, Phyllanthus niruri, Achyranthus aspera, Eucalyptus globulus* and *Xanthium strumarium*), rhizobacteral strains (*Azotobacter chroococcum* HT 54, HT 57, Mac 27, *Gluconacetobacter diazotrophicus* 33-47 and *Pseudomonas* spp. P36) and chemical (Carbosulfan 25 EC and Cartap hydrochloride 50 SP) were evaluated *in vitro* against juvenile mortality of *M. graminicola* at various dilutions *viz.,* 1:5, 1:10, 1:20, 1:40 and 1:80. Five ml of suspension containing approximately 100 freshly hatched second juveniles (J2s) of *M. graminicola* were taken in 50 mm size Petri plates. Measured quantity of stock solution (1:5, 1:10, 1:20, 1:40 and 1:80) was added to each Petri plate to make the resultant dilutions of 1:5, 1:10, 1:20, 1:40 and 1:80. Water alone was taken as check. Each dilution was replicated five times. These Petri plates were kept in BOD incubator at 25±1°C. Juvenile mortality after 24h exposure of the larvae to different dilutions of different extracts was recorded by counting living and dead J2s under stereoscopic binocular microscope. The immobilized larvae from each replicate were transferred to a Petri-dish containing distilled water and their revival was recorded under stereoscopic binocular microscope after 24h.

Computation of Mortality (%): Per cent juvenile mortality was computed with the following formula

$$\text{Juvenile Mortality (\%)} = \frac{\text{Number of juvenile killed/ immobilized}}{\text{Total no. of juvenile}} \times 100$$

For rice nursery

Out of treatments of rhizobacteria, phytotherapeutic substances and chemicals tested *in vitro*, one best treatment from each having highest J2s mortality of *M. graminicola* was combined in this experiment. Nursery growing pots of 5 kg soil capacity were filled with infested soil having initial nematode population (Pi) of 292 J2s/200 cc soil. Seeds of rice (var. PB 1121) were treated by dipping in *A. chroococcum* HT 54 culture @ 50 ml broth (10 kg seed) for 12h before sowing (T1-T9). The soil was drenched with 350 ml of the neem leaf extract (1:10 in T10-T17) and carbosulfan (1:40 in T19-T26) concentration by pouring the solution in each pot. The above treatments showed best results under *in vitro* condition. Organic amendments (neem and mustard cake) and bio-agent (*T. viride* and *P. fluorescens*) were added 10 days before sowing. Weighed amount of *T. viride* and *P. fluorescens* were mixed in farm yard manure (FYM) for enrichment. Two days waiting period was kept for proper multiplication. All other chemicals were added at the time of sowing. The pots without any chemicals, organic amendments and bio agents were treated as control. Each treatment was replicated five times and statistical design was CRD. The following treatments were maintained which were mentioned below.

Main treatments (best treatments from *in vitro*)

i. *Azotobacter chroococcum* HT 54
ii. *Azadirachta indica*
iii. Carbosulfan

Sub treatments

i. Neem cake @ 50g/pot
ii. Mustard cake @ 50g/pot
iii. Pseudomonas fluorescens @ 50 g/pot
iv. Trichoderma viride @ 50 g/pot

v. Furadan 3G (carbofuran) @ 200 mg/pot

vi. Caldan 4 G (cartap hydrochloride) @ 200 mg/pot

Total treatments (alone and in combination) are T1- HT 54 (ST*) + neem (SD*); T2- HT 54 (ST) + carbosulfan (SD); T3- HT 54 (ST) + neem cake @ 50g/pot; T4- HT 54 (ST) + mustard cake @ 50g/pot; T5- HT 54 (ST) + *Pseudomonas fluorescens* @ 50 g/pot; T6- HT 54 (ST) + *Trichoderma viride* @ 50 g/pot; T7- HT 54 (ST) + carbofuran @ 200 mg/pot; T8- HT 54 (ST) + cartap hydrochloride @ 200 mg/pot; T9- HT 54 (ST); T10- Neem (SD*) + carbosulfan (SD); T11- Neem (SD) + neem cake @ 50g/pot; T12- Neem (SD) + mustard cake @ 50g/pot; T13- Neem (SD) + *P. fluorescens* @ 50 g/pot; T14- Neem (SD) + *Trichoderma viride* @ 50 g/pot; T15- Neem (SD) + carbofuran @ 200 mg/pot; T16- Neem (SD) + cartap hydrochloride @ 200 mg/pot; T17- Neem (SD); T19- Carbosulfan (SD)+ neem cake @ 50g/pot; T20- Carbosulfan (SD)+ mustard cake @ 50g/pot; T21- Carbosulfan (SD) + *P. fluorescens* @ 50 g/pot; T22- Carbosulfan (SD) + *T. viride* @ 50 g/pot; T23- Carbosulfan (SD) + carbofuran @ 200 mg/pot; T24- Carbosulfan (SD) + cartap hydrochloride @ 200 mg/pot; T26- Carbosulfan (SD); T27- Neem cake @ 50g/pot+ mustard cake @ 50g/pot; T28- Neem cake @ 50g/pot + *P. fluorescens* @ 50 g/pot; T29- Neem cake @ 50g/pot+ *T. viride* @ 50 g/pot; T30- Neem cake @ 50g/pot + carbofuran @ 200 mg/pot; T31- Neem cake @ 50g/pot+ cartap hydrochloride @ 200 mg/pot; T32- Neem cake @ 50g/pot; T33- Mustard cake @ 50g/pot+ *P. fluorescens* @ 50 g/pot; T34- Mustard cake @ 50g/pot+ *T. viride* @ 50 g/pot; T35- Mustard cake @ 50g/pot+ carbofuran @ 200 mg/pot; T36- Mustard cake @ 50g/pot+ cartap hydrochloride @ 200 mg/pot; T37- Mustard cake @ 50g/pot; T38- *P. fluorescens* @ 50 g/pot+*T. viride* @ 50 g/pot; T39- *P. fluorescens* @ 50 g/pot+ carbofuran @ 200 mg/pot; T40- *P. fluorescens* @ 50 g/pot+ cartap hydrochloride @ 200 mg/pot; T41- *P. fluorescens* @ 50 g/pot; T42- *T. viride* @ 50 g/pot+ carbofuran @ 200 mg/pot; T43- *T. viride* @ 50 g/pot+ cartap hydrochloride @ 200 mg/pot; T44- *T. viride* @ 50 g/pot; T45- Carbofuran @ 200 mg/pot + cartap hydrochloride @ 200 mg/pot; T46- Carbofuran @ 200 mg/pot; T47- Cartap hydrochloride @ 200 mg/pot and T48- Untreated check.

Where, ST*- seed treatments and SD*- soil drenching

At the time of nursery transplanting, each plant was uprooted carefully from soil and recorded the observations *viz.,* plant growth characteristics (seedling length, fresh and dry seedling weight) and nematode multiplication (number of galls/seedling, number of eggs/seedlings and final nematode population in the soil/200cc soil was analyzed by Cobb's Sieving and Decanting technique, Cobb, 1918; Schnidler, 1961).

a) Number of galls/plant:-Roots were spread in the big sized Petri plate which contained water. Each visible gall/knot formed by the nematode penetration was counted on the whole root system with the help of a hand lens.

b) Number of eggs/plant: - The numbers of eggs/plant were counted after teasing the roots in water.

c) Final nematode population in the soil (200 cc soil):- For recording final soil population, each pot soil after depotting was analysed by Cobb's Sieving and Decanting method and nematodes extracted by Modified Baermann's Funnel technique (Christie and Perry, 1951). The extracted nematodes per ml nematode suspension were counted under stereoscopic binocular microscope with the help of counting dish and finally the soil population/kg soil was calculated.

For transplanted rice under screen house condition

From the nursery experiment, three best treatments sowing highest nursery growth parameters and minimum nematode galling (neem cake @ 50g/pot + *P. fluorescens*

@ 50 g/pot, *P. fluorescens* @ 50 g/pot + carbofuran @ 200 mg/pot, *T. viride* @ 50 g/pot + carbofuran @ 200 mg/pot along with one check) were selected for transplanting of rice seedling in infested soil kept earthen pots of 5 kg soil capacity for mid population evaluation. So, seedlings selected from these above mentioned treatments were combined with below mentioned treatment for integration. Pi for this experiment was found 292 J2/200 cc soil. The uprooted seedlings were transplanted in the pots where the soil was mixed with the treatments. Treated nursery of above mentioned treatments were combined with four further soil treatment before transplanting. Each treatment was replicated five times and statistical design was CRD.

Main treatments (best treatments from rice nursery)

i. Neem cake @ 50g/pot + *P. fluorescens* @ 50 g/pot
ii. *P. fluorescens* @ 50 g/pot+ carbofuran @ 200 mg/pot
iii. *T. viride* @ 50 g/pot+carbofuran @ 200 mg/pot

Main treatments: i. Carbofuran @ 200 mg/pot; ii. T40 (N); iii. Neem cake @ 25g/pot + *P. fluorescens* @ 25 g/pot & iv. *P. fluorescens* @ 25 g/pot + carbofuran @ 100 mg/pot

Total treatments (alone and in combination) are T1-T40 (N) + (neem cake @ 25g/pot + *P. fluorescens* @ 25 g/pot); T2-T40 (N) + (*P. fluorescens* @ 25 g/pot+ carbofuran @ 100 mg/pot); T3-T40 (N) + carbofuran @ 200 mg/pot; T4-T40 (N) alone; T5-T26 (N) + (*P. fluorescens* @ 25 g/pot + carbofuran @ 100 mg/pot); T6-T26 (N) + carbofuran @ 200 mg/pot; T7-T26 (N) alone; T8- T37 (N) + carbofuran @ 200 mg/pot; T9- T37 (N) alone; T10- Carbofuran @ 200 mg/pot and T11- Untreated check

Where, N -Treated nursery

Sixty days after transplanting, each plant was uprooted carefully from soil and recorded the observations such as plant growth characteristics (shoot and root length, fresh and dry shoot, root weight (g) and also on nematode multiplication such as number of galls/plant, number of eggs/plant, FNP in the soil and reproduction factor (RF).

Computation of reproduction factor (RF):- The reproduction factor was computed with the following formula.

$$\text{RF} = \frac{\text{Number of eggs + final nematode population in the soil}}{\text{Initial nematode population (Pi)}}$$

For transplanted rice under field condition

This experiment was conducted on *M. graminicola* infested field at Premnagar village, Hansi, district Hisar during kharif season, 2017-18. The experimental area had been used for continuous rice cultivation for more than five years. Soil samples were taken from each corner of the field for assessing Pi of *M. graminicola* which was 292 J2/200 cc soil. Experimental site was thoroughly ploughed to a fine tilth, harrowed and levelled. Two deep summer ploughings (DSP) were done during the month of June (16th and 26th) at 10 days interval. The normal package of practices like FYM and fertilizer was applied into entire field. The entire site was divided into 48 plots with a size of 2x2 m^2. As in experiment of rice nursery, the treated nursery (best three treatments) was transplanted in the soil of which was treated with five treatments along with one untreated check. In total, sixteen treatments were replicated five times with RBD layout.

Main treatments (best treatments from rice nursery)

i. Neem cake @ 50g/pot + *P. fluorescens* @ 50 g/pot
ii. *P. fluorescens* @ 50 g/pot+carbofuran @ 200 mg/pot
iii. *T. viride* @ 50 g/pot+carbofuran @ 200 mg/pot

Sub treatments of main field

i. Two deep summer ploughings (DSP) at 16th and 26th June, 2017

ii. Carbofuran @ 2.0 kg a.i./ha

iii. Neem cake @ 1 t/ha + *P. fluorescens* @ 2.5 kg/ ha

iv. *P. fluorescens* @ 2.5 kg/ha+carbofuran @ 1.0 kg a.i./ha

v. T40 (N)

Total treatments (alone and in combination) are T1- DSP+ carbofuran @ 2.0 kg a.i./ha; T2- DSP + T40 (N); T3- DSP + T26 (N); T4- DSP + T37 (N); T5- DSP; T6- T40 (N) + carbofuran @ 2.0 kg a.i./ha; T7- T26 (N) + carbofuran @ 2.0 kg a.i./ha; T8- T37 (N) + carbofuran @ 2.0 kg a.i./ha; T9- carbofuran @ 2.0 kg a.i./ha; T10- T40 (N) + neem cake @ 1 t/ha + *P. fluorescens* @ 2.5 kg/ ha; T11-T40 (N) +*P. fluorescens* @ 2.5 kg/ ha+carbofuran @ 1.0 kg a.i./ha; T12- T40 (N); T13-T26 (N) + *P. fluorescens* @ 2.5 kg/ha+carbofuran @ 1.0 kg a.i./ha; T14-T26 (N); T15-T37 (N) and T16- Untreated check

Where, N –treated nursery

This experiment was terminated at harvest after recording the yield data. At the end of the experiment, five randomly selected plants per plot were carefully uprooted and recorded the observations *viz.,* grain yield and also on nematode multiplication such as root-knot index, number of eggs/plant and FNP in the soil (200 cc soil).

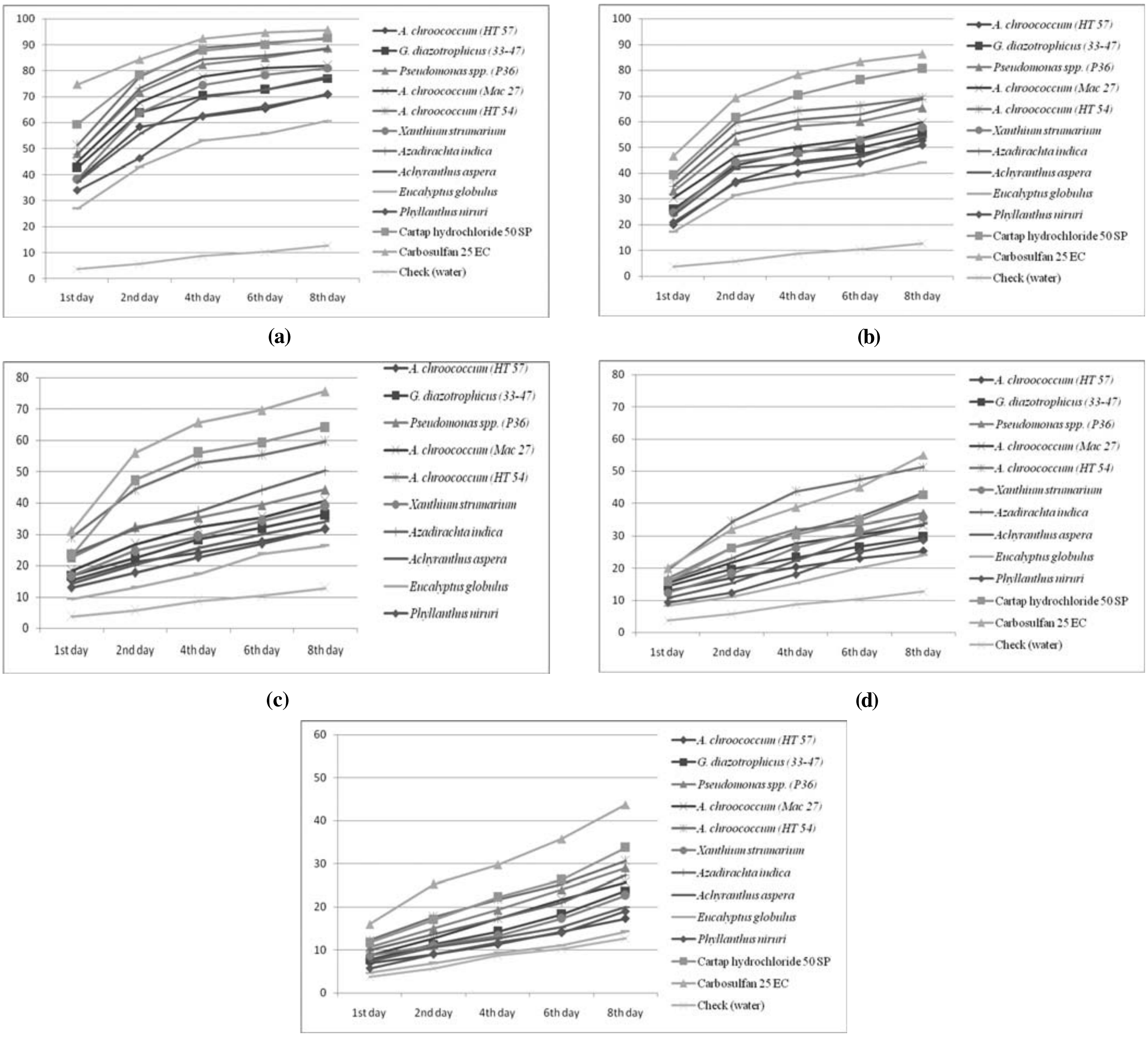
STATISTICAL ANALYSIS

The data obtained in all the experiments was analysed by using OPSTAT software available online at CCS HAU, Hisar, Haryana, India website.

RESULTS AND DISCUSSION

***In vitro*:** The data in Fig 1(a-e) clearly depicted the effects of different rhizobacteria, phytotherapeutic substances and chemicals on juvenile mortality of *M. graminicola* at different concentration levels *viz.,* 1:5, 1:10, 1:20, 1:40 and 1:80. Maximum and significantly higher juvenile mortality was found, where juvenile of *M. graminicola* were exposed to the chemicals viz., carbosulfan followed by cartap hydrochloride. Among the rhizobacterial strains and plant extracts, the maximum juvenile mortality was observed in *A. chroococcum* (HT 54) which was statistically at par with aqueous extracts of *A. indica*, as only 65.2 and 61.8 per cent juvenile mortality was found in these cases, respectively. All other rhizobacterial strains and aqueous extracts of plants significantly increased juvenile mortality at all the concentrations as compared to untreated check (16.1%). All the CFs resulted in juvenile mortality ranging from 19.7-65.2 per cent. After 24h of exposure, highest mortality was shown by *A. chroococcum* HT 54 followed by *Pseudomonas* spp. P 36. At 1:10 and 1:20 concentration also, mortality per cent was more in *A. Chroococcum* HT 54. Similar trend was observed with other concentration levels also. Mortality rate was found to be reduced upon diluting the CFs. Minimum and significantly lowest mortality was observed for *A. chroococcum* HT 57 in all the five dilutions as compared to check. All the five rhizobacterial strains were effective in nematode mortality (65.2%) at 1:5 concentration and least mortality (19.7%) was observed in 1:80 concentration irrespective of rhizobacterial strains.

The aqueous extract of *A. indica* was most consistent in terms of juvenile mortality as it was effective upto 1:10 dilution but showed low mortality at 1:80 dilution. The highest mortality of juveniles was observed at 1:5 dilution of aqueous extract of tested plants while lowest was observed at lowest dilution i.e. 1:80. Irrespective of aqueous extract of plants, per cent juvenile mortality increased significantly with increase in concentration of the aqueous extracts. Among chemicals, carbosulfan showed maximum juvenile mortality followed by cartap hydrochloride. The chemicals showed juvenile mortality upto 1:40 (upto 50% mortality) dilution but showed low



(e)

Fig. 1. Effect of different rhizobacteria, aqueous extract of phytotherapeutic substances and chemicals on the juvenile mortality of *M. graminicola*

mortality at 1:80 dilution. Similar trend was observed with other concentration levels also. Among all the exposure periods, maximum mortality was found after 24h at concentration of 1:5 (Fig 1a) irrespective of all the substances. Rate of mortality was directly proportionate to concentration of plant extracts. Mortality of *M. graminicola* increased with the increase in exposure period. However, the interaction between the exposure period and substances were found non- significant in nematode mortality at all concentrations.

For rice nursery: In rice nursery experiment, minimum and significantly lowest number of galls/seedling was obtained in treatments neem cake @ 50g/pot + *P.*

fluorescens @ 50 g/pot (23.33) followed by *T. viride* @ 50 g/pot + carbofuran @ 200 mg/pot (24.67), *P. fluorescens* @ 50 g/pot+carbofuran @ 200 mg/pot (27.67). Significantly minimum and lowest number of eggs was found in treatments neem cake @ 50g/pot+*P. fluorescens* @ 50 g/pot (1196.67) followed by *T. viride* @ 50+carbofuran @ 200 mg/pot (1321.67), *P. fluorescens* @ 50 g/pot + carbofuran @ 200 mg/pot (1393.33). Data in Table 1 indicated that minimum and significantly lowest FNP was found in neem cake @ 50 g/pot + *P. fluorescens* @ 50 g/pot (97.33) and this was statistically at par with of that of *T. viride* @ 50 g/pot + carbofuran @ 200 mg/pot (124.67). The maximum FNP was recorded in untreated check. In individual treatments, minimum FNP was observed in application of carbofuran @ 200 mg/pot application and maximum in neem application.

For screen house condition: Integration of nematode management practices was carried out by using treated seedling (nursery) transplanted into earthen pots in screen house condition. Three best nursery treatments along with one check were combined in this experiment. Significantly maximum and higher shoot length was observed in treatments T40 (N)+ neem cake @ 25g/pot+ *P. fluorescens* @ 25 g/pot (86.0 cm) followed by T26 (N)+ *P. fluorescens* @ 25 g/pot+ carbofuran @ 100 mg/ pot (84.0), T40 (N)+*P. fluorescens* @ 25 g/pot+

Table 1. Effect of integration of nursery treatments on reproduction and multiplication of rice root-knot nematode, *M. graminicola*

Treatments	No. of galls/seedling	No. of eggs/seedling	FNP/200cc Soil
HT 54 (ST)+Neem (SD)	139.00(11.81)	15163.33(122.85)	805.00(28.36)
HT54 (ST)+Carbosulfan (SD)	117.67(10.88)	10488.33(102.29)	629.67(25.10)
HT54 (ST)+Neem cake @ 50g/pot	74.00(8.62)	4723.33(68.68)	278.67(16.71)
HT 54 (ST)+Mustard cake @ 50g/pot	140.67(11.88)	15418.33(123.97)	821.00(28.67)
HT54 (ST)+*P. fluorescens* @ 50 g/pot	92.67(9.66)	6411.67(79.99)	381.67(19.56)
HT54 (ST)+*T. viride* @ 50 g/pot	45.67(6.79)	3070.00(55.32)	210.67(14.51)
HT54 (ST)+ Carbofuran @ 200 mg/pot	93.33(9.69)	6655.00(81.46)	348.33(18.69)
HT54 (ST)+Cartap hydrochloride @ 200 mg/pot	97.00(9.87)	7168.33(84.36)	412.00(20.31)
HT54(ST)	122.67(11.09)	11285.00(106.14)	665.67(25.81)
Neem (SD)+Carbosulfan (SD)	134.33(11.62)	14230.00(118.57)	778.33(27.91)
Neem (SD)+Neem cake @ 50g/pot	145.00(12.06)	17338.33(131.59)	900.00(30.01)
Neem (SD)+Mustard cake @ 50g/pot	124.00(11.16)	11588.33(107.28)	685.67(26.20)
Neem (SD)+*P. fluorescens* @ 50 g/pot	143.67(12.01)	16773.33(129.35)	861.67(29.36)
Neem (SD)+*T. viride* @ 50 g/pot	105.00(10.28)	7816.67(88.40)	471.00(21.72)
Neem (SD)+Carbofuran @ 200 mg/pot	98.00(9.94)	6890.00(82.83)	405.67(20.16)
Neem (SD)+Cartap hydrochloride @ 200 mg/pot	147.33(12.16)	17783.33(133.24)	923.33(30.40)
Neem (SD)	151.00(12.31)	18783.33(136.81)	953.33(30.88)
Carbosulfan (SD)+ Neem cake @ 50g/pot	35.00(5.98)	1906.67(43.03)	183.00(13.51)
Carbosulfan (SD)+ Mustard cake @ 50g/pot	57.33(7.59)	3526.67(58.98)	234.00(15.29)

Treatments	**No. of galls/seedling**	**No. of eggs/seedling**	**FNP/200cc Soil**
Carbosulfan (SD)+ *P. fluorescens* @ 50 g/pot	40.67(6.42)	2486.67(49.74)	196.67(14.02)
Carbosulfan (SD)+ *T. viride* @ 50 g/pot	30.67(5.60)	1741.67(41.71)	174.67(13.22)
Carbosulfan (SD)+ carbofuran @ 200 mg/pot	42.67(6.56)	2931.67(54.05)	206.00(14.35)
Carbosulfan (SD)+ Cartap hydrochloride @ 200 mg/pot	70.00(8.39)	4360.00(65.59)	257.00(16.03)
Carbosulfan (SD)	103.67(10.20)	7545.00(86.77)	430.00(20.75)
Neem cake @ 50g/pot+ mustard cake @ 50g/pot	72.33(8.52)	4528.33(67.21)	273.00(16.53)
Neem cake @ 50g/pot+ *P. fluorescens* @ 50 g/pot	23.33(4.91)	1196.67(34.49)	97.33(9.87)
Neem cake @ 50g/pot+ *T. viride* @ 50 g/pot	29.33(5.49)	1496.67(38.55)	169.67(13.03)
Neem cake @ 50g/pot+ carbofuran @ 200 mg/pot	37.67(6.19)	2136.67(46.20)	189.00(13.73)
Neem cake @ 50g/pot+ cartap hydrochloride @ 200 mg/pot	88.67(9.45)	5726.67(75.49)	321.67(17.96)
Neem cake @ 50g/pot	109.33(10.49)	8386.67(91.57)	508.67(22.57)
Mustard cake @ 50g/pot+ *P. fluorescens* @ 50 g/pot	32.67(5.78)	1878.33(43.31)	178.33(13.34)
Mustard cake @ 50g/pot+ *T. viride* @ 50 g/pot	90.67(9.55)	5811.67(76.05)	330.67(18.21)
Mustard cake @ 50g/pot+ carbofuran @ 200 mg/pot	53.00(7.31)	3453.33(58.46)	222.00(14.91)
Mustard cake @ 50g/pot+ cartap hydrochloride @ 200 mg/pot	82.33(9.09)	5253.33(72.25)	285.00(16.89)
Mustard cake @ 50g/pot	128.67(11.37)	12493.33(111.67)	703.00(26.53)
P. fluorescens@ 50 g/pot+*T. viride* @ 50 g/pot	66.33(8.13)	4046.67(63.09)	247.33(15.72)
P. fluorescens @ 50 g/pot+ carbofuran @ 200 mg/pot	27.67(5.33)	1393.33(37.26)	147.67(12.16)
P. fluorescens @ 50 g/pot+ cartap hydrochloride @ 200 mg/pot	48.33(9.98)	3223.33(56.43)	217.00(14.74)
P. fluorescens @ 50 g/pot	132.33(11.53)	13106.67(114.27)	766.67(27.70)
T. viride @ 50 g/pot+ carbofuran @ 200 mg/pot	24.67(5.05)	1321.67(36.25)	124.67(11.19)
T. viride @ 50 g/pot + cartap hydrochloride @ 200 mg/pot	62.00(7.88)	3901.67(62.22)	240.00(15.50)
T. viride @ 50 g/pot	110.33(10.54)	8841.67(93.83)	519.33(22.81)
Carbofuran @ 200 mg/pot + cartap hydrochloride @ 200 mg/pot	75.33(8.67)	4996.67(70.52)	277.33(16.67)
Carbofuran @ 200 mg/pot	107.33(10.39)	8185.00(89.95)	494.33(22.25)
Cartap hydrochloride @ 200 mg/pot	113.33(10.68)	9425.00(96.85)	613.33(24.78)
Untreated check	165.33(12.88)	22781.67(150.67)	1306.67(36.03)
CD (P=0.05)	(1.39)	(11.81)	(1.83)
SE(m)	(0.49)	(4.20)	(0.65)
C.V.	(9.37)	(8.94)	(5.61)

Figures in parenthesis are “n transformed values carbofuran @ 100 mg/pot (83.1). However, all the treatments significantly increased the shoot length as compared to untreated check (Table 2). In individual application, carbofuran @ 2.0 kg a.i./ha was superior over untreated check. Data on fresh shoot weight revealed that significantly maximum and higher fresh shoot weight was observed in treatments T40 (N)+ neem cake @ 25g/pot+ *P. fluorescens* @ 25 g/pot (16.2 g) followed by T26 (N)+*P. fluorescens* @ 25 g/pot+ carbofuran @ 100 mg/pot, T40 (N)+*P. fluorescens* @ 25 g/pot+ carbofuran @ 100 mg/pot. In case of dry shoot weight, maximum and significantly higher fresh shoot weight was recorded in treatments T40 (N)+ neem cake @ 25g/pot+ *P. fluorescens* @ 25 g/pot (2.5 g) followed by T26 (N)+*P. fluorescens* @ 25 g/pot+ carbofuran @ 100 mg/pot and T40 (N)+*P. fluorescens* @ 25 g/pot+ carbofuran @ 100 mg/pot.

Maximum and significantly higher root length was found in treatments T40 (N)+ neem cake @ 25g/pot+ *P. fluorescens* @ 25 g/pot (19.8 cm). Significantly maximum and higher fresh root weight was found in treatments of T40 (N)+ neem cake @ 25g/pot + *P. fluorescens* @ 25 g/pot (15.9 g) followed by T26 (N)+ *P. fluorescens* @ 25 g/pot + carbofuran @ 100 mg/pot, T40 (N)+ *P. fluorescens* @ 25 g/pot + carbofuran @ 100 mg/pot. In case of dry root weight, maximum and significantly higher dry root weight was found in treatments T40 (N)+ neem cake @ 25g/pot+ *P. fluorescens* @ 25 g/pot (2.5) followed by T26 (N)+*P. fluorescens* @ 25 g/pot + carbofuran @ 100 mg/pot and T2-T40 (N)+*P. fluorescens* @ 25 g/pot + carbofuran @ 100 mg/pot.

In mid season population development experiment, minimum and significantly lowest number of galls/plant

Table 2. Effect of various treatments on plant growth parameter of rice infested with *M. graminicola*

Treatments	**Shoot Length (cm)**	**Fresh shoot weight (g)**	**Dry shoot weight (g)**	**Root length (cm)**	**Fresh root weight (g)**	**Dry root weight (g)**
T40(N)+ (neem cake @ 25g/pot+ *P. fluorescens* @ 25 g/pot)	86.0	16.2	2.5	19.8	15.9	2.3
T40(N)+ (*P. fluorescens* @ 25 g/pot+ carbofuran @ 100 mg/pot)	83.1	14.9	2.0	18.3	14.8	1.8
T40(N)+ carbofuran @ 200 mg/pot	81.8	14.2	1.9	17.1	14.3	1.5
T40(N)	72.1	9.9	1.5	13.6	11.0	0.5
T26(N)+ (*P. fluorescens* @ 25 g/pot+ carbofuran @ 100 mg/pot)	84.0	15.4	2.2	19.2	15.4	2.0
T26(N)+ carbofuran @ 200 mg/pot	78.7	12.5	1.8	16.6	13.8	1.4
T26(N)	74.6	10.7	1.6	14.2	11.3	0.7
T37(N)+ carbofuran @ 200 mg/pot	77.7	12.0	1.7	15.6	12.1	1.1
T37(N)	70.8	9.2	1.3	13.0	10.5	0.5
Carbofuran @ 200 mg/pot	75.8	11.6	1.7	14.7	11.6	0.9
Untreated check	60.3	7.1	1.1	10.6	8.2	0.2
CD at 5%	9.5	2.4	0.6	3.7	3.2	0.5
SE(m)	3.2	0.8	0.2	1.3	1.1	0.2
C.V.	7.3	11.5	21.5	13.9	15.0	23.0

N -Nursery application; Initial nematodes population (INP): 292/200 cc soil was recorded in treatments T40 (N)+ neem cake @ 25g/pot+*P. fluorescens* @ 25 g/pot combination (45.7). This was statistically at par with of that of T26 (N)+ *P. fluorescens* @ 25 g/pot+carbofuran @ 100 mg/pot and T40 (N)+ *P. fluorescens* @ 25 g/pot+carbofuran @ 100 mg/pot. However, all the treatments significantly decreased the number of eggs/plant as compared to untreated check (Table 3). Minimum and significantly lowest number of eggs and FNP were observed in treatments combination of T40 (N)+ neem cake @ 25g/pot+ *P. fluorescens* @ 25 g/pot (3961.7 and 173.0) followed by T26 (N)+ *P. fluorescens* @ 25 g/pot+ carbofuran @ 100 mg/pot. Minimum RF was observed in treatment T40 (N)+ neem cake @ 25g/pot+*P. fluorescens* @ 25 g/pot followed by T26 (N)+*P. fluorescens* @ 25 g/pot+carbofuran @ 100 mg/pot combination. The value of RF was directly proportional to number of eggs/plant and final nematode population in the soil (Fig. 2).

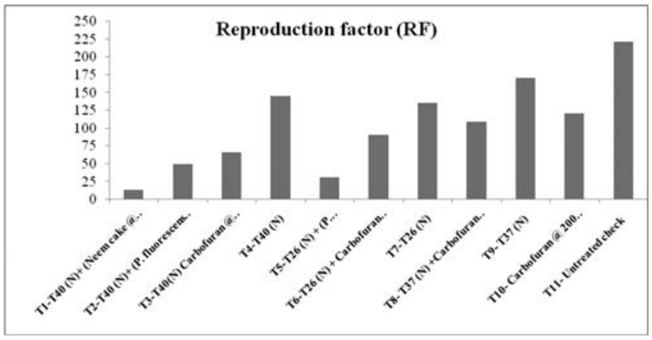


Fig. 2. Reproduction factor of rice root-knot nematode, *M. graminicola* in rice under screen house condition

Field condition: Integration of nematode management practices was carried out by using nursery treated seedling transplanted into treated fields. Three best nursery treatments along with one check were combined with field treatments (Table 4). This experiment was continued up to harvesting of rice under field condition. Maximum and significantly higher yield was found in

Table 3. Effect of various treatments on reproduction and multiplication of *M. graminicola*on rice

Treatments	No. of galls/plant	No. of eggs/plant	FNP/200cc soil
T40(N)+ neem cake @ 25g/pot + *P. fluorescens* @ 25 g/pot	45.7(6.8)	3961.7(62.6)	173.0(13.2)
T40(N)+ *P. fluorescens* @ 25g/pot+ carbofuran @ 100 mg/pot	70.7(8.5)	14192.7(118.5)	322.7(18.0)
T40(N)+ carbofuran @ 200 mg/pot	83.3(9.1)	18610.0(136.0)	390.0(19.8)
T40(N)	150.3(12.3)	41473.7(203.5)	887.0(29.8)
T26(N)+ *P. fluorescens* @ 25g/pot+ carbofuran @ 100 mg/pot	63.0(7.9)	8815.7(93.7)	258.0(16.1)
T26(N)+ carbofuran @ 200 mg/pot	96.0(9.8)	26182.0(161.7)	458.3(21.4)
T26(N)	131.7(11.5)	38948.3(197.2)	719.3(26.8)
T37(N)+ carbofuran @ 200 mg/pot	103.0(10.2)	31204.3(176.4)	595.0(24.3)
T37(N)	162.7(12.7)	48923.3(221.1)	1098.3(33.1)
Carbofuran @ 200 mg/pot	110.3(10.5)	34712.3(186.2)	650.0(25.5)
Untreated check	204.3(14.3)	63505.3(251.9)	1326.7(36.3)
CD at 5%	(1.8)	(17.7)	(3.4)
SE(m)	(1.8)	(17.7)	(3.4)
C.V.	(0.6)	(6.0)	(1.2)

Figures in parenthesis are “n transformed values; Initial nematodes population (INP): 292/200 cc soil; N -Nursery application treatments of T40 (N)+ DSP (51.25 q/ha) followed by T26 (N)+ DSP, T37 (N)+DSP (50.17). In individual application, carbofuran @ 2.0 kg a.i./ha was superior over untreated check and T26 (N) and T37 (N) on at par with carbofuran @ 2.0 kg a.i./ha. In this experiment, minimum and significantly lowest nematode reproduction and multiplication was found in treatments T40 (N)+DSP. This was statistically at par with of that of T26 (N)+ DSP. In individual treatments, minimum and significantly lowest nematode reproduction and multiplication were recorded in carbofuran @ 2.0 kg a.i./ha (Table 4). It is evident from the data in Table 4 that number of eggs/plant in all the treatments reduced significantly as compared to untreated check. Minimum and significantly lowest number of galls per plant was found in T40 (N)+ DSP (29.7) and this was statistically at par with of that of T40 (N) with DSP. The treated nursery treatments T37 (N) was statistically at par from T40 (N) and significantly different from T26 (N). The maximum number of eggs/plant was found in untreated check. In case of final nematode population, minimum and significantly lowest final nematode population was found in treatments T40 (N)+DSP (138.3) followed by T26 (N)+DSP, T37 (N)+DSP (203.3).

Table 4. Integrated management of *Meloidogyne graminicola* in transplanted rice

Treatments	Grain yield (q/ha)	No. of galls/ plant	No. of eggs/ plant	FNP/200 cc soil
DSP+carbofuran @ 2.0 kg a.i./ha	48.17	58.7(7.7)	5638.3(75.1)	268.3(16.4)
T40 (N)+DSP	51.25	29.7(5.5)	3551.7(59.6)	138.3(11.8)
T26 (N)+DSP	50.17	41.3(6.5)	4308.3(65.6)	203.3(14.3)
T37 (N)+DSP	48.92	53.7(7.4)	4873.3(69.8)	238.3(15.4)
DSP	38.42	93.7(9.7)	14880.0(121.8)	855.0(29.2)
T40 (N)+carbofuran @ 2.0 kg a.i./ha	41.92	78.7(8.9)	9613.3(98.0)	546.7(23.4)
T26 (N)+carbofuran @ 2.0 kg a.i./ha	43.08	75.3(8.7)	8123.3(89.9)	491.7(22.2)
T37 (N)+carbofuran @ 2.0 kg a.i./ha	44.58	74.7(8.7)	7590.0(87.1)	448.3(21.2)
carbofuran @ 2.0 kg a.i./ha	41.58	81.7(9.1)	10078.3(100.1)	586.7(24.2)
T40 (N)+neem cake @ 1t/ha+*P. fluorescens* @ 2.5 kg/ ha	46.83	68.3(8.3)	6258.3(79.1)	383.3(19.6)
T40 (N)+*P. fluorescens* @ 2.5 kg/ha+carbofuran @ 1.0 kg a.i./ha	47.42	62.0(7.9)	6081.7(78.0)	331.7(18.2)
T40 (N)	39.00	91.7(9.6)	13123.3(114.4)	838.3(29.0)
T26 (N)+*P. fluorescens* @ 2.5 kg/ha+carbofuran @ 1.0 kg a.i./ha	45.42	71.0(8.5)	6728.3(82.0)	425.0(20.6)
T26 (N)	40.92	83.0(9.1)	10646.7(102.9)	711.7(26.7)
T37 (N)	40.58	86.0(9.3)	11593.3(107.4)	768.3(27.7)
Untreated check	37.92	134.7(11.6)	24593.3(156.8)	1085.0(32.9)
C.D. at 5 %	7.1	(1.1)	(10.0)	(1.9)
SE(m)	2.5	(0.4)	(3.4)	(0.7)
C.V.	9.7	(7.5)	(6.4)	(5.2)

Figures in parenthesis are “n transformed values; Initial nematodes population (INP): 292/200 cc soil; N -Nursery application

For the management of rice root-knot nematode, a chemical method is expensive and hazardous which are causing ecological imbalance in nature. Though a number of management options are available to minimize the nematode damage individually but their integration is lacking under field conditions to lower down nematode density for a longer time span. Integrated management of nematode is need of the hour. Under this strategy, these components were applied starting from laboratory to nursery and lastly to the end point of rice cultivation in the main field. Kumar *et al.* (2018) reported that the PGPR, *Bacillus* strain of RKB-91 were the most effective bacteria at S/2 concentration in both cultures and CFs respectively at 48h, followed by *Bacillus* spp. RKB-65. The present work also facilitated the comparative analysis of the effect of CFs on mortality of *M. graminicola* at different concentrations (1:5, 1:10, 1:20, 1:40 and 1:80). Other related studies revealed that presence of toxic metabolites in the CFs of *P. fluorescens*, *T. viride*, *B. subtilis* and *Paecilomyces lilacinus* that induced significant levels of mortality of banana lesion nematodes (Shanthi and Rajendran, 2010). According to Using plant extracts in controlling PPNs has shown by several authors (Satyal *et al.,* 2012). Per cent of *M. graminicola* viable J2 was decreased with increased in exposure time from 24-196h in each concentration. The present study also revealed that mortality rate increased with increase in exposure period and concentration of the aqueous extracts.

In rice nursery experiment, minimum and significantly lowest nematode reproduction and multiplication was obtained in treatments like neem cake @ 50g/pot + *P. fluorescens* @ 50 g/pot followed by *T. viride* @ 50 g/pot + carbofuran @ 200 mg/pot. The present results were consonance with the findings of Ziaul (2013) who reported that the soil application and root dip of *P. fluorescens* or *T. harzianum* + carbofuran was found most effective in increasing yield of rice and suppressed the gall formation, egg mass production and soil population of *M. graminicola.* Priya (2015) reported that *T. viride* had given lowest nematode population, least galling, and higher yield followed by *P. fluorescens* and *B. subtillis*. Pathak and Kumar (2003) observed that *T. viride* was more effective in suppression of nematode population. Similar alleviation of nematode disease intensity has been exhibited by a number of rhizobacteria. *P. fluorescens* and *B. megaterium* were found to reduce root galling and penetration of J2 of *M. graminicola* in rice seedlings (Anitha and Rajendran, 2005a; Padgham *et al.,* 2005). Another reason of lesser nematode infection is the bacterial growth on root surface. Once the bacterium occupies the root surface, it grows by using the root exudates and excretes various metabolites. Thus, a micro-chemical environment, not favourable for the penetration of PPNs is developed causing diminished the attractiveness among nematodes towards root. Ammonium ions produced by rhizobacteria such as *A. chroococcum* were reported to be strong repellent of *M. incognita* juveniles (Castro *et al.,* 1990). Reduced nematode penetration and root galling in rhizobacteria-inoculated seedling is probably related to the nematoxic metabolites produced by these bacteria, which reduced egg hatch and motility of juveniles. Thus, fewer J2 were available to invade host plant roots, producing fewer root galls. Further, disruption in the movement of infective juveniles due to fatty acids (Djian *et al.,* 1991), reduced attractiveness by root tips due to ammonia (Castro *et al.,* 1990) was excreted by these bacteria. The ability of rhizobacteria to colonize root surface probably was delayed and reduced root invasion and egg deposition, which resulted into reduced nematodes population and thus affecting the total reproduction in comparison to the untreated check.

The results found by Javed *et al.* (2007) and Jain and Gupta (1998) are in total conformity with the results of the present studies. Organic amendments, especially neem cake suppressed the *M. graminicola*. The nematode population levels under organic amendment treatments might have changed due to changes in soil properties, nutrients released to plants increase in predators and parasitic microorganisms, toxic metabolites released from organic amendment's breakdown or health of the host crop (Akhtar and Malik, 2000). Ammonia released during decomposition of the amendments were toxic to PPNs including root-knot nematodes (Khan *et al.,*1974; Mian *et al.,* 1982) which might be partially involved in suppressing *M. graminicola* and reducing root-knot development in rice roots. Moreover, organic amendments might have improved the soil structure, fertility and water holding capacity of soil so that general plant health and tolerance to PPNs attack was improved (Akhtar and Malik, 2000). Therefore, incorporation of neem cake (50 g/pot) can improve rice seedling health in *M. graminicola* infested soil. Since, neem cake would be a low cost nematode management alternative for resource poor rice growing farmers.

The efficacy of organic amendments against PPNs might be attributed to the stimulation of micro-organisms that are capable of parasitizing eggs or other developmental stages of PPNs (Gul *et al.,* 1990). These substances may also accelerate proliferation of the microbial forms which also produce metabolites toxic to nematodes. Organic acids and ammonical nitrogen are produced during the microbial decomposition of organic matters in soil resulting in an increased pH value and stimulates the process of nitrification and production of nitrates which are toxic to PPNs present in the soil (Rodri-quez-Kabana, 1986). This is perhaps obvious because, being a bio-degradable chemical, the efficacy of carbofuran on *M. graminicola* declines 20 days after its application in rice (Krishnaprashad and Rao, 1982). On the other hand, use of organic amendments along with nematicides proved to be most effective for managing *Heterodera oryzae* in rice (Prasad *et al.,* 1986). Carbofuran is a systemic nematicide and is absorbed by plant roots and its root-dip application has proved effective against large number of PPNs, especially endoparasites (Prasad *et al.,* 2006). Soil application of carbofuran probably suppressed the nematode juveniles (J2) present in the root zones and attacking the fresh lateral roots.

Similar results were also found by Sharma and Pandey (2009), when bio-agents were applied in the soil, they enhanced plant growth parameters. These bio-agents were self-propagating under favourable conditions and therefore, may remain in soil for long period and also produce enzymes such as chitinases which are capable of rupturing nematode egg shells contributing to infect the nematodes (Gortari and Hours, 2008). *T. viride* are well proven and are considered as an economically viable and eco-friendly alternative to chemical nematicides against root-knot nematode in different crops (Ramakrishnan and Rajendran, 2010). It was discussed in nursery experiment that neem cake boosted the growth of rice seedlings which gave the best results in terms of highest plant growth parameters when combined with other treatments. The effectiveness of *P. fluorescens* in enhancing plant growth parameters may be attributed to the property of these bio-agents which may benefit plant growth by providing growth regulators or by producing toxic metabolites which inhibit PPNs and exclude other deleterious microorganisms. In its addition, PGPR strains usually have been found to increase the root length and root biomass and this better developed root system may increase the mineral uptake in plants (Khalid *et al.,* 2004). The application of cartap hydrochloride was least effective in enhancing the plant growth parameters as well as in the reduction of nematode parameters due to its low dose as well as ineffective active ingredient. Minimum and significantly lowest number of galls was recorded in treatmentsT40 (N)+neem cake @ 25g/pot+ *P. fluorescens* @ 25 g/pot combination (Table 3). This was statistically at par with of that of T26 (N)+ *P. fluorescens* @ 25 g/pot+ carbofuran @ 100 mg/pot and T40 (N)+ *P. fluorescens* @ 25 g/pot+ carbofuran @ 100 mg/pot. Significantly minimum and lowest number of eggs was found in treatments T40 (N)+ neem cake @ 25g/pot+ *P. fluorescens* @ 25 g/pot. However, all the treatments significantly decreased the number of eggs/ plant as compared to untreated check. Minimum and significantly lowest number of eggs was observed in treatments combination of T40 (N)+ neem cake @ 25g/ pot+ *P. fluorescens* @ 25 g/pot followed by T26 (N)+

P. fluorescens @ 25 g/pot+ carbofuran @ 100 mg/pot. Minimum RF was observed in treatments T40 (N)+ neem cake @ 25g/pot+ *P. fluorescens* @ 25 g/pot followed by T26 (N)+ *P. fluorescens* @ 25 g/pot+ carbofuran @ 100 mg/pot combination. The value of RF was directly proportional to number of eggs/plant and final nematode population in the soil (Fig 2).

Our results are also in an agreement with those of Anitha and Rajendran (2005b) who reported that *P. fluorescens* reduced nematode population on roots as compared to other bio-control agents. *P. fluorescens* are natural inhabitants on the root surface and primary consumers of root exudates rich in amino acids which are converted to ammonia to maintain a micro-zone around the growing roots that would be suppressive to pathogens including PPNs. The reduction in number of galls/plant may be due to the failure of majority of the encumbered juveniles to penetrate the host root. The growth promoting and PPNs managing capabilities of neem cake and *T. viride* has been discussed in previous experiments but need further elaborations. In the present study, the pronounced effect of *T. viride* might be due to production of antibiotics in the soil and also due to competition with nematodes for space etc. But when neem cake was integrated with carbofuran, it also gives effective control. Reduction in infestation of rice by *M. graminicola* has been also observed by several workers (Sharma-Poudyal *et al.,* 2002) and improvement in rice plant growth by addition of different organic amendments. Our results are in conformity with those of Kumar and Khanna, 2006. Javed *et al.,* 2007 suggested that neem cake might possess active ingredients that show differential effects on the development of *Meloidogyne* spp. Among the organic amendments, neem cake treated plants had minimum nematode population which are in agreement with the findings of Prasad *et al.,* 2005, who recorded that neem cake effectively reduced the nematode population as compared to other organic amendments. The reduction of nematode population in soil amended with organic materials (neem cake) may be due to the marked increase in number of fungi, nematodes, mites and other microorganisms that are parasitic or predacious on different stages of PPNs or due to the stimulation of selected micro-organisms capable of decomposing the proteins or other materials that make up nematode cuticle or other structures. These results may be attributed to the fact that application of neem cake not only established the growth of antagonistic fungi in the rhizosphere, but also reduced the PPNs infection significantly. With reference to reduction of nematode population by neem cake similar results were recorded by Prasad *et al.,* 2005 who reported that the neem cake treated plants had minimum nematode population. The action of soil micro-organisms on the organic materials during the decomposition process can produce a wide range of chemicals such as ammonia, nitrites, hydrogen sulphide, organic acids and enzymes. These chemicals are known to possess nematicidal properties that affect egg hatch and juveniles mortality of *M. graminicola*.

In the present investigation, two deep summer ploughing (DSP) were applied at an interval of ten days period in June which reduced *M. graminicola* population significantly after every ploughing. Khan and Singh (2008) recorded that DSP minimized PPNs and saprozoic nematodes in plots as comparison to control fields. The defence enzymes cause biochemical and physiological changes which are directly inhibitory to PPNs (Paul and Kumar, 2003). Other studies also reported that suppression of nematode multiplication by *P. fluorescens* was due to its capability of altering root exudates which could alter nematode behavior and lower nematode population in root system as found by Oostendrop and Sikora, 1989. Pankaj *et al.,* 2010 also reported that soil application of carbofuran, phorate, isazophos, cartap, quinalphos significantly reduced galling of *M. graminicola* at 1 kg a.i/ha or above and the greatest reduction in galling occurred with carbofuran at 2 kg a.i./ ha. Data on rice grain yield, minimum and significantly higher grain yield of rice was recorded in treatments of T40 (N)+DSP followed by T26 (N)+DSP, T37 (N)+DSP,

DSP+carbofuran @ 2.0 kg a.i./ha. The increase in rice grain yield was proportional to the nematode parasitism, i.e., as the nematode reproduction increased, the yield was correspondingly decreased. DSP helps to reduce the initial economic injury level of *M. incognita*, which is very important as damage to crop yield is depend upon the initial nematode population (Pi). It is notable in the present investigation that still it was more than the economic threshold level (292 j_2/200 cc soil) at the time of transplanting of rice. Three DSP (May-June) at the interval of two weeks caused reduction in *M. incognita* population density and dropped the initial inoculums level to 2.4 j_2/g soil (Satyandra *et al.,* 2011). The increased yield in the plants treated with *P. fluorescens* may be attributed to the increased plant growth due to enhanced root colonization by the growth promoting bacteria due to the suppression of the PPN population in soil and root.

CONCLUSION

An ideal approach for management of rice root-knot nematode is to integrate the different management practices so as to suit the regional conditions. The increasing interest in neem and its products in recent years have resulted in the development of cheaper, safer and eco-friendly nematicides which can be used alone and in combinations with other components of nematode management such as bio-control agents. Such integrated methods (combination of treated nursery with neem cake and *P. fluorescens* along with deep summer ploughing in the main field before transplanting) of nematode management will be much safer, economical, effective and highly practicable. This work is a contribution to the knowledge of the current situation in the bio-pesticides sector where bacterial (PGPR) formulations of different types are developed and traded. Such findings when included in package and practices of the university/ state will be of immense benefits for the farmers because rice, being a most important crop, is highly susceptible to rice root-knot nematode.

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Grafting for the Management of *Meloidogyne incognita* in Brinjal

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An attempt was made for the management of root knot nematode, *Meloidogyne incognita* in brinjal through grafting. Thirteen *Solanum* species were screened against the *M. incognita*. Of these, *S. torvum* and *S. khasianum* showed resistant and moderately resistant reaction against *M. incognita* and remaining species showed highly susceptible reactions against *M. incognita. S. torvum* and *S. khasianum* were taken as rootstocks. Liliya and Kokila were taken as scions. Grafting of susceptible and cultivated hybrids, Liliya and Kokila were grafted on resistant rootstock *S. torvum* and on moderately resistant rootstock *S. khasianum.* Cleft grafting method was adopted for grafting. The highest success rate of grafting was recorded on Liliya grafted on *S. torvum*, followed by Kokila grafted on *S. torvum*. Liliya and Kokila grafted on *S. torvum* showed maximum reduction in number of galls, egg mass per root system, final nematode population and rate of reproduction with increased number of fruits per plant, fruit weight and total fruit yield per ha followed by Liliya and Kokila grafted on *S. khasianum* grafted plants. Delayed flowering and fruiting was observed in grafted plants as compared to non grafted plants.

Key words: Grafting, *Solanum* spp. and *Meloidogyne incognita*

Brinjal (*Solanum melongena* L.) is known as "King of Vegetables" and belongs to the family Solanaceae. It is also called as Egg plant and is mostly cultivated in the tropical and temperate regions of the world. Brinjal fruit is primarily consumed as cooked vegetable and it contain proteins, vitamins, fibre, carbohydrates, fats and some other nutrients (Anonymous, 2007). Brinjal has been cultivated in India for last 4,000 years, although it is often thought of as a Mediterranean or mid-Eastern vegetable. In India, 13154 ('000 MT) brinjal production has been recorded while that in Assam it is 324.97 ('000 T) during 2021-22 (Anonymous, 2021).

Brinjal is subjected to the attack by number of pests affecting leaves, stems, fruits and roots. Among the different pests, root-knot nematode, *Meloidogyne incognita* is one of the most destructive species (Dhawan and Sethi, 1976) and causes 10.00-42.00 per cent loss in yield of brinjal under Indian conditions (Anonymous, 2017). In Assam, 17.42% loss in yield of brinjal due to root-knot nematode, *M. incognita* has been recorded (Anonymous, 2013). There are different options to manage this nematode. The use of chemical nematicides has been and well become restricted due to ecological and human health hazards. Among the non-chemical methods for managing this nematode, the use of resistant cultivars is one of the effective and eco-friendly methods, but resistant varieties of eggplant are not that available in the country.

A number of wild relatives of *Solanum* spp. have been recorded to be resistant to root knot nematodes (Rahman *et al*., 2002). Some of these resistant *Solanum* spp. have been used as rootstocks and grafted with scions of compatible eggplant to manage the root-knot nematodes (Rahman *et al*., 2002). To strengthen grafting technology, selection of rootstocks resistant to root-knot nematode and efficacy for grafted eggplants against the root knot nematode disease is of urgent necessity. Therefore, the present study on "grafting for the management of *M.incognita* in brinjal was under taken to identify the resistant rootstocks against *M. incognita* and not only to evaluate the grafting compatibility between resistant rootstocks and cultivated eggplant varieties but also to determine the yield performance of the grafted plants under field conditions.

MATERIALS AND METHODS

The present investigation was carried out into two different aspects, A. Screening of *Solanum* species against *Meloidogyne incognita* and B. Grafting for the management of *Meloidogyne incognita* in brinjal. The details of the materials and methods are described below:

Screening of *Solanum* species against *Meloidogyne incognita*

A pot experiment was conducted in the Net house, Department of Nematology, AAU Jorhat-13 to screen *Solanum* species against *M. incognita.*

Thirteen seeds of *Solanum* species *viz., Solanum torvum, S. violaceum, S. khasianum, S. melongena* varieties *viz.,* Kuchia, HAB 901, BSS-793, DEB 701 and the hybrids *viz*., Liliya and Kokila were obtained from the Department of Horticulture, Assam Agricultural University, Jorhat. The other hybrids namely, KSP-1229, KSP-1072, KSP-1164 and brinjal variety, PPL were obtained from Jorhat market.

The seeds were soaked in 100 PPM GA_3 solution for 24 hrs before sowing to facilitate good and early germination. Treated seeds were sown in plastic protrays containing mixture of coco-peat and vermicompost in 1:1 ratio and later 3weeks old seedlings were transplanted in earthen pots (500 cc capacity) containing sterilized soil mixture (Soil , dried cow dung and sand in the ratio of 2:1:1) in net house conditions at Department of Nematology, Assam Agricultural University, Jorhat. After 3days of transplanting, pots were inoculated with freshly hatched second stage juveniles (J_2) of *M. incognita* at the rate of $1J_2$/cc of soil.

Treatment Details

The experiment was laid down in the completely randomized design (CRD) with 13 treatments and 4 replications. The details of the treatments are as follows:

T_1= *Solaum torvum,* **T_2**= *S. melongena* var. Kuchia, **T_3**= *S. melongena* var. HAB 901, **T_4**= *S. violaceum,* **T_5**= *S. khasianum,* **T_6**= *S. melongena* Hyb. KSP-1229, **T_7**= *S. melongena* var. BSS-793, **T_8**= *S. melongena* Hyb. *KSP-1072,* **T_9**= *S. melongena* Hyb. KSP-1164, **T_{10}**= *S. melongena* var. DEB 701, **T_{11}**= *S. melongena* var. PPL, **T_{12}**= *S. melongena* Hyb. Liliya, **T_{13}**= *S. melongena* Hyb. Kokila. The DEB-701 was used as susceptible check (SC).

Observation recorded

After 45 days of inoculation, plants were uprooted carefully, roots were washed free of soil and data on number of galls per root system, egg masses per root system, final nematode population in soil were recorded. Reaction of the plants for resistance/susceptibility against *M. incognita* was worked out on the basis root knot index (0-5 rating scale) given by Sasser *et al*. (1984) based on the number of galls per root system in which **1**= No galls (Highly resistant), **2**=1-10 galls per root system (Resistant), **3**= 11-30 galls per root system (Moderately resistant), **4**= 31-100 galls per root system (Susceptible) and **5**= >100 galls per root system (Highly susceptible).

Grafting for the management of *Meloidogyne incognita* in brinjal

A field experiment was conducted at Experimental Farm, Department of Horticulture, AAU, Jorhat, during 2021.

From screening trail results as described above, *Solanum torvum* (showing resistant reaction against *M. incognita*) and *S. khasianum* (showing moderately resistant reaction against *M. incognita*) were selected as rootstocks for grafting. The highly susceptible hybrids *i.e*., Liliya and Kokila were selected as scions for their popularity and acceptance among the farmers of Assam. The rootstock seeds were sown 4 weeks before the scion seeds. Before sowing, the seeds were soaked in 100 PPM GA_3solution for 24hr before sowing to facilitate good and early germination. Seeds were sown in plastic protrays containing mixture of coco-peat and vermicompost in 1:1 ratio and later transplanted in poly bags.

The 20-25 days old scion *i.e*, Liliya and Kokila were grafted on two different rootstocks namely *S. torvum,* and *S. khasianum* (50-60 days old) by using cleft grafting. Grafting was carried out in net house at Horticulture farm, AAU, Jorhat where scion and rootstock were cut at 45^0 and joined by using polythene wrapper. The grafts were kept in net house and moistened with light irrigation. The grafting percentage of the successful grafts was estimated by using formula given below:

$$\text{Grafting percentage} = \frac{\text{Successful grafted seedlings}}{\text{Total no. of seedlings grafted}} \times 100$$

Two weeks old grafted plants were used for the field experiment.

Preparation and layout of experiment

The experimental field was brought into the tilt by one ploughing, 2-3 harrowing and 1 levelling. The stubbles of the previous crop, weeds and grasses were removed. Plot size of 3.50m length and 3.00m width were prepared. A spacing of 70 cm (row to row) and 60 cm (plant to plant) was maintained. The experiment was laid in randomized block design (RBD) with 6 treatments and 4 replications**.** Before layout of the experiment, the initial population of *M. incognita* in soil was estimated and same was recorded 253.00J_2/ 200cc of soil.

The details of the treatments are given below:

T_1= Liliya grafted on *S. torvum*

T_2= Liliya grafted on *S. khasianum*

T_3= Kokila grafted on *S. torvum*

T_4= Kokila grafted on *S. khasianum*

T_5= Liliya (scion-1)

T_6= Kokila (scion-2)

Maintenance of field experiment and observations

Field operations like weeding, application of manure, fertilizers, insecticides, irrigation and harvesting were done according to package of practices given by AAU., Jorhat. The observations like days to flowering and fruiting, fruits per plant, fruit weight (g), fruit yield per plant (kg), total fruit yield (q/ha) whereas, number of galls and egg masses, final nematode population and rate of reproduction were recorded after harvesting of crop. Furthermore, plant height (cm) was recorded at 30, 60 and 90 days after planting (DAP).

Statistical analysis

The data collected were subjected to statistical analysis by Fischer's method of analysis of variance. Significance of variance among the data were calculated out by calculating the "F" value and comparing it with tabulated value of "F" at 5 % level of probability. Further the treatments were compared among themselves by calculating critical difference (CD) at 0.05 probabilities by using Duncan's multiple range test (DMRT)

RESULT AND DISCUSSION

The results of the pot experiment reveal that (Table 1) all the tested *Solanum* species showed varying degrees

Table 1. Reaction of Solanum spp. against *Meloidogyne incognita*

Sr. No.	*Solanum* spp.	Number of galls	Number of egg masses	RKI	Reaction	FNP /500 cc soil	Rate of reproduction
1.	*Solaumtorvum*	7.00e	5.75g	2.00d	R	214.07d	0.42c
2.	Kuchia	118.75cd	27.75def	5.00a	HS	827.67 bc	1.65b
3.	HAB 901	190.75a	70.00a	5.00a	HS	1146.70ab	2.29a
4.	*S. violaceum*	107.75cd	24.00ef	4.50b	HS	727.37bcd	1.45bc
5.	*S. khasianum*	24.75e	11.00fg	3.00c	MR	357.68cd	0.71c
6.	KSP-1229	123.75bcd	64.75ab	4.25b	HS	1440.19a	2.87a
7.	BSS-793	150.50abc	38.50cde	5.00a	HS	773.21bc	1.54b
8.	*KSP-1072*	92.50cd	36.00 cde	4.50b	HS	1069.31ab	2.13a
9.	KSP-1164	93.75cd	37.50cde	4.50b	HS	1056.43ab	2.10a
10.	DEB 701 (SC)	115.00d	32.75 cde	5.00a	HS	1197.33ab	2.39a
11.	PPL	178.25ab	42.00cd	5.00a	HS	914.62ab	1.82ab
12.	Liliya	145.00abcd	38.25cde	5.00a	HS	1216.52 ab	2.42a
13.	Kokila	177.50ab	48.25bc	5.00	HS	1100.37ab	2.19a
	S.Ed (±)	19.84	6.24	0.24		185.77	0.51
	CD (P=0.05)	56.75	17.87	0.49		531.48	1.05

Figures within parenthesis are square root transformed values
R= Resistant, MR = Moderately Resistant, HS = Highly Susceptible, SC = Susceptible Check

of resistant and susceptible reactions against *M. incognita.* Out of thirteen *Solanum* species, *S. torvum* was found to be resistant whereas, *S. khasianum* was found to be moderately resistant against *M. incognita*. However, the rest of the *Solanum* species like Kuchia, Liliya, Kokila, HAB-901, KSP-1229, BSS-793, KSP-1072, KSP-1164, PPL, DEB-701 and *S. Violaceum* were found to be highly susceptible against *M. incognita.* Seren and Devran, (2021) also reported that *S. torvum* to be resistant to *M. incognita.* Ali *et al.* (1992) also screened 8 wild *Solanum* species like *S. khasianum, S. torvum, S. toxicarium, S. indicum, S. integrifolium, S. surattense, S. Mammosum* and *S. Sisymbriifolium* against *M. incognita* and reported that *S. torvum*, *S. Khasianum* and *S. Toxicarium* to be resistant to *M.incognita*. Unlike their findings wherein *S. Khasianum* was reported to be resistant against *M.incognita,* in the present investigation, *S. khasianum* was found to be moderately resistant which might be due to effect of environment and differences in genetic make-up among the *Solanum* species (Kamran *et al.,* 2012). Rahman *et al*. (2002) screened 6 wild *Solanum* species, *S. santwongsci*, *S. integrifolium*, *S. indicum, S. torvum, S. sisimbriifolium* and *S. khasianum* against *M. incognita* and reported *S. torvum* and *S. sisimbriifolium* found to be resistant whereas, *S. khasianum* showed moderately resistant reaction against *M. incognita*, thus confirm the results of the present investigation.

In the present study, it was observed that *S. torvum* and *S. khasianum* showed <1 rate of reproduction of *M. incognita*. However, the other *Solanum* species were showed >1 rate of reproduction of *M. incognita* and which were found to be highly susceptible to *M.*

incognita. Similarly, Ocal and Devran (2019) observed that *S. torvum* cv. Hawk was resistant to *M. incognita* with the gall index 0.8 and the reproduction factor 0.034 after 8 weeks with the initial inoculum of 1000 J_2 per pot. Williamson and Kumar (2006) reported that, in the moderately resistant plants *M. incognita* fail to produce enough functional feeding sites in the host after invasion due to hypersensitive responses from the host which leads in failure to develop as reproducing females. From the above experiment, *S. torvum* (showing resistant reaction against *M. incognita*) and *S. khasianum* (showing moderately resistant reaction against *M. incognita*) were selected as rootstocks for grafting. The highly susceptible hybrids *i.e*., Liliya and Kokila were selected as scions for their popularity and acceptance among the farmers of Assam.

As far as grafting percentage (Table 2) is concern, maximum percentage of success of grafting was recorded in Liliya grafted on *S. torvum* followed by Kokila grafted on *S.torvum* and least was observed in Liliya grafted on *S. khasianum*, followed by Kokila grafted on *S. khasianum*. Low compatibility of *S. khasianum* rootstock may be due to low callus formation between rootstock and scion which leads to low survival rate (Kumar *et al.,* 2017). Ali *et al.* (1994) also observed 70-80 % success rate when eggplant grafted with non tuberous *Solanum* rootstocks. Maximum survival rate of grafted plants with *S. torvum* rootstock was reported by Rahman *et al.* (2002). Kumar *et al.* (2017) also observed 67.50% and 66.50% compatibility when Pusa hybrid-6 and Pusa Shyamala grafted on *S. torvum* which are similar to the results of the present investigation where success rate of 65.29 % in Liliya grafted on *S. torvum* and 63.88 % in Kokila grafted on *S.torvum* were recorded.

Table 2. Grafting successes of brinjal hybrids with wild *Solanum* rootstocks

Brinjal hybrids	***Solanum* rootstocks**		
	S. torvum	***S. khasianum***	**Mean (%)**
Liliya	65.29	53.42	59.35
Kokila	63.88	56.00	59.94
Mean (%)	64.58	54.71	59.64

The results of the field experiment reveal that there were significant differences in plant height (Table 3) among the treatments at all the stages of observation *i.e.,* at 60 and 90 days after planting (DAP). At all the stages of observation, the rate of growth was found to be more in grafted plants as compared to non grafted plants of Liliya and Kokila. This might be because of efficient absorption of water and nutrients by the wild *Solanum* rootstocks (Young, 1989).

Data regarding days to flowering and fruiting (Table 3) reveal that non grafted plants bloomed earlier than grafted plants. Early flowering and fruiting was recorded in Kokila (T_6) and Liliya (T_5). In grafted plants, the minimum days to flowering and fruiting was recorded in Kokila grafted on *S.torvum* (T_3) followed by Liliya grafted on *S. torvum* (T_1) and maximum days to flowering and fruiting was recorded in Liliya grafted on *S. khasianum* (T_2) followed by Kokila grafted on *S. khasianum* (T_4). Kumar *et al.* (2017) reported that, in grafted plants, the formation of graft union between rootstock and scion are necessary for the translocation of food materials from root system to apical parts. It is delayed by around a week that leads to the delayed flowering in grafted plants. Further, because of delayed graft union, scions of the grafted plants took longer time for flowering and fruiting (Rahman *et al.,* 2002). Similar results of delayed flowering in grafted plants were recorded by Matsuzoe *et al.* 1990; Ali *et al.* 1994; Suthar *et al.* 2005 and Kumar *et al.*, 2017.

The data on fruit parameters (Table 4) *viz.,* number of fruits per plant, fruit weight, fruit yield per plant and total fruit yield/ha reveal that in general, significantly higher fruit parameters were recorded in all the treatments with grafted plants as compared to the treatments with

Table 3. Plant height, days to flowering and fruiting in grafted and non grafted plants

Treatments	Plant height (cm)			Days to	
	30 DAP	60 DAP	90 DAP	Flowering	Fruiting
T_1: Liliya grafted on *S. torvum*	33.14b	59.68a	71.67a	65.25b	76.25b
T_2: Liliya grafted on *S. khasianum*	30.27b	52.32c	64.37b	69.93a	80.31a
T_3: Kokila grafted on *S. torvum*	37.06a	58.06a	70.69a	64.50b	75.06b
T_4: Kokila grafted on *S. khasianum*	30.67b	52.21c	60.57b	69.56a	80.00a
T_5:Liliya (scion-1)	30.07b	42.03d	51.42c	60.75c	70.68c
T_6:Kokila (scion-2)	28.69c	42.37d	53.09c	59.37c	69.75c
S.Ed (±)	1.57	2.58	2.03	1.13	0.65
CD (P=0.05)	3.37	5.55	4.37	2.43	1.39

Table 4. Fruit parameters in grafted and non grafted plants

Treatments	Fruits per plant	Fruit weight (g)	Fruit yield per plant (kg)	Total fruit yield (q/ha)
T_1: Liliya grafted on *S. torvum*	20.12a	67.53a	1.35a	319.45a
T_2: Liliya grafted on *S. khasianum*	15.62b	59.25b	0.92c	209.96c
T_3: Kokila grafted on *S. torvum*	19.25a	65.98a	1.27b	298.78b
T_4: Kokila grafted on *S. khasianum*	16.25b	60.23b	0.97c	224.24c
T_5:Liliya (scion-1)	13.06c	54.34c	0.70d	148.04d
T_6:Kokila (scion-2)	13.62c	55.71c	0.75d	154.55d
S.Ed (±)	0.77	1.58	0.05	11.58
CD (P=0.05)	1.67	3.41	0.11	24.69

Table 5. *Meloidogyne incognita* infection and multiplication in grafted and non grafted *Solanum* species

Treatments	Number of galls	Number of egg masses	FNP (200 cc soil)	Rate of reproduction
T_1: Liliya grafted on *S. torvum*	6.50c	2.75b	119.60d	0.46d
T_2: Liliya grafted on *S. khasianum*	18.00b	7.25b	190.65c	0.75c
T_3: Kokila grafted on *S. torvum*	6.75c	3.25b	126.60d	0.49d
T_4: Kokila grafted on *S. khasianum*	19.25b	7.75b	202.50c	0.79c
T_5:Liliya (scion-1)	89.75a	42.00a	653.25a	2.58a
T_6:Kokila (scion-2)	87.00a	44.00a	600.08b	2.36b
S.Ed (±)	11.97	2.75	15.41	0.06
CD (P=0.05)	25.51	5.78	32.38	0.13

non grafted plants. The maximum fruit parameters were recorded in the treatments where *S. torvum* was used as rootstock (T_1 and T_3). Mai and Abawi (1987) reported that severe galling and significantly reduces water and nutrient translocation efficiency of the roots that lead to reduction in yield. Flower inducing substances are controlled by photoperiod and the graft union facilitates the early movement of flower inducing substances there by increased number of fruit set percentage which in turn increase the number of fruits as reported by Kumar *et al.* (2016) in grafted eggplant.

In the present investigation, the data on number of galls, egg masses per root system, nematode population and rate of reproduction (Table 5) show significant reduction in the treatments with grafted plants as compared to treatments with non grafted plants. Curuk *et al.* (2009) studied the effect of grafting in eggplant against *M. incognita* in Turkey and observed that when eggplant cultivar 'Faselis' grafted on resistant rootstock '*S. torvum'* and planted in a soil infected with *M. incognita* which causes to augmented the protection against pathogen infestations.

Benjamin *et al.* (2018) who recorded 0.07 to 0.64 as reproduction factor of *M. incognita* in resistant and tolerant rootstocks of *Solanum viz.*, *S. aethiopicum*, *S. macrocarpon,S. lycopersicum* "Mongal F1" and *S. lycopersicum* "Samrudhi F1". Similarly, Ocal and Devran (2019) also reported 0.034 as reproduction factor of *M. incognita* in*S. torvum* cv. Hawk. These findings are similar to the results of the present investigation where, the number of galls and egg masses were recorded to be less in plants grafted on resistant rootstocks such as *S. torvum* and *S. khasianum.* However, Khan (1994) concluded that nematode resistance in host plants was manifested by reduced rates of nematode reproduction, egg masses and consequently, low nematode population densities than that of a susceptible one, thus, confirm the results of the present investigation.

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Efficacy of Essential Oils from Geranium (*Pelargonium graveolens*) and Citronella (*Cymbopogon nardus*) and Their Major Compounds Against *Meloidogyne graminicola* in Pots and Field Conditions

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Pot and field experiments of *Pelargonium graveolens* (geranium), *Cymbopogon nardus* (citronella) oils and their major metabolites (citronellol and citronellal) were carried out for their nematicidal activity against *Meloidogyne graminicola* (root-knot nematode) at 500 and 1000ppm concentration using drenching and root dipping methods with direct seeded and transplanted rice. Results of the pot experiments showed the variable nematicidal activity of essential oils and pure compounds. Citronella oil (3.33 galls/seedling) and citronellal (3.33 galls/seedling) showed promising nematode controlling activity followed by geranium oil (3.67 galls/seedling) and citronellol (4.33 galls/seedling) at 1000 ppm on 30 days after inoculation (DAI) and were at par with carbofuran 3G (3.33 galls/seedling) and velum prime (3.33 galls/seedling), used as positive controls but found better than negative control (14.67 galls/seedling) in pot experiment. The plant growth of rice seedlings followed the pattern as citronella oil (35.0cm) > geranium oil (34.67cm)> citronellol (31.67cm) > citronellal (31.67cm) @ 1000 ppm at 30 DAI, compared to carbofuran (33.67cm) and velum prime (33.33cm). These results from pot experiment showed the promising activity of oil and compounds similar to positive controls. Citronella and geranium oils were further evaluated in a sick plot (1.0mx1.0m) at two concentrations (500 and 1000ppm) using drenching and seedling dip methods together with commercial nematicides. Results also showed that the plant growth (shoot length) of the rice seedlings in treatment with oil (@ 1000 ppm) were at par with carbofuran 3G and velum prime treatments but higher than negative control. The number of galls in citronella oil (0.6/seedling), carbofuran (0.6/seedling) were recorded less compared to velum prime (1.0/seedling) at 1000 ppm and negative control (3.8/seedling) under field conditions on 30day after showing (DAS). This study showed that the citronella and geranium oils could be an alternative to the commercial nematicides for controlling of *M. graminicola* up to 30 days after showing in direct seeded rice nursery.

Keywords: Essential oils, Nematicidal activity, citronellol and citronellal

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In India, rice (*Oryza sativa* L.) is one of the staple food crops, cultivated in almost all states, covering about 43.8 mha with a production of 177.6 million tonnes (FAOSTAT 2021) but rice in field is severely affected by plant parasitic nematode, *Meloidogyne graminicola* leading to loss in crop yield. It has been reported that *M. graminicola* is prevalent in major rice producing countries. In India, infestation of *M. graminicola* has been reported from several states and has caused upto 50-90% loss in grain yield (Pankaj *et al.*, 2010; Pankaj *et al.*, 2015). Its infestation in rice plants also showed stunting growth and chlorosis with characteristic terminal swellings/galls on the roots (Jain et al., 2012). The management of *M. graminicola* in rice nursery level is carried out using synthetic nematicides such as carbofuran and velum prime but since past decades use of synthetic nematicides is being discouraged due to their toxicity to non-target organisms, human being and environment (Ntalli, and Caboni, 2012). The cultivation of rice in field without application of synthetic nematicides may severely affect rice production. Therefore, development of environmentally benign and target specific nematicides as an alternative to toxic synthetic nematicides of biological origin, is urgently required.

Plant based nematicides are considered to be effective and safe to the environment, human health, non-targeted organisms and are biodegradable. Currently, farmers are using only synthetic nematicides for the management of nematodes in field due lack of effective bionematicides in the market. It has been reported that the intercropping of tomato with *Tagetes* (Hooks *et al*., 2010), use of neem-based products (neem seed cake), neem leaf mulch and use of *Crotalaria* species are being practiced to reduced galls and egg mass numbers of *M. incognita* in field (Oka, 2010; Collange *et al*., 2011). Nematicidal activity of crude plant extracts and essential oils has been reviewed (Ntalli and Caboni, 2012) which showed the promising effect of both crude plant extracts and essential oils against different nematodes. Essential oils are also reported to play an important role in the protection of the plants from insect-pests and diseases (Bakkali *et al*., 2008). Earlier studies showed that the essential oils possess nematicidal activity against *Meloidogyne hapla* (Douda *et al*., 2010), *Bursaphelenchus xylophilus* (Barbosa *et al*., 2010), *M. javanica* (Oka, 2001; Onifade *et al*., 2008), *M. incognita* (Leela *et al*., 1992), and *M. graminicola* (Ajith *et al*., 2020; 2021). Considering the damaging potential and economic loss caused by *M. graminicola* in rice, the pot and field experiments of the essential oils from *Pelargonium graveolen*s (geranium) and *Cymbopogon nardus* (citronella) and their major metabolites (citronellol and citronellal) were carried out for nematicidal activity against *M. graminicola* (root-knot nematode).

MATERIALS AND METHODS

The soil of the field of Nematology Division, ICAR-IARI, New Delhi was used for all the experimental purposes. The soil was mixed thoroughly with sand in the ratio 3:1 and sieved through 20 mesh sieves for removal of debris. The soil-sand mixture was steam-sterilized at 1.0546 kg/cm^3 pressure for 4 h and stored in a well labeled small polythene bags, exposed to open sunlight by spreading a polythene sheet for aeration. Three random samples from sterilized soil were processed by Cobb's modified sieving and Baermann's funnel technique and examined to ensure that the sterilized soil was free from plant-parasitic nematodes.

Maintenance of nematode culture

The *M. graminicola* population used in the study was originally isolated from the heavily infected rice nursery at ICAR-IARI, New Delhi, India. A pure culture of an Indian isolate of *M. graminicola* was maintained on rice plants (*O. sativa* cv. Pusa Basmati 1121), grown in the soil-less culture. Egg masses were collected from galled roots and were kept for hatching on a double-layered tissue paper supported on a sieve of wire gauze in a Petri dish containing distilled water. Freshly hatched second-stage juveniles (J2s) were used for subsequent experiments.

Extraction of second-stage juveniles (J2s)

The second stage juveniles (J2s) of *M. graminicola* were extracted by removing root galls followed by placing excised galls on a double folded tissue paper placed on wire mesh in the Petri dish filled with water just touching the tissue paper to keep it moist. The whole setup was covered with another Petri dish and was incubated at room temperature (25-29°C) for a period of 48 to 96 h. After 48 h, the nematode suspension was collected in a beaker. This suspension was kept undisturbed at room temperature (27-28°C) for 1-2 h, allowing the nematodes to settle down at the bottom of the beaker. The nematode suspension was concentrated by decanting excess water and the J2 population was counted under the stereoscopic binocular microscope. An average of three aliquots of 1 mL each was calculated.

Natural geranium and citronella oils were purchased from the M/s Shiv Sales Corporation, New Delhi, Tween-80 were purchased from CDH while citronellal and citronellol were separated from the citronella oil using column chromatography. Distilled water used for preparation of test solution was purchased from Merck while tap water was using for the dilution of the test solutions during application in pot or field.

Preparation of test solutions

The stock solutions (1.0%) of geranium oil, citronella oil, citronellol and citronellal were prepared in distilled water containing Tween-80 (4.0%) by mixing ingredients for half hour using lab stirrer. Two concentration (500 & 1000ppm) of test solutions were prepared from the stock solution by serial dilution and were used for the pot experiment followed by field trials for their nematicidal activity against *M. graminicola*. Two commercial nematicides, carbofuran and velum prime (1000 and 500ppm) were used as positive controls while distilled water with Tween-80 (4.0%) and water were used as negative controls in pot experiments. In field trial, carbofuran was used as positive control @ 3.0g/ha and velum prime 34.48% SC as per recommended dose/ha.

Root dip treatment in pots

The soil (1150.0 g) was filled in the pot (size 10x10 cm). The roots of 21 days old five rice seedlings were dipped separately in 50 mL formulation (500 and 1000 ppm) of essential oils and pure compounds and positive (carbofuran & velum prime) and negative (Tween 80, 4.0% & distilled water) controls for 20 min. The seedlings were removed and transplanted in each pot (5 seedlings/pot). The root zones of each seedling of the treatment were inoculated with nematodes (400 J2s/1mL) having a total of about 2000 nematodes per pot. Each experiment was carried out in triplicates. The observations for the height of seedlings and number of galls in the roots per seedling were recorded on 7, 14 and 30 days after transplantation (DAT).

Drenching in field

Field experiment was conducted in a sick plot (1.0x1.0m) at Division of Nematology, ICAR-Indian Agricultural Research Institute, Pusa, New Delhi, in duplicate. Based on the results of pot experiment, geranium oil and citronella oil were further tested at 500 and 1000ppm under field conditions by applying test solution (150 ml/line) on the soil followed by sprinkling paddy seeds and covering the seeds with soil. The watering of the experimental field was carried out as recommended. Ten rice seedlings were randomly uprooted from each plot, washed and the heights of the seedlings and number of galls per seedling were measured and counted on 30 days after showing (DAS). Carbofuran (3.0 g/ha) and Velum prime (34.48% SC) were used positive controls as per recommended dose.

Statistical method

The data recorded on the shoot length of the seedlings and number of galls per seedling from pot and field experiments in triplicate was statistically analyzed using the SPSS v22.0 software and results were presented as mean±SD.

RESULTS AND DISCUSSION

Geranium (*Pelargonium graveolens*) and citronella (*Cymbopogon nardus*) are commercially grown in India for production of geranium and citronella oils, used in perfumery and medicinal purposes (Agarwal, 2008). However, the use of these oils and their secondary metabolites were never explored for the management of nematodes in agriculture. Analysis of the oils from *P. graveolens* and *C. nardus* has indicated the presence of citronellol and citronellal as major compounds. Both oils and citronellol and citronellal were evaluated for their nematicidal activity at two different concentration (500 and 1000 ppm) to determine their effects on galls formation in the roots of rice seedling, inoculated with J2s of *M. graminicola* and heights of the rice seedlings using root dipping method in pot experiment (Table 1). Results showed that the seedling treated with citronella oil showed the presence of 4.33 galls and 3.33 galls/seedling in the roots of rice seedlings at 500 and 1000 ppm

Table 1. Effect of essential oils and pure compounds (as root dip) on seedling growth and root gall formation inoculated with *M. graminicola*

Test sample	Conc. (ppm)	Shoot length of rice seedling (in cm) and number of galls/seedlings					
		7 DAI		14 DAI		30 DAI	
		Shoot length (cm)	No. of galls	Shoot length (cm)	No. of galls	Shoot length (cm)	No. of galls
Citronella oil	1000	19.16±0.76	1.33±0.58	23.17±1.040	2.00±0.00	35.00±1.00	3.33±0.58
	500	16.00±1.00	1.67±0.58	20.50±2.291	2.67±0.58	31.00±1.00	4.33±0.57
Geranium oil	1000	26.00±1.00	1.33±0.58	29.67±0.58	2.67±0.58	34.67±0.58	3.67±0.58
	500	18.00±1.00	1.67±0.58	22.00±1.00	2.67±0.58	27.00±1.00	5.33±0.58
Citronellal	1000	19.67±1.52	0.67±0.57	24.50±0.50	2.00±0.00	30.00±1.00	3.33±0.58
	500	18.33±0.57	1.67±0.58	23.00±1.00	3.00±0.00	26.00±1.00	4.33±0.57
Citronellol	1000	24.667±0.58	1.333±0.58	27.67±0.58	2.67±0.58	31.67±1.52	4.33±0.57
	500	20.33±0.58	1.67±0.58	24.67±1.52	3.67±0.58	29.00±1.00	5.67±0.57
Carbofuran 3G	500	25.00±1.00	1.67±0.58	30.33±0.58	3.00±0.00	33.67±1.53	4.33±0.58
Velum prime	500	24.67±0.58	2.67±0.58	28.00±0.00	3.67±0.58	33.33±0.58	4.67±0.58
Control (c)		17.00±1.00	4.67±0.58	22.00±1.00	8.66±0.58	25.00±1.00	14.67±0.57
Water+Tween-80(4%)	Tween-80 (4.0%)						

Positive control: (a) (b) and negative control: (c); *Values given in the table is mean values of three replicates IN=inoculated, DAI=days after inoculation

concentration while those treated with geranium oil had 5.33 galls and 3.67 galls/seedling at similar concentration on 30 days after inoculations compared to positive control, carbofuran (4.33 galls/seedling) and velum prime (4.67 galls/seedling) at 500 ppm but number of galls/seedling were recorded higher (14.67/seedling) in negative control (4.0% Tween-80) with less seedling height (25.0cm) compared to test sample and positive controls. Similarly, the seedlings treated with citronellal and citronellol at 500 and 1000ppm showed the maximum number of 5.67 galls and 4.33 galls/seedling on 30 DAI with significant shoot length 30.0 cm and 31.67cm compared to negative control (25.0cm). In pot experiment, both oil and pure compounds showed encouraging and comparable efficacy similar to commercial nematicides (carbofuran and velum prime) and better than negative control, in terms of controlling of gall formation in the roots of rice and height of seedlings.

Based on the results of the pot experiments, the geranium and citronella oils were further evaluated in the field trial in *M. graminicola* infested (sick) plots (1.0 m^2) at two concentration (500 and 1000ppm) during 2019-20 and 2020-21 using drenching method to record their efficacy in natural conditions up to 30 days after sowing (DAS). The irrigation of the plots was carried out as recommended. The average heights (Fig. 1) of the rice seedlings were found higher in higher concentration of oils due to lesser galls/seedling (Fig. 2) in all the treatments, but citronella oil treatment at showed better height (27.0 cm) on 30 DAS closed to carbofuran and velum prime at similar concentration (1000 ppm) due to presence of

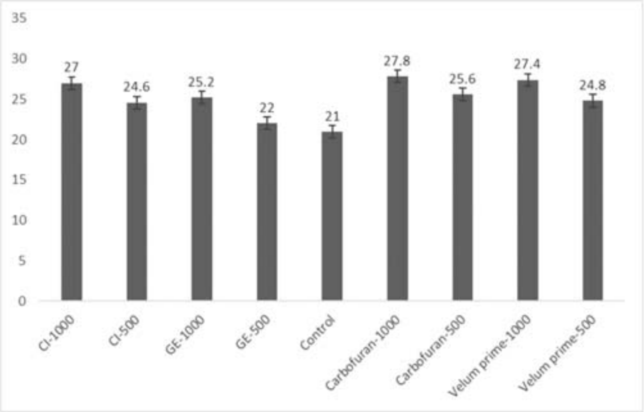

Fig. 1. Effect of citronella (CI) and geranium (GE) oils on shoot height (in cm.) per seedling of direct seeded rice (cv.PB-1121) against *M. graminicola* (30DAS)

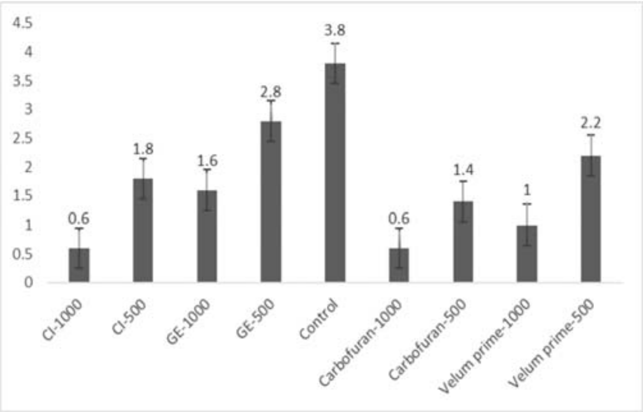

Fig. 2. Effect of citronella (CI) and geranium (GE) oils on gall formation (galls/seedling) in roots of direct seeded rice seedling (cv.PB-1121) against *M. graminicola* (30DAS)

fewer number of galls but better than negative control in which average rice seedling height was found to be 21.0cm. Comparing the effect of concentration of citronella oil and geranium oil on plant height citronella oil showed better growth (24.6 and 27.0 cm) compared to geranium oil (22.0 and 25.2cm) at 500 and 1000ppm. Earlier number of studies showed that the essential oils and their metabolites possessed nematicidal activity under in-vitro conditions (El-Nuby, 2021; Faria *et al*., 2021) but none of the study was carried out to evaluate them in field condition against *M. graminicola* except recently studies on essential oil and their major compounds against nematode in both pot and field conditions (Ajith *et al*. 2020; 2021).

These results showed that efficacy of the oils against rice root-knot nematode were concentration dependent. Citronella oil showed promising nematode controlling activity under both pot and field conditions. The pot experiment also indicated that the geranium oil, citronellal and citronellol were effective and could be useful substitute to carbofuran or velum prime, if used at and above 1000 ppm concentration. Thus, oils and compounds have the potential to be used as bionematicide, however large-scale field trials are further recommended to establish their efficacy in different geographical conditions against *M. graminicola*.

CONCLUSIONS

Geranium and citronella oils together with citronellal and citronellol showed promising efficacy in controlling of galls formation in the roots of rice seedlings in pot experiment similar to commercial nematicides. The efficacy of citronella oil and geranium oil was also found comparable to the commercial nematicides in field trial carried out during 2019-20 and 2020-21 in terms of nematode controlling agents. Citronella and geranium oils could be a new and effective alternative as nematode controlling agent to the synthetic nematicides against *M. graminicola* in rice nursery up to 30 DAS in direct seed rice using drenching method in natural farming, however large-scale field trial is further suggested to establish its efficacy for the development of natural nematicides.

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Utilization of Agri-Based Wastes against *Meloidogyne incognita* infecting Cucumber in Polyhouse

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ABSTRACT: An experiment was carried out in the poly-house to find out the efficacy of agri-based wastes against root-knot nematode, *Meloidogyne incognita* infecting cucumber. Agri-based wastes *viz.,* tea waste (TW), tobacco churi (TC), poultry manure (PM), water hyacinth powder (WHP), lantana leaf powder (LLP) and neem cake (NC) were applied @ 20 g and 40 g per plant as soil application before sowing to manage *Meloidogyne incognita* on Cucumber in naturally infested polyhouses. Yield of cucumber significantly increased with the application of organic amendments over untreated check (Control) in root-knot nematode infested polyhouse. After analysis of all treatments, maximum yield (3.180 kg/plant) was obtained with the application of Tobacco Churi at 40 g per plant followed by tea waste (3.060 kg per plant) and Poultry manure (2.890 kg per plant) at the rate 40 g per plant as compared to control (1.215 kg/plant). Among all the treatments, the highest yield (3.390 kg/plant) was recorded with neem cake at 50 g/plant which was kept as standard check.

Key words: Root-knot nematode, cucumber, protected cultivation, organic amendments

Cucumber (*Cucumis sativus* L.) is a very useful and nutritious vegetable and a good source of vitamins and kind of minerals. The production of cucumber under protected cultivation is the most popular technology throughout the world. It is a typical subtropical crop and grows best under conditions of high temperature, humidity, light intensity and it is highly susceptible to both abiotic and biotic stresses. Cucumber grows fast and develops an abundant plant canopy in poly-house. The plants are soft and highly susceptible to various pests. The devasting pests is root-knot nematode, *Meloidogyne incognita* in polyhouse due to favourable environmental conditions (Desaeger and Csinos, 2006; Bhati and Baheti, 2020a), causing great damage to cucumber (Sharma *et al.* 2007; Sharma *et al.* 2009; Rao *et al.,* 2015; Bhati and Baheti, 2020b).

The crop losses (21.3%) caused by plant parasitic nematodes amounting to Rs.102,039.79 million (1.58 billion USD) annually in India. Whereas, root-knot nematode (*Meloidogyne* spp.) is responsible for causing 75.83% of the estimated losses. In cucumber, it causes 12.00% losses with estimated monetary loss of Rs. 110.46 million per annum in open field (Kumar *et al.,* 2020) and 66.84% losses on cucumber in poly-house (Bhati and Baheti, 2021). Simultaneously, 22.45 to 45.50% avoidable yield losses caused *M. incognita* in okra (Baheti and Bhati, 2017).

Plant parasitic nematodes need to be managed in poly-house in order to maintain both quality and quantity of cucumber. Different approaches are used to prevent and manage nematodes. Beyond good crop practices, polyhouse growers often use more pesticides and those inputs to agriculture have contributed significant improvements in crop productivity and it attracts to farmers (Sharma *et al.* 2008). However, the health consciousness of the people about 10 to continuous use of the chemicals also restricts use of chemicals in vegetable protection. Under such circumstances exploitation of agri-based wastes (organic amendments) to reduce the nematodes seems to be the most appropriate alternative to chemicals. The organic amendments suppress the plant parasitic nematodes through a number of ways such as antibiosis, production of organic acids, increasing population of natural enemies, improved tolerance of plants and plant growth. The use of agri-based wastes *viz.,* tea waste (TW), tobacco churi (TC), poultry manure (PM), water hyacinth powder (WHP), lantana leaf powder (LLP) and neem cake (NC) against nematodes are method to change waste into wealth. The use of these agri-based wastes for nematode management was found to be a good alternative method for waste management in agriculture.

MATERIALS AND METHODS

This experiment was conducted at naturally infested poly-houses of progressive farmers during *kharif* 2016 and 2017.

Selection of experimental site

Survey was under taken to identified and locates root-knot nematode, *M. incognita* infested polyhouse before laying out of experimental trial. Two cucumber growing progressive farmer's of two different locations selected in which they have well established poly-house and naturally infested with *Meloidogyne incognita* having an initial nematode population of 1350 and 1360 juveniles/100 cc soil during 2016 & 17, respectively.

Identification of root-knot nematode species

Root samples were brought to the laboratory and washed carefully in running tap water to remove adhering soil particles. Egg masses with females were detached from infected roots with the help of teasing needle and forceps under stereoscopic binocular microscope. Egg masses were kept in water at least for 24 hrs for hatching and females were picked up for identification of nematode species. The perineal patterns of females were prepared for the species identification as described by Taylor and Netscher (1974). Observations of such several patterns were recorded and the nematode species was identified as *M. incognita* (Eisenback *et al.,* 1980).

Planning of Sowing

After layout and proper treatment, sowing of cucumber was done in the month of July through dibbling method and labeled properly. The cucumber variety "Mini-angle" was used which is highly susceptible to *M. incognita*. Spacing for each plant was maintained at recommended level for better growth of the plants under protected cultivation. Some seeds also sown in pro trays for gap filling.

Source of Agri-based wastes

The Agri-based wastes (organic amendments) tea waste was collected from road side tea stalls, tobacco churi from tobacco sellers, poultry manure from poultry farms, water hyacinth leaves from ponds and lantana leaf collected from fields. The leaves were dried in shade and powdered for application.

Use of application of treatments

To test the efficacy of potential organic amendments *viz.,* tea waste (TW), tobacco churi (TC), poultry manure (PM), water hyacinth powder (WHP) and lantana leaf powder (LLP) were applied at 20 and 40 g/plant against *M. incognita* on cucumber in poly-house. Treated (Neem cake 50 g/plant) and control were also maintained for comparison of results. Completely randomized block design were used for lay out with five replications.

Observations

Observations on number of galls/5 g root, egg masses/ 5 g root, eggs & juvenile/egg mass, final nematode population per 100 cc soil, vine length (meter), vine weight (kilo gram) were recorded. The yield data (kg per plant) were recorded from first picking of cucumber to till harvest of experiment. Picking of cucumber was done at time to time as required and collected separately in well labeled cloth bags and weighed treatment wise to obtained yield record data.

Statistical Analysis

All the experiments in poly-house were conducted in a completely randomized design. All the experiments were conducted twice using the same treatments and data of the two trials were pooled for presentation. In the end of experiments, recorded data were statistically analysed for elucidation of findings using regression analysis with Excel 2016. The critical difference (CD) was found out for comparison of treatments data, where the 'F' test was found significant at 5 percent level of significance. Summary tables of recorded data along with SEm± and CD were worked out and presented.

RESULTS AND DISCUSSION

Organic amendments play an important role to improve the physical, biological and chemical properties of the soil which enhances the plant growth of agri-horticultural crops. It reduces pest and soil pathogens including nematodes. Looking at the benefits of organic amendment, it has been tested for the management of root-knot nematode, *Meloidogyne incognita* on cucumber in poly house at farmer's field. The observations on number of galls/5g roots, egg masses/5g roots, eggs and larvae/egg mass, final nematode population per 100 cc soil, vine length (meter), vine weight (kilo gram) and yield kg/plant were recorded and presented in Table 1-4 and illustrated with Fig. 1-2.

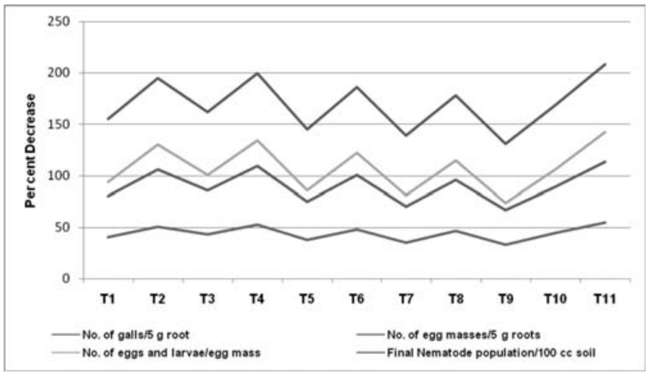

Fig. 1. Impact of organic amendments on reproduction of *Meloidogyne incognita* infecting cucumber in poly-house

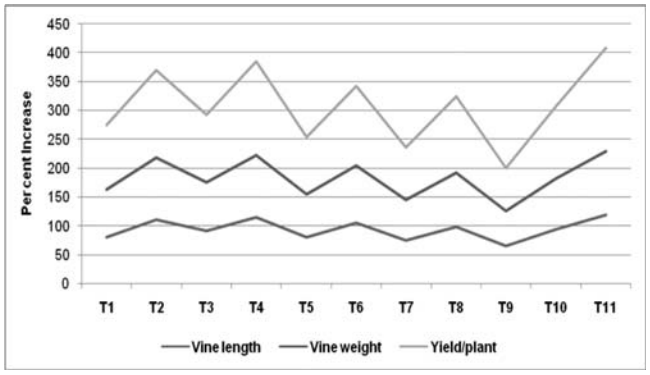

Fig. 2. Impact of organic amendments on plant growth of cucumber infested with *Meloidogyne incognita* in poly-house

Effect of treatments on nematode reproduction

Results of pooled analysis revealed that number of galls per 5g were roots significantly reduced with the organic amendment over untreated check. Among organic amendments, tobacco churi applied at 40g/plant was found to be the best and produced minimum galls (34.80 / 5g roots) followed by tea waste at 40g/plant (36.00 galls / 5g roots) and poultry manure at 40g/plant (38.00 galls / 5g roots) as compared to control (73.70 galls per 5g roots) . Soil application of neem cake at 50 g per plant (33.20 galls per 5g roots) which was kept as standard check found to be the best and it was at par with tobacco churi 40g/plant. The similar trend was observed with respect to egg masses/5g roots, eggs and larvae/egg mass, final nematode population/100 cc soil.

Table 1: Effect of organic amendment against root-knot nematode, *M. incognita* on cucumber in poly-house

Treatments	Galls/5 g root			Egg masses/5 g roots			Eggs and larvae/ egg mass			Final Nematode population/ 100 cc soil		
	2016	**2017**	**Pooled**	**2016**	**2017**	**Pooled**	**2016**	**2017**	**Pooled**	**2016**	**2017**	**Pooled**
T_1 Tea waste at 20 g/plant	43.20	44.00	43.60	37.40	38.60	38.00	219.00	213.40	216.20	735.80	728.40	732.10
T_2 Tea waste at 40 g/plant	35.80	36.20	36.00	28.00	28.80	28.40	191.00	188.00	189.50	667.80	672.20	670.00
T_3 Tobacco churi at 20 g/plant	41.00	42.60	41.80	35.40	36.80	36.10	211.80	214.80	213.30	721.80	734.80	728.30
T_4 Tobacco churi at 40 g/plant	34.20	35.40	34.80	26.40	27.60	27.00	186.00	192.00	189.00	660.00	666.60	663.30
T_5 Poultry manure at 20 g/plant	45.20	46.20	45.70	39.20	40.00	39.60	224.80	221.60	223.20	770.00	764.20	767.10
T_6 Poultry manure at 40 g/plant	37.40	38.60	38.00	30.20	29.60	29.90	198.80	194.20	196.50	682.20	686.80	684.50
T_7 Water hyacinth powder at 20 g/plant	47.20	48.00	47.60	40.60	41.40	41.00	222.00	225.20	223.60	788.00	794.40	791.20
T_8 Water hyacinth powder at 40 g/plant	38.80	39.40	39.10	32.20	31.60	31.90	202.00	205.00	203.50	694.00	698.60	696.30
T_9 Lantana leaf powder at 20 g/plant	48.20	49.80	49.00	41.00	42.80	41.90	232.00	234.20	233.10	804.00	807.20	805.60
T_{10} Lantana leaf powder at 40 g/plant	39.00	41.80	40.40	34.40	35.80	35.10	207.60	209.80	208.70	707.60	702.80	705.20
T_{11} Neem cake at 50 g/plant	32.80	33.60	33.20	25.60	26.40	26.00	181.60	175.60	178.60	651.80	644.40	648.10
T_{12} Check	72.80	74.60	73.70	64.80	60.80	62.80	246.00	256.00	251.00	1885.00	1893.60	1889.30
SEm±	2.116	2.029	2.072	2.078	2.046	2.062	11.230	11.210	11.22	32.566	32.080	32.323
CD ($P \leq 0.05$)	6.030	5.783	5.906	5.924	5.830	5.877	32.009	31.952	31.980	92.819	91.434	92.126

Data are the average value of five replications

Table 2: Effect of organic amendment on plant growth of cucumber infected with root-knot nematode, *M. incognita* in poly-house

Treatments	Vine length (m)			Vine weight (kg)			Yield kg/plant		
	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled
T_1 Tea waste at 20 g/plant	2.536	2.496	2.516	0.675	0.678	0.676	2.600	2.552	2.576
T_2 Tea waste at 40 g/plant	2.908	2.938	2.923	0.771	0.768	0.769	3.050	3.070	3.060
T_3 Tobacco churi at 20 g/plant	2.670	2.640	2.655	0.680	0.685	0.682	2.660	2.638	2.649
T_4 Tobacco churi at 40 g/plant	2.960	2.990	2.975	0.776	0.771	0.773	3.150	3.210	3.180
T_5 Poultry manure at 20 g/plant	2.532	2.482	2.507	0.651	0.647	0.649	2.424	2.408	2.416
T_6 Poultry manure at 40 g/plant	2.828	2.858	2.843	0.746	0.741	0.743	2.920	2.860	2.890
T_7 Water hyacinth powder at 20 g/plant	2.468	2.418	2.443	0.631	0.626	0.628	2.338	2.316	2.327
T_8 Water hyacinth powder at 40 g/plant	2.768	2.738	2.753	0.715	0.720	0.717	2.850	2.812	2.831
T_9 Lantana leaf powder at 20 g/plant	2.328	2.286	2.307	0.591	0.596	0.593	2.178	2.080	2.129
T_{10} Lantana leaf powder at 40 g/plant	2.720	2.700	2.710	0.694	0.699	0.696	2.755	2.710	2.732
T_{11} Neem cake at 50 g/plant	3.024	3.054	3.039	0.779	0.783	0.781	3.405	3.375	3.390
T_{12} Check	1.410	1.370	1.390	0.369	0.374	0.371	1.228	1.202	1.215
SEm ±	0.085	0.088	0.086	0.025	0.015	0.020	0.079	0.059	0.069
CD ($P \leq 0.05$)	0.241	0.251	0.246	0.070	0.044	0.057	0.225	0.167	0.196

Data are the average value of five replications

Experimental results (Table 3) showed that soil amendment with tobacco churi at 40 g/plant has reduces the egg masses to the tune of 56.94% over untreated check [control] followed by tea waste (54.71%) and poultry manure (52.36%) at 40 g/plant. Maximum reduction (58.54%) in egg masses per 5 g roots were obtained with the application of neem cake at 50 g/plant. Soil amendment with tobacco churi at 40 g/plant, decreased the final nematode population /100cc soil to the tune of 64.90% over untreated check. It was observed 64.54% with tea waste and 63.78% with poultry manure at 40 g/plant. Highest reduction (65.70%) of final nematode population was noticed with application of neem cake at 50 g/plant.

The results obtained in present investigation are also in accordance with findings of Srivastava *et al.* (1971) who tested different oil-cakes against *M. javanica* on tomato and brinjal. They observed that neem cake was found to be the most effective for the management of nematodes. Khan *et al.* (1979) recorded that the application of neem, groundnut, mahua and castor cakes significantly suppressed *M. incognita* in the okra. Ramkrishanan *et al.* (1997) conducted a field experiment for the control of root-knot nematode on okra with various organics including neem and mustard cake as soil amendments. Maximum reduction in nematode reproduction factor was recorded in soil amended with neem cake. A study was conducted by Nchore *et al.* (2011) in greenhouse as well as in field to determine the efficacy of cattle manure, goat manure, *Tithonia diversifolia* and agro-industrial wastes of tea (*Camellia sinensis),* pyrethrum and vegetable waxy resins for the management of root-knot nematodes on *Solanum nigrum* and found better results for suppression of RKN population and reproduction. Mehta *et al.* (2015) evaluated the efficacy of neem (*Azadirachta indica*), aak (*Calotropis procera*) and water hyacinth (*Eichhornia crassipes*)

Table 3: Changes in reproduction of root-knot nematode, *M. incognita* on cucumber under polyhouse through organic amendment

Treatments	Galls/5 g root*			Egg masses/5 g roots*			Eggs and larvae/ egg mass*			Final Nematode population/ 100 cc soil*		
	2016	**2017**	**Pooled**	**2016**	**2017**	**Pooled**	**2016**	**2017**	**Pooled**	**2016**	**2017**	**Pooled**
T_1 Tea waste at 20 g/plant	40.66	41.02	40.84	42.28	36.51	39.40	10.98	16.64	13.81	60.97	61.53	61.25
T_2 Tea waste at 40 g/plant	50.82	51.47	51.14	56.79	52.63	54.71	22.36	26.56	24.46	64.57	64.50	64.54
T_3 Tobacco churi at 20 g/plant	43.68	42.90	43.29	45.37	39.47	42.42	13.90	16.09	15.00	61.71	61.20	61.46
T_4 Tobacco churi at 40 g/plant	53.02	52.55	52.78	59.26	54.61	56.94	24.39	25.00	24.70	64.99	64.80	64.90
T_5 Poultry manure at 20 g/plant	37.91	38.07	37.99	39.51	34.21	36.86	9.76	12.03	10.90	59.15	59.64	59.40
T_6 Poultry manure at 40 g/plant	48.63	48.26	48.44	53.40	51.32	52.36	19.19	24.14	21.67	63.82	63.73	63.78
T_7 Water hyacinth powder at 20 g/plant	35.16	35.66	35.41	37.35	31.91	34.63	8.62	13.44	11.03	58.20	58.05	58.13
T_8 Water hyacinth powder at 40 g/plant	46.70	47.18	46.94	50.31	48.03	49.17	17.89	19.92	18.91	63.18	63.11	63.15
T_9 Lantana leaf powder at 20 g/plant	33.79	33.24	33.51	36.73	29.61	33.17	5.69	8.52	7.11	57.35	57.37	57.36
T_{10} Lantana leaf powder at 40 g/plant	46.43	43.97	45.20	46.91	41.12	44.02	15.61	18.05	16.83	62.46	62.89	62.68
T_{11} Neem cake at 50 g/plant	54.95	54.96	54.95	60.49	56.58	58.54	26.18	31.41	28.80	65.42	65.97	65.70
T_{12} Check	-	-	-	-	-	-	-	-	-	-	-	-

* % decrease over check

Table 4: Changes in plant growth parameters of cucumber under polyhouse infested with *M. incognita* through organic amendment

Treatments	Vine length*			Vine weight*			Yield/plant*		
	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled
T_1 Tea waste at 20 g/plant	79.86	82.19	81.03	82.74	81.00	82.09	111.73	112.14	111.93
T_2 Tea waste at 40 g/plant	106.24	114.45	110.35	108.66	104.96	107.13	148.37	155.40	151.85
T_3 Tobacco churi at 20 g/plant	89.36	92.70	91.03	83.98	82.66	83.71	116.61	119.46	118.02
T_4 Tobacco churi at 40 g/plant	109.93	118.25	114.09	109.90	105.76	108.20	156.51	167.05	161.72
T_5 Poultry manure at 20 g/plant	79.57	81.17	80.37	76.14	72.68	74.69	97.39	100.33	98.84
T_6 Poultry manure at 40 g/plant	100.57	108.75	104.66	101.95	97.81	100.13	137.80	137.93	137.86
T_7 Water hyacinth powder at 20 g/plant	75.04	76.50	75.77	70.83	67.13	69.17	90.39	92.67	91.52
T_8 Water hyacinth powder at 40 g/plant	96.31	99.85	98.08	93.56	92.00	93.13	132.08	133.94	133.00
T_9 Lantana leaf powder at 20 g/plant	65.11	66.86	65.99	60.01	58.96	59.75	77.35	73.04	75.22
T_{10} Lantana leaf powder at 40 g/plant	92.91	97.08	95.00	87.82	86.39	87.48	124.32	125.45	124.89
T_{11} Neem cake at 50 g/plant	114.47	122.92	118.70	110.77	109.02	110.22	177.26	180.78	179.01
T_{12} Check	-	-	-	-	-	-	-	-	-

* % increase over check

leaf powder against *H. zeae* on maize and observed reduction in nematode reproduction. The organic amendments also reduced nematode reproduction with combination of other management practices (Bhati *et al.,* 2021).

The suppression of root-knot nematode with organic amendment under protected cultivation may be because of the several factors *i.e.* production of volatile fatty acids, phenols, ammonia, organic acid, amino acids etc. Hence decomposition of organic materials are highly toxic to nematodes because it produce microbial metabolites.

Effect of treatments on plant growth and yield

The survival of plant parasitic nematodes depend on host plant, temperature, humidity, soil environment and change in any one of these factors influences the nematode survival directly as well as indirectly. Therefore, in the present investigation agri-based wastes tested for the management of root-knot nematode, *Meloidogyne incognita* on cucumber under protected cultivation as soil application.

Results exhibited that maximum 2.97 m vine length of cucumber in protected cultivation was found with tobacco churi at 40 g/plant followed by 2.923 and 2.843 meter vine length observed with tea waste and poultry manure at 40 g/plant, respectively. Minimum vine length (1.39 m) was observed in control. Among all the treatments, neem cake at 50 g per plant (3.03 m) gives better response to increase in vine length of cucumber. The similar trend was exhibited with respect to vine weight and yield (kg/plant) of cucumber.The application of tobacco churi at the rate of 40 g / plant increase vine weight 108.20% of cucumber under protected cultivation followed by 107.13 and 100.13% with tea waste and poultry manure at 40 g/plant, respectively. Whereas, maximum yield (3.180 kg/plant) was obtained with the application of tobacco churi at 40 g/plant followed by tea waste (3.060 kg/plant) and poultry manure (2.890 kg/plant) at the rate 40 g per plant as compared to untreated check (1.215 kg/plant).

The results of present investigation are in accordance with the results of several previous workers who reported the effectiveness of organic amendments to enhance the crop growth parameters in nematode infested areas. Muhammad *et al*. (2001) reported that organic amendments of soil with neem cake, mustard cake, farm yard manure and poultry manure at 25 g/kg of soil significantly enhanced the growth of green gram. Randhawa *et al*. (2002) observed that soil amendment with neem cake significantly increased okra yield. Patel *et al.* (2003) reported that application of neem, castor, mahua, mustard, piludi and karanj cakes, farm yard and poultry manures significantly increased the plant growth of cotton. Neem, karanj and mustard oil-cakes tested by Baheti *et al.* (2019) for the management of root-knot nematode on okra. All treatments were found to be effective for the management of root-knot nematode. It will enhance the crop yield over untreated check [control].

CONCLUSION

These findings support that application of organic amendments will reduce the nematode population and it will enhance the plant growth in nematode prone areas. This might be possible due to the fact that organic amendments improve the physical properties of soil, increase the population of natural enemies of plant parasitic nematode and increase the activity of beneficial microbes in soil.

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Application of Green Synthesis in Silver Nanoparticles for Root Knot Nematode Management on Tomato

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ABSTRACT: Synthetic nematicides are the major control options employed for the management of plant parasitic nematodes in tomato plants. Residual pesticide has created great concern for human health. Therefore, there is the need for intensive research on alternative control measures to keep nematode population at low level without environmental consequences. Plantain peel extract mediated silver nanoparticles and other organic extracts from the peels were investigated for their nematicidal potential by comparing with the standard carbofuran. Air-dried plantain peels were grinded in to powder and divided into four parts, one part was extracted with ethanol, two parts were extracted with n-hexane. A part of the n-hexane extract was derivatized, while the remaining powder was used directly as soil amendment. The ethanol extract was used as reducing agent in the synthesis of silver nanoparticles. The nanoparticles were characterised with UV-visible spectroscopy and SEM which shows that the particles are oval and planar in nature. Significant (P=0.05) reduction in *M. incognita* population and root gall index was observed in tomato plants treated with silver nanoparticles. There was a direct relationship between yield and dosage of treatment application. Plantain peel extract nanoparticles may serve as a potential bio-nematicide for *M. incognita* as an alternative to the synthetic nematicide, therefore a contribution to environmental sustainability.

Key words: *Meloidogyne incognita*; tomato; nematicides; environmental pollution; silver nanoparticles; waste utilisation.

Plant parasitic nematodes (PPNs) are a major constraint to crop production (Ibrahim *et al.,* 2010). The major root-knot nematode in tropical and sub-tropical countries are *Meloidogyne arenaria*, *incognita* and *javanica* (Moens *et al.,* 2009), and among these, the most important is *M. incognita* (Sasser, 1980;Qiao*et al.,* 2012), a sedentary endoparasite of over 3000 plant species (Hanson *et al.,* 2010) worldwide. Tomato, globally cultivated for its fleshy fruit is a high value vegetable crop with rich sources of vitamins, minerals and essential nutrients (Van der Vossen *et al*., 2004; Karen, 2007), which are highly important for the body metabolism. Tomato production is greatly hindered by root knot nematode (*Meloidogyne incognita*) a cosmopolitan pest and obligate parasites of several crops in Nigeria (Ogwulumba *et al.,* 2011; Fabiyi, 2020; Fabiyi, 2021).The tomato roots are injured extensively by *M. incognita* through the production of galls in the root tissue with extensive feeding (El-Sherif *et al.,* 2007; Safdar *et al*.,2012), which reduces the movement of water and nutrients from the root to the shoot, weakening the plant and providing entry for other soil microbes which predisposes the tomato plants to rot and wilt inducing pathogens (Williamson and Gleason, 2003; Jones *et al.,* 2013). Infections are usually severe, which eventually translates to low yield at harvest. This accounts in part for poor tomato yield in Nigeria (Ogwulumba *et al.,* 2011). In severe cases death of the plants and yield loss of about 30% has been recorded (Shakil *et al.,*2012). Synthetic nematicides are used effectively to suppress root-knot nematode populations in tomato fields, but their detrimental effect on the agro-ecological system and beneficial soil-dwelling microbes cannot be overemphasized (Bahadur *et al.,* 2006; Zasada *et al.,* 2010).This has spurred research into several alternative methods of control (Hashem and Abo-Elyousr, 2011; Ntalli and Caboni, 2012; Atolani *et al.,* 2014a; Atolani et al., 2014b). In this context, investigations were made into different extracts from plantain peel. Plantain (*Musa paradisiaca*) generates solid wastes after harvest and consumption in the household. The peels are about 33% of the fruit and it is considered as waste (Velumani, 2016). This constitutes environmental pollution when allowed to decay in the open and public places, thus posing serious health risk and culminating into diseases and epidemics (Cairncross and Feachem, 1993;Pichtel, 2005; Ezejiofor *et al.,* 2013). A number of plant materials and agricultural wastes have been examined and established to be effective in plant parasitic nematode control (Oka and Yerumiyahu, 2002; Fabiyi *et al.,* 2020a; Fabiyi, 2021). In this study, plantain peel mediated silver nanoparticles, n-hexane extract, derivatised n-hexane extract and dried powdered plantain peels were assessed as possible options in *M. incognita* control.

MATERIALS AND METHODS

Preparation and Extraction of Plantain Peels

Plantain peels were collected from restaurants in Ilorin metropolis; they were diced into tiny pieces and air-dried for six weeks. The dried plantain peels which weighed 3803g was divided into four equal parts, two parts were extracted separately with n-hexane, apart was grinded into powder, while another part was extracted in ethanol. A part of then- hexane extract was decanted, filtered and concentrated under vacuum while the other part was only filtered and used for derivatisation. The crude n-hexane extract and powdered material was coded PLTP/Hex and PLTP/PWD(Plantain peel n-hexane extract and Plantain peel powdered material respectively)

Derivatisation of Plantain Peel Extract

10 ml of methanol was added to 200 ml of plantain peel extract. This was shaken vigorously in a separating funnel. A colloidal mixture was observed, it was allowed to settle and then filtered, 5 ml of conc. H_2SO_4 was added to the filtrate, and the mixture was refluxed for 2hr.The reaction mixture was cooled diluted with water and extracted with dichloromethane. The dichloromethane extract contain methyl benzoate. The dichloromethane was removed under vacuum and the substance was codedPLTP/DER (Plantain peel derivatised).The procedure was repeated several times to achieve enough quantity for application.

Synthesis of Silver nanoparticles

0.1M of $AgNO_3$ solution of silver nitrate was prepared following the method of Fabiyi *et al.*(2018). 10 ml of this was added to 500ml of ethanol extract of plantain peel. The mixture was stirred continuously at room temperature using the magnetic stirrer for an hour, while stirring there was colour change from golden brown to chocolate brown, and finally to a dark brown solution. After twenty-four (24) hours there was no more colour change. The silver nanoparticles were dispensed into centrifuge tubes and centrifuged at 5000rpm for thirty minutes; the pellets were later dispersed into distilled water and was coded PTLP/Nano (Plantain peel silver nanoparticles)**.**

Characterization of Silver nanoparticles

The UV-vis spectrum of nanoparticles was recorded on DU 730 UV-Vis spectrophotometer (Beckman Coulter, USA) by taking 0.1 ml of the sample and diluting it with 2 ml distilled water at intervals of 15 minutes for two hours. The time of reaction was measured in the region ranging from 200- 1000 nm, while the morphology and size distribution of the synthesized silver nanoparticles was characterised using scanning electron microscope (SEM)JSM 7800F prime- JSM 7200F.

Field Experiment

Experiments were conducted in two planting seasons between the months of August to December in the year

2016 and 2017, at the University of Ilorin teaching and research farm Lat 8^{p}, 29^{1} N of the Equator; Long: 4^{p}, 40^{1} E of the Greenwich Meridian. The experimental field was 30 m x 25 m in size, this was harrowed and sixty beds of 1.3 m^{2}raised to a height of 15 cm each were made. The experiment was a randomised complete block design (RCBD). There were five treatments applied at four dosages (levels) and each of these was replicated three times. Tomato 'Roma' seeds were raised in the nursery and the seedlings were transplanted to the field at two weeks after emergence at a spacing of 25 cm apart and 50 cm between the rows. Pure populations of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949raised on *Celosia argentea* (L.) were extracted using the method of Hussey and Baker (1973), the extracted eggs and juveniles served as source of inoculums. Approximately 500 eggs and juveniles per ml of *M. incognita* were inoculated around the base of each tomato plant (Fabiyi, 2019) at a week after transplanting. Treatments were applied at a depth of 1.5 inch below the ground at the base of each tomato plant a week after inoculation at 0, 30, 60 and 90mls which is equivalent to 60,120 and 180mM respectively for the silver nanoparticles, while plantain peel derivatized and plantain peel n-hexane extract was at 0, 30, 60 and 90 ml. Plantain peel powder was applied at 0, 30, 60 and 90 g and carbofuran at 0.5kg ai/ha,1.0kg ai/ha and 1.5kg ai/ha. The plantain peel n-hexane extract was emulsified with a surfactant before application. Weeds were removed manually throughout the planting season. Vegetative growth was monitored over a period of eight weeks by data on numbers of leaves and plant height. At harvest, tomato yield was weighed, while nematode population in root and soil was counted. The method of Bridge and Page, (1980) was employed for root gall rating on a scale of 0-10, where 0- root knots absent, 1- very small invisible knots, 2-small but very visible with main roots clean, 3-knots largely visible with main roots clean, 4-large root knots abound with main roots clean, 5-50% of roots affected, with knots on main root, 6-main root knotted, 7-a handful of main root knotted, 8-all parts of main root knotted, few clean roots visible, 9-severe root knots on root system, 10-severe root knots all over, plant may be dead. Differences among mean values were analysed by anova and means were separated with Duncan's multiple range test.

RESULTS

Spectroscopy

The stability and formation of silver nanoparticles was confirmed from the UV-Vis spectroscopy with a Surface Plasmon Resonance (SPR)absorption band at ë 415 nm, while the changes in colour during the reaction also affirm that nanoparticles have been formed. The SEM micrograph shows the conglomeration, size and shape of the nanoparticles at x 10,000 and x 20, 000 magnifications with nano size 10nm and 5 nm respectively. Figs 13 & 14 shows the micrograph of the plantain peel extract mediated silver nanoparticles. The result depicts oval and planar shaped nanoparticles.

Field Experiment

The results of the various treatments from plantain peel extract and dosages of treatment applications are depicted in figures 1 to 12. Significantly (P=0.05) taller plants, more leaves and heavier fruits were observed in experimental beds treated with plantain peel mediated silver nanoparticles as against those with plantain peel powder and plantain peel n-hexane extract (Figs. 1, 3 &5), while the untreated control plots had short stunted plants, fewer numbers of leaves and light weight fruits. At fourteen weeks after planting (WAP), tomato plants treated with plantain peel mediated silver nanoparticle (PLTP/Nano) had longer vine length 75.03cm (Fig.1) as against the untreated (0) control plots which had short vine length(44.17cm)on the average (Fig. 2).Similarly, numbers of leaves increased significantly over the weeks. Tomato plants treated with highest dosage (90ml) of treatment applications produced more numbers of leaves (Fig. 4). Lower yield per plant was recorded in tomato

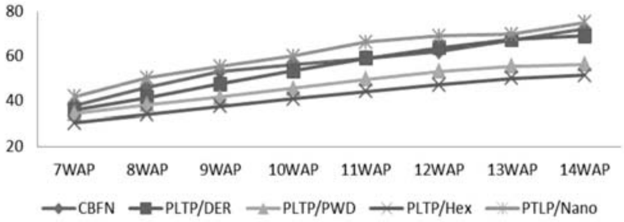

Fig. 1. Effect of different extracts of plantain peels on plant height of tomato

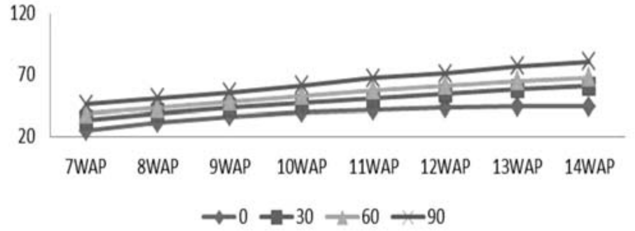

Fig. 2. Effect of different doses of plantain peel extracts on height of tomato

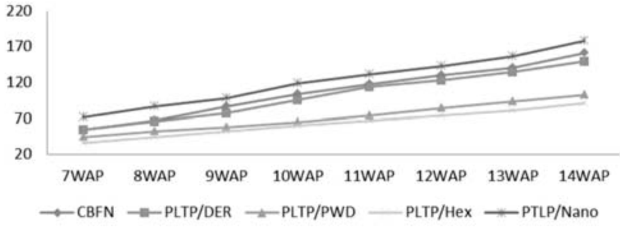

Fig. 3. Effect of different extracts of plantain peels on numbers of leaves of tomato

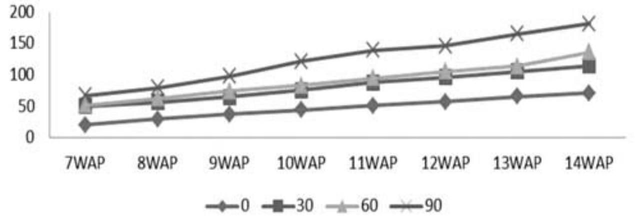

Fig. 4. Effect of different doses of plantain peel extracts on leaf number of tomato

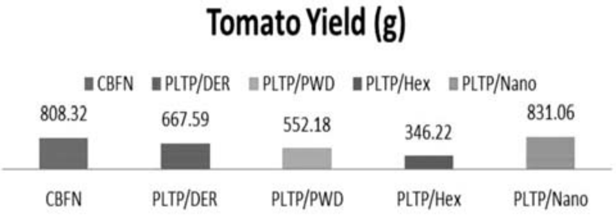

Fig. 5. Effect of different extracts of plantain peels on yield of tomato

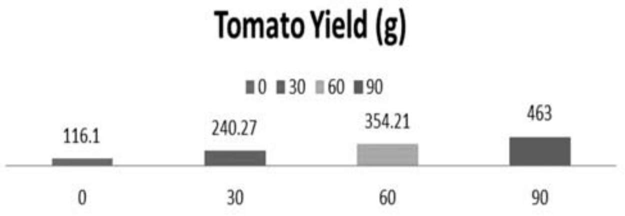

Fig. 6. Effect of different doses of plantain peel extracts on yield of tomato

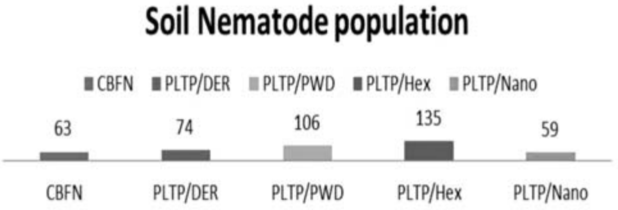

Fig. 7. Effect of different extracts of plantain peels on soil nematode population of tomato plants

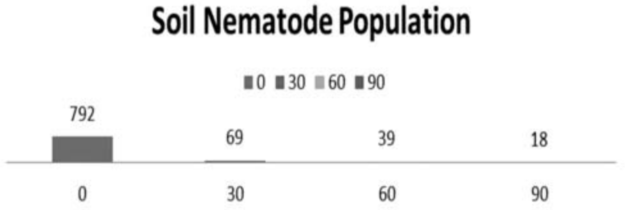

Fig. 8. Effect of different doses of plantain peel extracts on soil nematode population of tomato plants

plants treated with plantain peel hexane extract (PLTP/Hex). Carbofuran (CBFN) and plantain peel extract mediated silver nanoparticles (PLTP/Nano) treated tomato plants had higher yield (Fig. 5) per plant. Population of *Meloidogyne incognita* in soil and roots of treated tomato plants was significantly (P=0.05) lower as opposed to the untreated control (0) plants (Figs.8 &10). The highest dosage of treatment application(90ml)

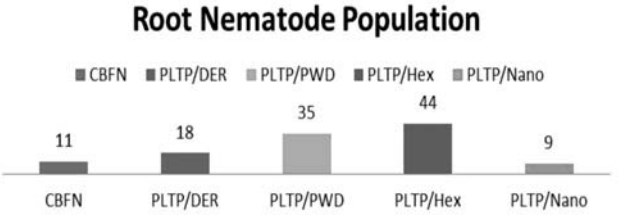

Fig. 9. Effect of different extracts of plantain peels on root nematode population of tomato plants

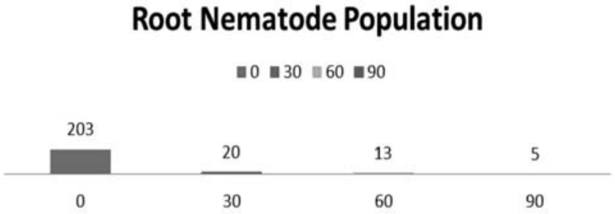

Fig. 10. Effect of different doses of plantain peel extracts on root nematode population of tomato plants

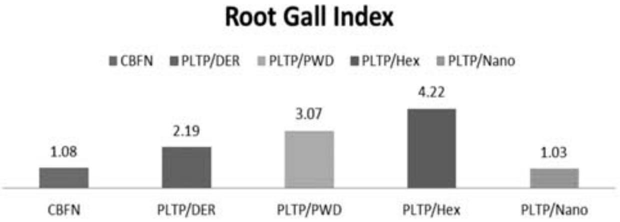

Fig. 11. Effect of different extracts of plantain peels on gall index of tomato plants

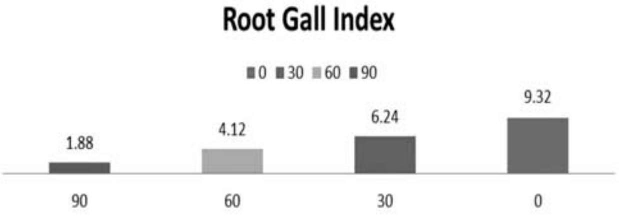

Fig. 12. Effect of different doses of plantain peel extracts on root gall index of tomato plants

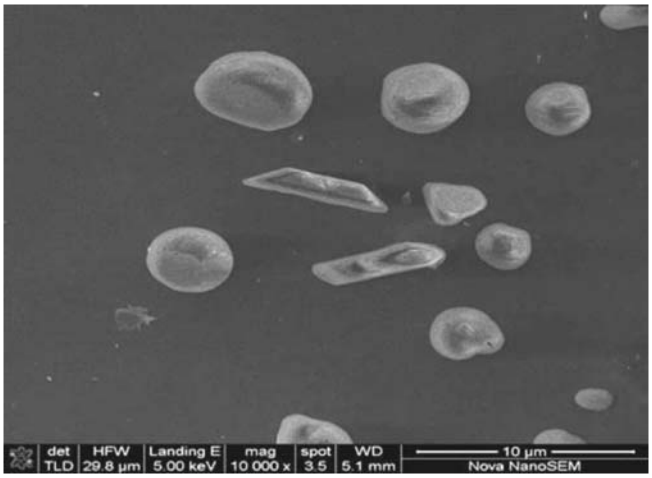

Fig. 13. S.E.M. micrograph of Plantain peels silver nanoparticles at x 10,000 magnifications

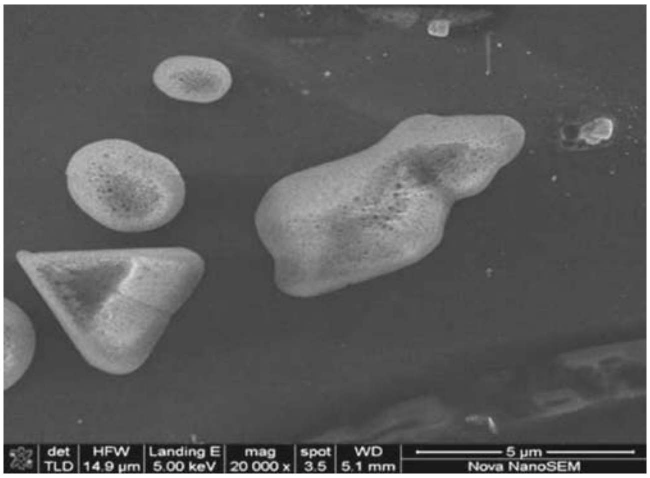

Fig. 14. S.E.M. micrograph of Plantain peels silver nanoparticles at x 20, 000 magnifications

significantly suppressed nematode population in soil and root. Reduced growth, lower yield and higher root gall index was observed in (0) untreated plants (Figs 4, 6 &12). Each treatment significantly reduced gall formation and gall index on tomato roots. Generally, the plantain peel mediated silver nanoparticles had a significantly positive effect on the growth parameters and was comparable to carbofuran, with the ability to reduce nematode population in root and soil (Figs. 7 & 9). The highest dosage (90 ml) of treatment application (P=0.05) significantly improved vegetative growth. Maximum reduction of nematode population was achieved in the plantain peel mediated silver nanoparticles at 90mls.

DISCUSSION

The ethanol extract of plantain peel act as a reducing and stabilising agent for the silver nanoparticles formed. The observed result could be attributed to the phytochemical constituents in the peels of plantain. Several researchers have reported a number of secondary metabolites in plantain peels. Akinsanmi *et al.,* (2015) reported the presence of tannin, saponin, alkaloids, phenols and flavonoids in the peels of plantain, which was corroborated by Velumani, (2016). Phytochemicals are known to act as reducing, capping and stabilising agents in green and bio- based synthesis of silver nanoparticles (Barros *et al.,* 2018), while the reducing and stabilising agents are responsible for the size, distribution and morphology of nanoparticles (Zhang *et al*., 2006). The ability of banana peel extract to act as a reducing and stabilizing agent was established by Aminuzzaman *et al.,* (2017) in their study on the preparation of copper oxide nanoparticles, in the same vein Bankar *et al.,*(2010) and Sunardi *et al.,*(2017) employed banana peel extract as a reducing and stabilising agent in the synthesis of gold nanoparticles and Zero Valent Iron (nZVI) respectively, while efficient antimicrobial activity was displayed by the gold nanoparticles on fungal and bacterial cultures. The silver nanoparticles exhibited significantly better nematicidal activity which is comparable to the standard carbofuran. This corroborates the findings of Fabiyi and Olatunji (2018) on the application of silver nanoparticles in the management of *M. incognita* on groundnut plants. Likewise, silver nanoparticles was reported to reduce nematode population and improve rice growth (Fabiyi *et al.,* 2020b). The efficacy of nanoparticles has been reported to be dependent on characteristics such as shape, size and surface morphology (Gurunathan *et al*., 2009). The antimicrobial effect of silver nanoparticles towards *S. aureus* was studied by Helmlinger *et al.,* (2016). They demonstrated that the release of silver ions and shape of silver nanoparticles has a direct effect on the microbial activity of silver nanoparticles. Kokila *et al.,* (2015) documented the bactericidal effect of banana peel mediated silver nanoparticles against gram negative and gram-positive bacteria. The insecticidal properties of hematite (á-Fe2O3) nanoparticles were reported by Hassan *etal.,* (2018) a 100% mortality was recorded in 72 hours with an increase in concentration. Similarly, the broad-spectrum antibiotics of nickel and nickel oxide nanoparticles against *E. coli* and *Bacillus subtilus* was reported by Din *et al.,* (2018). The population of *C. elegans* was reduced to less than 50% within 72 hours of exposure to size-turnable gold nanoparticles; a 60% reduction in brood size of the worm was also observed (Chun-Chin Hu *et al*., 2018). Methyl benzoate the derivatized product also exhibited nematicidal activity than other treatments, Pandey (2011), reported 99% mortality of *M. incognita* juveniles at500 µg/ mlafter24hours with benzoates. Similarly, Xingkai Cheng *et al.,* (2015), reported the effectiveness of emamecting benzoate on plant parasitic nematodes. They observed greater than 70% mortality of *M. incongita* juveniles and a corresponding reduction in root gall index in the green house and field trials. Agricultural wastes as soil amendments offer alternative method of control in plant parasitic nematode (PPN) management. Simple phenolics and flavonoids have been reported widely as nematicidal compounds (D'Addabbo *et al.,* 2010). Agricultural waste compost materials have been widely used in nematode control (Fabiyi, 2018).Plantain peels contain a good percentage of carbon while decaying, municipal wastes and plant materials with high nitrogen content have been documented to reduce nematode population in soil (Porazinska *et al.,* 1999; Wang *et al.,* 2007; Omoniyi *et al.,* 2017). Decomposing plant materials release toxic compounds such as nitrogen compounds and organic acids whichhave adverse effects on nematodes and have been used widely in the control of plant parasitic nematodes (McSorley, 2011; Thoden *et al.,* 2011). The antifungal property of plantain peel extract was reported by Okorondu *et al.,* (2012) and Prakash *et al.,* (2017), while the antibacterial activities of banana peel was observed by Ehiowemwenguan *et al.,* (2014)and Suraj *et al.,* (2015). Incorporation of plantain peels into the soil by farmers is economically viable, and this will amount to a valuable utilization of plantain wastes while at the same time enhancing solid waste management and sustaining the environment by reducing pollution, which arises from the improper disposal of plantain peels.

CONCLUSION

The use of plantain peel mediated silver nanoparticles will go a long way in reducing the environmental pollution of synthetic nematicides. Nematode management systems with the use of agricultural wastes can be implemented in tomato production. Plantain peels are a potential source of nutrients and phytochemicals which can enrich the soil while reducing proliferation of crop damaging soil micro-organisms. Amending the soil with plantain peels will enhance soil structure and improve soil fertility.

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Effect of Physico-Chemical Properties of Soil on Rice Root-Knot Nematode (*Meloidogyne graminicola*) in Major Rice Growing Districts of Karnataka, India

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ABSTRACT: The present study was conducted to find out the relationship of physico-chemical properties *viz.,*pH, electrical conductivity (EC), organic carbon (OC), soil moisture, nitrogen, phosphorus, potassium and micro nutrients content of rice growing soil with incidence of rice root-knot nematode (*Meloidogyne graminicola*) of rice. To investigate the effect of physicochemical properties of soil on incidence of Rice root-knot nematode (*Meloidogyne graminicola*)in major rice growing districts of Karnataka state*viz.,*Shivamogga, Chikmagaluru, Davanagere, Mandya, Kodagu, Udupi and Karnataka. Systematic surveys were during *Kharif*-2015 and 2016 in major rice growing districts and estimated nematode population and analyzed physicochemical properties of soil under laboratory. The results revealed thatin Chi-kadadkatte village of Davanagere district, V.C Farm of Mandya district, Tuduru, Beguvalli, Hasudi village of Shivamogga, Karagunda, Lakkavalli of Chickmagaluru and Mikere village of Kodagu district recorded highest incidence with nematode population ranging between 586.33 to 841.00 J2 / 200 cc of soil and Root Knot Indices(RKI) varying from 3 to 4. These locations had sandy loamy soil, with acidic soil pH, lower soil organic carbon, phosphorous and potassium and higher nitrogen and moisture content. Analyses of correlation of coefficient showed that population of *M. graminicola* had significantly positive correlation with soil moisture, nitrogen, soil organic carbon at ($P \leq 0.01$) while significantly negative correlation with other physico chemical properties of soil viz., soil pH, soil EC, phosphorus, potassium, sulpher, zinc and manganese. The results of the present study show that the sandy loamy soil, with acidic soil pH, lower soil organic carbon, higher nitrogen, lower phosphorous and potassium and higher moisture play a major role in survival of *M. graminicola* and influence in incidence of root-knot disease incidence in rice.

Keywords: *M. graminicola*, physicochemical properties of soil, rice, rice root-knot nematode, soil pH, soil organic carbon survey, soil

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Rice (*Oryza sativa* L.) is one of the most important staple food crops of India and is a major source of calories for about 60 per cent of world population and influences the livelihoods and economies of several billion people especially concentrated in Asia, Latin America, Middle East, and the West Indies. For centuries, rice has shaped asian societies and their cultures. Asian cultures are partly cultures of rice and many asian societies relate to rice beyond the satisfaction of basic needs. Rice crop is affected by several biotic and abiotic stresses, of which, plant-parasitic nematodes constitute an important component (Pankaj *et al.,* 2010; Sehgal and Narasimhamurthy, 2021). Over 200 species of plant parasitic nematodes have been reported to be associated with rice (Prot and Rahman, 1994) and are becoming increasingly important in the rapidly changing production system of rice (Coyne and Plowright, 2000). Rice root-knot nematode, *M. graminicola* Golden and Birchfield 1965 has emerged as a pest of international importance (De Waele and Elsen, 2007). In rice, the environmental conditions and type of agro-system determine the community of nematodes present and, consequently, the species profile of parasitic nematodes can differ from region to region (Prot and Rahman, 1994). Many nematode species have been described in association with rice, but only a few of these have significant detrimental effects (Kyndt*et al.,* 2014). *M. graminicola*is one of the most prevalent plant parasitic nematode in rice agro-systems. It is considered to be a major threat to rice agriculture, particularly in Asia, where changes in agricultural practices in response to environmental (climate change) and socioeconomic conditions have led to a dramatic increase in populations (De Waele and Elsen, 2007).

M. graminicola is a devastating plant pathogen, and is therefore classified as a quarantine pest in many countries. Rice is the most important host for *M. graminicola*, but the nematode has a wide range of alternative hosts (Bridge *et al.,* 2005). This nematode is frequently found associated with other cereals, as well as dicotyledonous and grass plants, including many weeds commonly found in rice fields that may constitute a major reservoir of nematodes (Rich *et al.,* 2009). The population densities of *M. graminicola*, fluctuate throughout the year. The most important factors responsible for these fluctuations are various soil factors such as, soil moisture, soil temperature and soil pH; climatic factors namely, rainfall and air temperature; host-plant growth stage; and crop cycle duration (Wallace, 1973, Robbins and Baker, 1974, Kyi*et al.,* 2001, Siddiqui, 2007). Hence, present studies were conducted with the objective to determine the effect of physicochemical properties of soil on incidence of rice root-knot nematode in major rice growing districts of Karnataka.

MATERIALS AND METHODS

Survey

Systematic surveys were conducted during 2015 and 16 (Fig. 1). To find out the incidence and to estimate nematode population (*M. graminicola)* in soil and the part of the soil was used for analyses of physicochemical properties of soil. While surveying, the following parameters were recorded such as, soil types, variety, sources of irrigation, soil moisture, GIS/GPS information (Latitude and longitude) of the surveyed location.

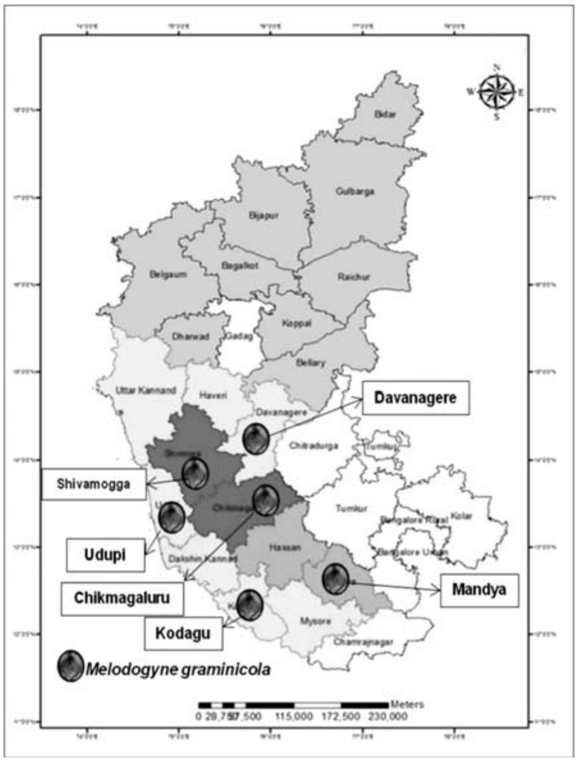

Fig. 1. Location of sampling sites from different district of Karnataka

Collection of sample

During the survey, rice plants in both nursery and main field showing uneven patches with yellowing, stunted growth, reduced tillering with galls on roots were observed. The plants in infested patches were dried up early. Such plants were selected for sampling.

Estimation of nematode population and Root Knot Index

From the collected composite soil sample 200cc was thoroughly mixed in 2 L water and stirred the suspension that allow the heavy soil particles to settle down for about 5 sec. nematodes were extracted by using Cobb's sieving and decanting technique (Cobb, 1918) followed by Modified Baermann's funnel method (Whitehead and Hemming, 1965) and Root knot index was recorded according to the number of galls per root system in which 0 = No galls (Immune) 1 =1-2 galls / root system (Resistant), 2=3-10 galls/root system (Moderately resistant) 3= 11- 30 galls /root system (Moderately susceptible) 4= 31-100 galls/root system (Susceptible) and 5=>100 galls/root system (Highly susceptible) (Taylor and Sasser, 1978).

Soil analysis: During survey soil samples collected from different locations were marked and packed in polythene bags brought to the laboratory (Organic Farming Research Centre, ZAHRS, Shivamogga) and estimated physico-chemical properties of soil such as soil pH, soil organic carbon, soil EC, major and micronutrients *viz.* available nitrogen, phosphorous, potassium, sulphur, zinc, copper, iron and manganese by using standard methodology given below in Table 1.

Soil moisture: This was expressed in percentage on dry weight basis. A known amount of soil sample was taken from the different locality during survey and it was dried in oven at 80°C for 48 h and weighed again. The percentage moisture content was calculated by using the following equation

$$\text{Soil moisture}(\%) = \frac{\text{Loss of weight on drying}}{\text{Dry weight of soil}} \times 100$$

Statistical Analysis of data

The data obtained from the present investigations were subjected to statistical analysis. By using SPPSS 15.0 software.

Ethical Approval and/or Informed Consent

This article does not contain any studies with human participants or animals by any of the authors.

RESULTS

The results revealed that the incidence and population level of nematodes varies from location to location. Maximum nematode population 586.33 to 841.00 J_2/ 100 cc of soil observed in Chi-kadadkatte village (14°12'06.6"N/ 75°67'29.3"E) of Davanagere district,

Table 1. Method used to study the chemical properties of soils

Soil properties	Method of estimation
pH(1:2)	Blackman's glass electrode pH meter (soil: water: 1:2.5)
$EC_{(1:2)}$ ($dS\ m^{-3}$)	Solubridge conductivity meter (soil: water: 1:2), Jackson (1967)
OC (%)	Walkley and Black's (1934) wet oxidation method as described by Jackson, 1967.
Available N (Kg ha^{-1})	Alkaline potassium permagnate (Subbiah and Asija, 1956)
Available P (Kg ha^{-1})	Sodium bicarbonate extractable P (Olsen *et al.,* 1954)
Available K (Kg ha^{-1})	Neutral normal ammonium acetate extraction method (Jackson, 1973).
Available S (Kg ha^{-1})	Turbidometric (Jackson, 1973)
Available micronutrients' (mg kg^{-1})	
Fe	DTPA extractable using AAS as described byLindsay and Norvell (1978)
Cu	
Zn	

V.C Farm village (12°31’12.4"N/ 76°53’58.5"E) of Mandya district, Tuduru (13°71’54.9"N/75°37’49.2"E), Beguvalli (13°70’46.2"N/ 75°40’24.2"E), Hasudi (13°92’19.8"N/ 75°64’50.3"E) village of Shivamogga, Karagunda (13°24’85.2"N/ 76°20’44.5"E), Lakkavalli (13°69’86.9"N/75°54’43.0"E) village of Chickmagaluru district and Mikere of Kodagu district and least nematode population 210.56 and 240.66 J_2 100 cc of soil was observed in Kumsi of Shivamogga and Vadeyarhatturru of Davanagere district. This study was conducted to know the survival strategies of *M. graminicola* under different soil types, soil pH, soil EC, soil organic carbon, different soil moisture regime and other chemical properties of soil, *viz.,* major nutrients namely, nitrogen, phosphorous, potassium, sulphurand micro nutrient such as iron, zinc, copper and manganese. The results were presented in Table 2 and 3.

Effect of physicochemical properties of soil on population of *M. graminicola*

Effect of soil type, soil pH, EC and soil moisture level on incidence of M. graminicola

Effect of soil type

The locations having sandy loamy soil *viz.,*Chi-kadadkatte of Davanagere, Hosudi, Beguvalli and Thuduru of Shivamogga, Karagunda and Lakkavalli of Chickmagaluru, V.C.Farm, Akkihebbal of Mandya, Mekeri and Kaggodu of Kodagu district recorded highest nematode population ranging from 486.00-841.00 J2/200cc of soil. However, the locationsKumsi and Konehossur of Shivamogga and Megaramakki of Chickmagaluruhaving clay loamy soil recorded least nematode population 210.56-354.65 J2/200cc of soil (Table 2).

Effect of soil pH

The Locations Chi-kadadkatte of Davanagere, recorded highest nematode population (841.00 J2/200cc of soil) having soil pH (4.41) similarly, Hosudi, Beguvalli and Thuduru of Shivamogga recorded nematode population ranging between (612.33-748.52 J2/200cc of soil) with pH range (5.29-7.39). However, the same trend was observed in Karagunda (13°24’85.2"N/ 76°20’44.5"E) and Lakkavalli (13°69’86.9"N/ 75°54’43.0"E) of Chickmagaluru, V.C.Farm (12°31’12.4"N/ 76°53’58.5"E), Akkihebbal (12°62’26.9"N/76°38’97.7"N) of Mandya district, Mekeri (12°39’23.3"N/75°73’42.4"E) and Kaggodu (12°40’03.6"N/75°78’07.5"E) village of Kodagu district recorded highest nematode population ranging from (465.66-658.66 J2/200cc of soil) with pH ranging between 6.07-7.94. However, Gowthampura village (14°15’35.5"N/75°21’72.5"E) ofShivamogga district, Doddakunduru(13°66’90.0"N/75°67’26.2"E) village of Chickmagaluru district and Kolagadalu (12°37’75.4"N/ 75°61’49.8"E) village of Kodagu district recorded least nematode population (256.66, 310.25 and 352.33) respectively with soil pH ranging from 8.03-8.73. From the result it is evident that the pH range 4.41-7.94 favors the survival of *M. graminicola* population (Fig. 2).

Effect of soil EC

The highest nematode population was recorded in Chi-kadadkatte of Davanagere (841.00), Hosudi (748.52), Beguvalli (612.33) of Shivamogga, V.C.Farm (806.33), Chotnahalli (486.33) and Akkihebbal (654.68) of Mandya with soil EC ranging between 0.040-0.144 dsm^{-1}. However, the least nematode population was recorded in Kumsi and Gowthampura of Shivamogga and Chinnasamudra of Davanagere district ranging between (210.56-256.66 J2/200cc of soil) with soil EC rangining from 0.06-0.33 dSm^{-1} (Fig. 3).

Effect of soil moisture on survival of M. graminicola

Results from theTable 2 and Figure4 revealed that the highest nematode population was recorded at soil moisture ranging from (24.00-33.64%). In Chi-kadadkatte

Table 2. Effect of soil types, soil pH, soil EC and soil moisture on survival of *M. graminicola*

Sl. No.	District	Taluk	Village	Latitude/Longitude	Soil type	Variety	Soil pH	Soil EC (dSm^{-1})	OC (%)	Soil moisture (%)	Nematode population/ 200cc of soil
1	Davanagere	Honnali	Chi-kadadkatte	14°12’06.6"N/ 75°67’29.3"E	Sandy loam	Jyothi	4.41	0.042	1.65	32.00	841.00
2			Vadayarahatturu	14°12’48.1"N/ 75°63’02.6"E	Sandy loam	Jyothi	6.15	0.057	0.67	24.21	240.66
3			Doddethinahalli	14°13’82.8"N/ 75°59’53.4"E	Sandy loam	SannaAkki	5.73	0.049	0.59	25.30	324.60
4		Davanagere	Malebennur	14°34’98.8"N/ 75°74’02.6"E	Sandy loam	Jyothi	6.43	0.084	0.71	20.13	273.25
5			Chinnasamudra	14°35’89.4"N/ 76°07’90.7"E	Sandy loam	Ankursona	5.96	0.061	0.53	22.65	244.52
6			Dodderahalli	14°23’94.2"N/ 75°60’36.1"E	Sandy loam	MTU 1001	6.17	0.079	0.74	21.33	310.00
7	Shivamogga	Shivamogga	Kumsi	14°05 92.6"N/ 75°39’10.3"E	Clay loam	JJL	5.71	0.033	0.56	18.20	210.56
8			Konehossur	14°10' 07.0"N/ 75°28’75.4"E	Clay loam	Bhagyajyothi	5.84	0.036	0.51	23.45	286.33
9			Gowthampura	14°15’35.5"N/ 75°21’72.5"E	Sandy loam	JGL	8.73	0.220	1.17	22.21	256.66
10		Theerthahalli	Hosudi	13°92’19.8"N/ 75°64’50.3"E	Sandy loam	Jyothi	5.29	0.040	0.48	28.32	748.52
11			Beguvalli	13°70’46.2"N/ 75°40’24.2"E	Sandy loam	MTU 1001	6.68	0.136	0.56	25.60	612.33
12			Thuduru	13°71’54.9"N/ 75°37’49.2"E	Sandy loam	Jyothi	7.39	0.196	0.67	24.44	586.33
13	Chikmagaluru	N.R. Pura	Karagunda	13°24’85.2"N/ 76°20’44.5"E	Sandy loam	Jyothi	7.94	0.143	0.71	27.60	658.66
14			Megaramakki	13°41’49.0"N/ 75°46’06.9"E	Clay loam	Jyothi	8.19	0.237	1.08	21.33	354.65
15		Tarikere	Doddakunduru	13°66’90.0"N/ 75°67’26.2"E	Sandy loam	BPT Sona	8.06	0.170	1.10	20.00	310.25
16			Lakkavalli	13°69’86.9"N/ 75°54’43.0"E	Sandy loam	IET	6.14	0.087	0.42	26.56	586.33
17			Rangenahalli	13°70’59.5"N/ 75°69’86.2"E	Sandy loam	Jyothi	6.12	0.074	0.51	19.50	342.66
18	Mandya	Mandya	V.C.Farm, ZARS	12°31’12.4"N/ 76°53’58.5"E	Red Sandy	Jyothi	5.60	0.050	0.45	29.33	806.33
19			Chottanahalli	12°66'28.4"N/ 76°96'14.2"N	Sandy loam	Jyothi	6.07	0.070	0.53	24.22	486.23
20		K.R. Pet	Akkihebbal	12°62'26.9"N/ 76°38'97.7"N	Sandy loam	Jyothi	6.87	0.144	0.60	33.64	654.68
21	Kodagu	Madikere	Kaggodu	12°40’03.6"N/ 75°78’07.5"E	Sandy loam	Jyothi	6.98	0.143	0.65	25.63	486.33
22			Mekeri	12°39’23.3"N/ 75°73’42.4"E	Sandy loam	Jyothi	5.12	0.250	0.63	24.00	582.00
23			Kolagadalu	12°37’75.4"N/ 75°61’49.8"E	Sandy loam	Jyothi	8.03	0.211	1.16	22.00	352.33
24	Udupi	Bhramhavara	Santhekatte	13°45’02.8"N/ 74°92’43.2"E	Sandy loam	Jyothi	7.89	0.179	0.81	20.11	310.66
25			Innanje	13°24’67.3"N/ 74°77’01.1"E	Sandy loam	Jyothi	6.98	0.143	0.58	24.38	465.66
26			Mandarthi	13°49’67.9"N/ 74°80’97.7"E	Sandy loam	Jyothi	7.36	0.156	0.67	22.56	367.33

Table 3. Effect of chemical properties of soil on survival of *M. graminicola*

Village	Available (N)	Available (P)	Available (K)	Available (S)	Available (Zn)	Available (Fe)	Available (Cu)	Available (Mn)	J2 population/ 200cc of soil
		(kg/ha)				(ppm)			
Chi-kadadkatte	282.24	18.67	112.08	6.01	1.36	42.84	19.00	2.21	841.00
Vadayarahatturu	238.34	39.50	259.66	7.88	1.34	42.60	39.56	4.09	240.66
Doddethinahalli	200.70	50.14	205.23	7.20	0.93	50.36	43.00	4.85	324.60
Malebennur	225.79	41.55	288.96	7.48	1.57	47.76	34.62	4.24	273.25
Chinnasamudra	206.98	37.96	237.75	8.62	1.08	39.44	33.26	4.60	244.52
Dodderahalli	181.89	31.80	251.60	9.14	1.29	44.16	37.04	3.37	310.00
Kumsi	206.98	36.42	188.43	8.36	1.11	40.78	27.15	3.55	210.56
Konehossur	175.62	38.78	224.58	7.79	0.94	38.72	27.44	4.13	286.33
Gowthampura	351.23	57.30	467.58	8.14	1.99	19.84	14.62	2.32	256.66
Hosudi	263.42	35.14	191.25	8.37	1.01	53.14	48.13	4.31	748.52
Beguvalli	388.86	34.11	236.28	14.05	1.23	45.66	47.36	3.71	612.33
Thuduru	257.15	36.91	270.68	13.18	1.34	38.60	35.84	3.25	586.33
Karagunda	269.70	41.29	333.31	10.98	1.47	28.52	18.08	3.03	658.66
Megaramakki	156.80	64.38	356.97	13.48	1.68	24.88	15.08	2.44	354.65
Doddakunduru	200.70	53.09	336.67	11.88	1.52	20.86	15.13	2.27	310.25
Lakkavalli	169.34	34.88	217.73	9.28	1.19	45.20	30.92	3.48	586.33
Rangenahalli	188.16	37.70	233.05	9.08	1.02	42.40	39.87	3.05	342.66
V.C.Farm, ZARS	370.05	32.83	195.01	6.05	1.12	48.80	43.88	3.78	806.33
Chottanahalli	200.70	37.19	225.39	10.20	1.23	44.16	35.20	3.48	486.23
Akkihebbal	219.52	43.35	317.86	12.03	1.49	46.20	41.14	2.10	654.68
Kaggodu	257.15	45.65	336.13	11.83	1.48	49.96	25.04	2.49	486.33
Mekeri	351.23	40.01	233.32	10.05	1.22	39.90	31.21	3.87	582.00
Kolagadalu	294.78	63.86	383.98	17.25	1.44	24.00	19.37	2.32	352.33
Santhekatte	225.79	39.50	348.10	14.65	1.30	29.25	24.55	1.69	310.66
Innanje	282.24	33.86	277.54	11.00	1.43	40.40	43.22	2.43	465.66
Mandarthi	269.70	42.68	300.25	13.63	1.32	33.46	25.77	3.01	367.33

of Davanagere, Hosudi, Beguvalli and Thuduru of Shivamogga, Karagunda and Lakkavalli of Chickmagaluru, V.C.Farm, ZARS, Akkihebbal of Mandya, Mekeri and Kaggodu of Kodagu district with population ranging from 486.00-841.00 J2/200cc of soil. Whereas lowest nematode population was recorded in Kumsi and Gowthampura of Shivamogga and Chinnasamudra of Davanagere district ranging between (210.56-256.66 J2/200cc of soil) with soil moisture ranging from 18.20-22.65 percent.From the result it is evident that the moisture range (24.00-33.64 %) favors the growth of nematode population and higher nematode population was noticed in this regime. Whereas, the moisture level (18-22 %) less favors the nematode population.

Effect of other chemical properties of soil on M. graminicola population

Effect of soil organic carbon (OC)

Highest nematode population was recorded in Chi-kadadkatte village of Davanagere district (841.00), Hosudi (748.52), Beguvalli (612.33) village of Shivamogga district, V.C.Farm, ZARS (806.33), Chotnahalli (486.33) and Akkihebbal (654.68) village of Mandya district with soil OC ranging from 0.42-1.65 Per cent. (Table 2; Fig. 5).

Effect of available Nitrogen (Kg/ha)

The data presented in Table 3 indicated that where the fields *viz.,* Chi-kadadkatte of Davanagere, Hosudi, Beguvalli of Shivamogga, V.C.Farm, Chotnahalli and Akkihebbal of Mandya district received the higher dose of nitrogen (200.70- 388.86 kg/ha) the population varies in the range of 486.23- 841.00 J2/200cc of soil. However, the fields received lower dose of nitrogen less than 200 kg/ha recorded least nematode population (Fig. 6).

Effect of available Phosphorus (Kg/ha)

The highest nematode population ranging (486.23-841.00 J2/200cc of soil) was observed in the fields having lowest phosphorus in Chi-kadadkatte (18.67 kg/ha) of Davanagere, Hosudi (35.14), Beguvalli (34.11) of Shivamogga, V.C.Farm (32.83), Chotnahalli (37.19) and Akkihebbal (43.35) of Mandya respectively (Fig. 7).

Effect of available Potassium (Kg/ha)

Significant differences was observed among the level of potassium on effect of nematode population (Fig. 8) where the fields recorded highest nematode population (465.66-841J2/200cc soil) received lower dose of potassium ranging from 112.08-277.54 kg/ha. Similarly, the fields where recorded least nematode population was received higher dose of potassium ranging from 300.25-469.58 kg/ha.

Effect of available sulphur (Kg/ha)

The results indicated in the Table 3 revealed that there was no influence of sulphur on nematode population. The highest nematode population was observed in both lower as well as higher dose of sulphur received fields (Fig. 9).

Effect of available micronutrients (Zn, Fe, Cu and Mn)

The perusal of the data given in the Table 3 and Figs. 10,11,12 and 13 clearly shown that there was no influence of nematode population was observed with respect micronutrients, highest or lowest of nematode population was observed at irrespective level of micronutrients.

Relationship between physicochemical properties of soil with nematode population

Correlation studies of important physical and chemical properties of soil with nematode population revealed that soil pH was negatively correlated with nematode population (r=0.333**, R^2= 0.115) at ($P \leq 0.01$) level of significance, Soil EC was negatively correlated non significance (r= -0.055, R^2 = 0.002) with nematode

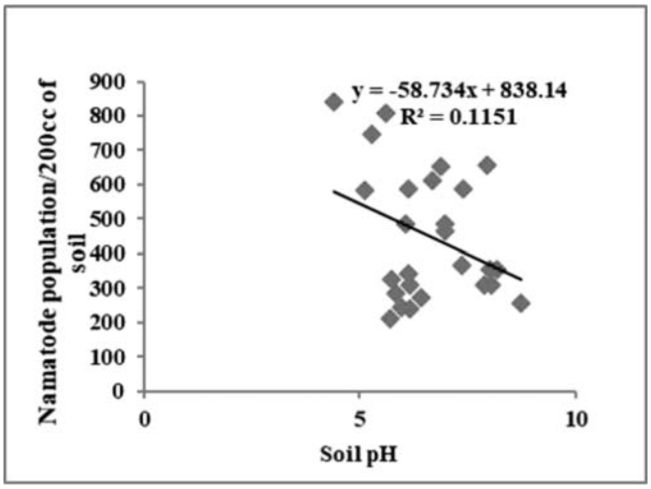

Fig. 2. Effect of soil pH on nematode population

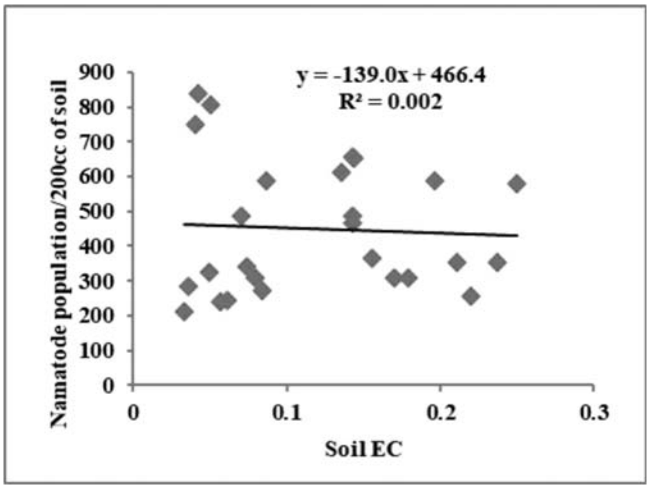

Fig. 3. Effect of soil EC on nematode population

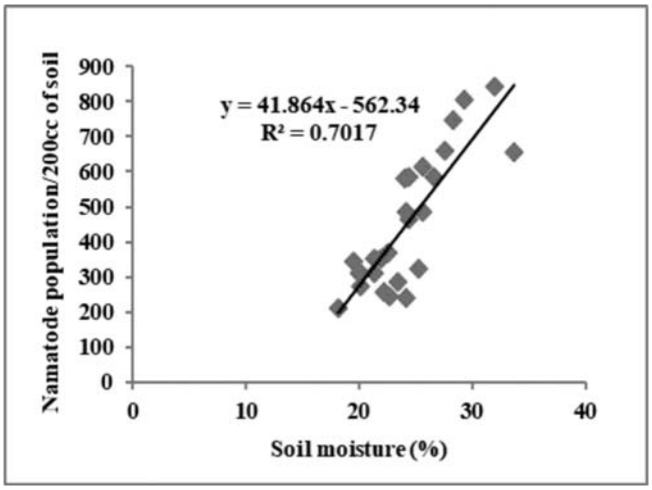

Fig. 4. Effect of soil moisture on nematode population

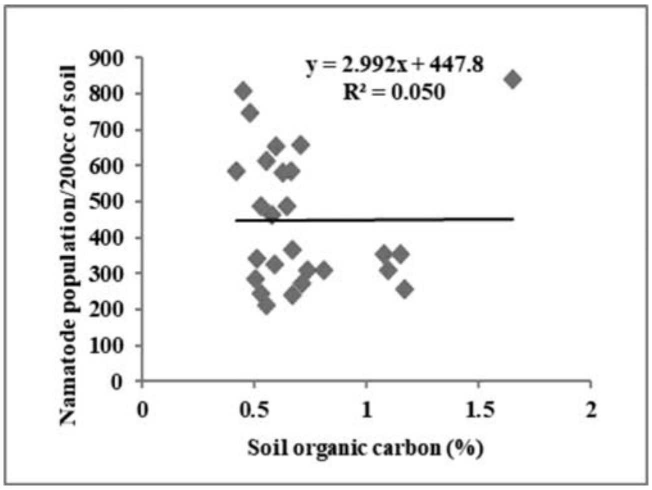

Fig. 5. Effect of soil OC on nematode population

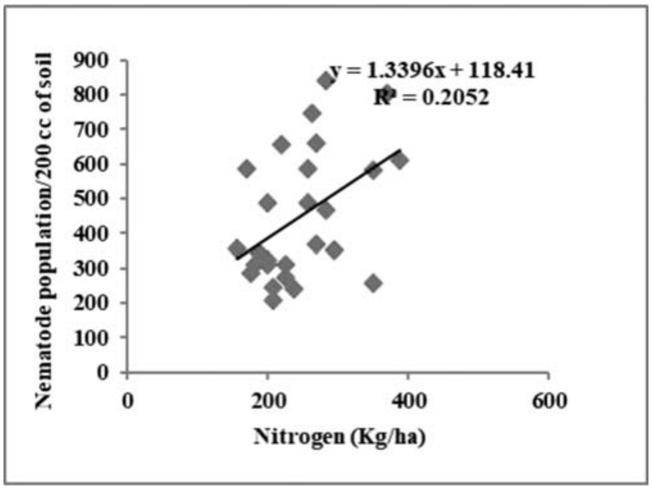

Fig. 6. Effect of Nitrogen on nematode population

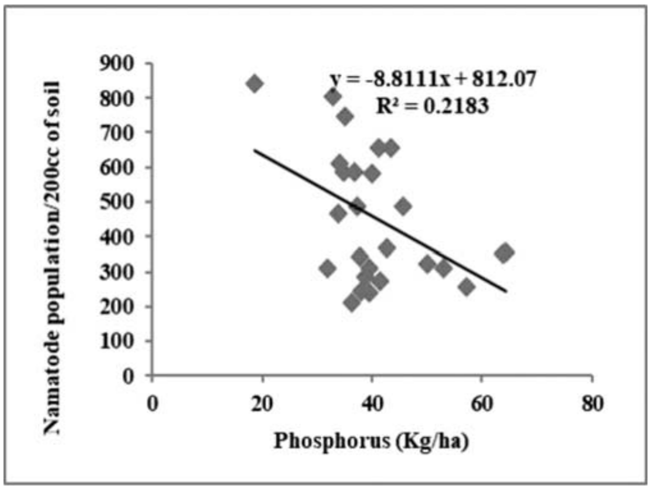

Fig. 7. Effect of Phosphorus on nematode population

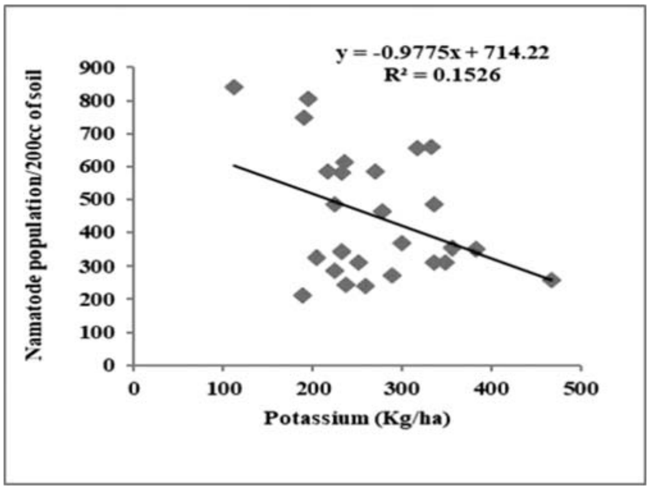

Fig. 8. Effect of Potassium on nematode population

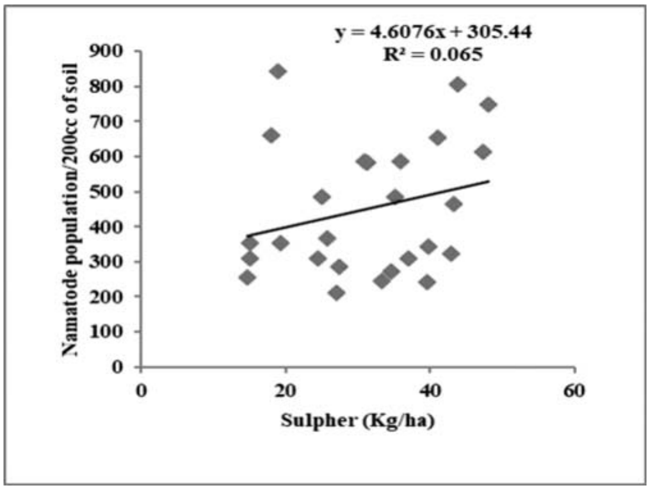

Fig. 9. Effect of Sulphur on nematode population

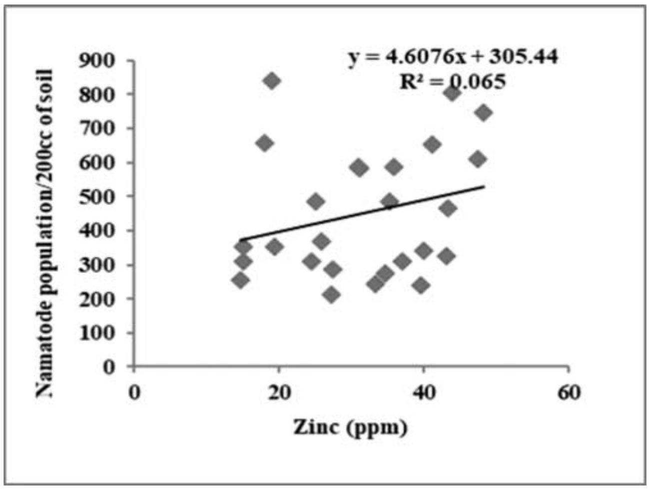

Fig. 10. Effect of Zinc on nematode population

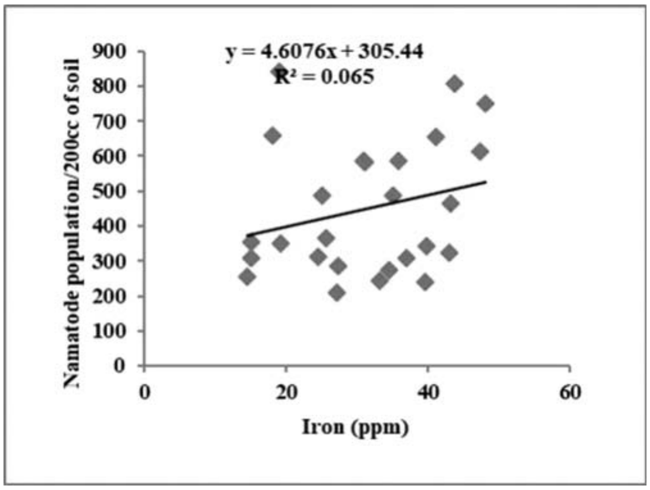

Fig. 11. Effect of Iron on nematode population

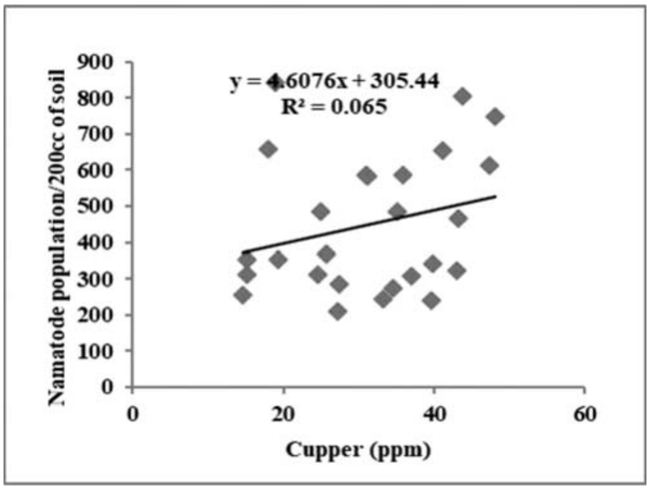

Fig. 12. Effect of Copper on nematode population

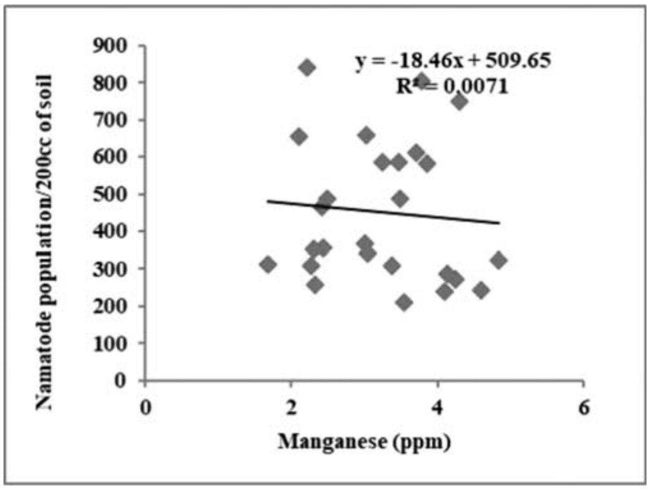

Fig. 13. Effect of Manganese on nematode population

population, Soil moisture was positively correlated non significant at ($P \leq 0.01$) level (r=0.836, R^2= 0.701), Soil organic carbon was positively correlated with nematode population at ($P \leq 0.01$) level of significance (r=0.005**, R^2 =0.050). However available nitrogen was positively correlated significant (r= 0.450*, R^2 =0.205) at (Pd” 0.05)level of significance, available phosphorous was negatively correlated significant at (r= -0.462*, R^2 = 0.218) at (Pd” 0.05) level of significance, potassium was negatively correlated with nematode population (r=-0.396*, R^2 = 0.152) at (Pd” 0.05) level significance, sulphur, zinc and manganese were negatively correlated non significant (r=-0.084, R^2 = 0.065), (r=-0.109, R^2 = 0.011) and (r=-0.085, R^2 = 0.007) at both ($P \leq 0.05$) and ($P \leq 0.01$) level significance. Whereas, Iron and cupper were positively correlated non significant (r=0.377, R^2 = 0.141) and (r=0.255, R^2 = 0.065) at both ($P \leq 0.05$) and ($P \leq 0.01$) level significance which depicted in the Table 4 and 5.

DISCUSSION

The increase in nematode populations with season would be probably due to moisture and soil pH has been previously reported (Jordaan *et al.,*1989). Higher soil moisture is favorable for nematode multiplication. Root-knot nematode population increased when the pH was in the range of 5-7.0 and as the pH goes more than 7 nematode population decrease but the pH do not show direct influence on nematode population(Siddiqui,2007). Prot and Rahman (1994) who also reported that large nematode population densities in light soil as compared to heavy soil may be the result of better growth and multiplication of the nematodes favored by light soil. Soriano *et al.* (2000) also found that greater damage to rice varieties in sandy soils than in clay soil. (Ravindra *et al.,* 2014) who observed that soil pH range between 6.5 to <8.5 in the districts *viz.,* Davanagere, Dakshina Kannada, Udupi, Uttar Kannada, Mysore, Kodagu and

Table 4. Correlation matrix of relationship between physicochemical properties of soil and population of *M. graminicola*

Variables	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}
Soil pH (X_1)	1.000												
Soil EC (X_2)	0.716*	1.000											
Soil moisture (X_3)	-0.335	-0.186	1.000										
Soil OC (X_4)	0.288	0.357	0.003	1.000									
Nitrogen (X_5)	0.017	0.300	0.303	0.115	1.000								
Phosphorus (X_6)	0.717*	0.606*	-0.376	0.252	-0.101	1.000							
Potassium (X_7)	0.923*	0.749*	-0.313	0.292	0.083	0.775*	1.000						
Sulpher (X_8)	0.674*	0.707*	-0.205	0.139	0.071	0.478*	0.578*	1.000					
Zinc (X_9)	0.706*	0.650*	-0.063*	0.596	0.203	0.497*	0.770*	0.300	1.000				
Iron (X_{10})	-0.773*	-0.660*	0.434*	-0.573*	-0.012	-0.629*	-0.717*	-0.484*	-0.583*	1.000			
Cupper (X_{11})	-0.524*	-0.460*	0.286*	-0.695	0.120	-0.477*	-0.522*	-0.258	-0.571*	0.791*	1.000		
Manganese (X_{12})	-0.592*	-0.565*	-0.024*	-0.587*	-0.067	-0.248	-0.550*	-0.534*	-0.632*	0.564*	0.552*	1.000	
Nematode population (X_{13})	-0.333**	-0.055	0.836	0.005**	0.450*	-0.462*	-0.396*	-0.084	-0.109	0.375	0.258	-0.088	1.000

*= Significant at $P \leq 0.05$ %; **= Significant at $P \leq 0.01$

Table 5. Correlation co-efficient of *M. graminicola* population in relation to physicochemical properties of soil

Physico-chemical parameters	Correlation co-efficient	Allometric equation
Soil pH (X_1)	0.115*	Y = -58.73x + 838.1
Soil EC (X_2)	0.002	Y = -139.0x + 466.4
Soil moisture	0.701	Y = 41.86x - 562.3
Soil OC (X_3)	0.050	Y = 2.992x + 447.8
Nitrogen (X_4)	0.205	Y = 1.339x + 118.4
Phosphorus (X_5)	0.218	Y=-8.811x + 812.0
Potassium (Kg/ha) (X_6)	0.152	Y=-0.977x + 714.2
Sulpher (Kg/ha) (X_7)	0.065	Y = 4.607x + 305.4
Zinc (X_8)	0.011	Y=-84.42x + 560.7
Iron (X_9)	0.141	Y = 7.551x + 153.1
Cupper (X_{10})	0.065	Y = 4.607x + 305.4
Manganese (X_{11})	0.007	Y=-18.46x + 509.6

*Values are significant at 0.05 significant levels

Haveri favours the incidence of rice root-knot nematode with root-knot index of 3.0 and (Rao and Israel, 1971a) J2 invasion was greatest in soils at 32% moisture content; development and egg mass production were greatest at 20 to 30% soil moisture and soil pH of 3.5. The highest nematode population was found in Chi-kadadkatte of Davanagere (841.00), Hosudi (748.52), Beguvalli (612.33) of Shivamogga, V.C.Farm (806.33), Chotnahalli (486.33) and Akkihebbal (654.68) of Mandya with soil organic carbon ranging from 0.45-1.65 %, higher nitrogen (200.70- 388.86 Kg/ha) lowest phosphorus (18.67-43.35 kg/ha) and lower potassium (112.08-277.54 kg/ha). The places received higher dose of nitrogenous fertilizers may leads to development of incidence; the results are in line with the findings of (Wei *et al.,* 2012). Who reported the higher level of nitrogen present in the soil commonly reduces soil pH and causes ammonium and aluminum toxicity or introduces sufficient salt to harm soil biota. (Rao and Israel, 1971b) who found that addition of nitrogen up to 40 kg/ha to the soil resulted in increased reproduction of *M. graminicola*. Application of additional phosphorus either alone or in combination with nitrogen also favored nematode development. (Pokharel *et al.,* 2004) reported that nematode induced rice yield reduction was low when plots were supplied with nitrogen and phosphorus as compared to control plots.

The results obtained in this study helps to understand the influence of physicochemical properties of soil on survival of *M. graminicola* in rice.

CONCLUSIONS

Physicochemical properties of soil like, soil pH, EC, organic carbon, soil moisture, major and micro nutrients such as nitrogen, phosphorus, potash, sulphur, zinc, iron, cupper and manganese play a major role on incidence and survival of *M. graminicola*in rice. The results of the present study show that where the locations having sandy loamy soil, with acidic soil pH, lower soil organic carbon, greater nitrogen, lower phosphorous and potassium and higher moisture content had higher nematode population and disease incidence is more. The result obtained in this study gives overview of influence of physicochemical properties of soil on survival and incidence of root-knot disease of rice

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Morphometrics of Plant Parasitic Nematodes Associated with *Trigonella corniculata*

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ABSTRACT: Kasuri methi (*Trigonella corniculata* L.) also known as Nagauri pan fenugreek is a leguminous seed spice crop and dried leaves are used as a spice to add aroma, fibers and flavor to the food products. Soil and plant samples were collected from farmer's field of 5 village's *viz.,* Inana (27^{0}07'41.5"N; 73^{0}49'24.8"E), Mundawa (27^{0}01'34.7"N; 73^{0}48'31.8"E), Kuchera (26^{0}59'14.89"N; 73^{0}58'15.89"E), Butati (26^{0}55'37.2"N; 73^{0}58'55.5"E) and Deshwal (26^{0}50'17.3"N; 73^{0}52'58.4"E) of Nagaur district of Rajasthan (India) on the basis of critical symptoms (yellowing, stunting, patchy growth, root galls and wilting) during 2019-2020. After processing and extraction of samples, it is found that all fields of *Trigonella corniculata* were highly infested with *Meloidogyne incognita, Pratylenchus thornei* and *Xiphinema americanum*. The population of the nematodes was identified on the basis of morphological characters and morphometrics.

Key words: *Trigonella corniculata*, *Meloidogyne incognita, Pratylenchus thornei, Xiphinema americanum*

Kasuri methi (*Trigonella corniculata* L.) is a herbaceous, bushy, slow growing annual leguminous crop. It's a spice crop and mostly dried leaves are used as a spice to add aroma, fibers and delicious flavor to the food and bakery items. It is the richest source of proteins, minerals and fibers especially iron, calcium and vitamins. India is known as a home of spices and it is the world largest producer, consumer and exporter of spices which are being cultivated widely in the country over different agro- climatic zones.

The main seed spices growing districts of Rajasthan are Nagaur, Barmer, Jodhpur, Pali, Jalore and Sirohi. Among them Nagaur district has the largest cultivated area of kasuri fenugreek and it's also known as a Nagauri pan fenugreek and grown in abundance in Nagaur district as a major rabi season crop (Kumawat *et al.,* 2018). Kasuri fenugreek grown in Nagaur is unique in itself with a special fragrance and is known in all over the World. It's very profitable crop and farmers are very conscious to maintain the quality of leaves.

Plant-parasitic nematodes cause on an average 12.3% losses annually in 40 major crops at a global level (Sasser and Freckman, 1987). Root-knot nematode, *Meloidogyne incognita* is a polyphagous pest of more than 3000 hosts and due to sedentary endoparasitic nature induced root galls and caused great losses to crops. Overall, plant-parasitic nematodes cause 21.3% crop losses amounting to Rs. 102,039.79 million (1.58 billion USD) annually in India and root-knot nematodes (*Meloidogyne* spp.) alone are responsible for 75.83% of the total estimated losses (Kumar *et al.,* 2020).*Trigonella corniculata* reported as a host of root-knot nematode (Bhati *et al.* 2021). Lesion nematode, *Pratylenchus thornei* is migratory endoparasite in nature and damage to cortical parenchyma tissues of roots, it causes cavity formation due to migration and feeding within the roots (Mountain and Patrick, 1959). In wheat crop, it has caused extensive root damage (Thompson *et al.,* 2012) and caused up to 40 % yield losses (Nicol and Ortiz- Monasterio, 2004) and in chickpea, it causes 29 % yield loss (Dwivedi *et al.,* 2008). Dagger nematode, *Xiphinema americanum* is migratory ectoparasite in nature and capable of feeding even up to cells lying deeper in the vascular bundles. They are better known for being the only group of phytonematodes, which transmits plant viruses (Hewitt et al., 1958). It caused 1-5% economic losses in soybean, 5-10 % in apple, apricots, peach and 15-20% in fig and grape (Koenning *et al.,* 1999).

MATERIALS AND METHODS

Collection of samples

The survey was conducted in the Nagaur district of Rajasthan and 50 soil and root samples collected from 5 different locations *viz.,* Inana (27^{0}07’41.5"N; 73^{0}49’24.8"E), Mundawa (27^{0}01’34.7"N; 73^{0}48’31.8"E), Kuchera (26^{0}59’14.89"N; 73^{0}58’15.89"E), Butati (26^{0}55’37.2"N; 73^{0}58’55.5"E) and Deshwal (26^{0}50’17.3"N; 73^{0}52’58.4"E). Most of the soil type of this region is sandy loam and found favorable for nematodes. The nematode infested plant of *Trigonella corniculata* identified on the basis of critical symptoms like yellowing, stunting, patchy growth, root galls and wilting, they are pulled out with the help khurpi and collect galled roots. Soil samples were taken with soil probes to a depth up to 25 cm from the rhizosphere of cropped plants and details of samples collection are given in Table 1.

Processing of samples

To identification and characterization of nematode species, pure culture of juveniles of root-knot nematode recovered from the egg mass obtained from the roots, mature females recovered from the galls of roots and the males recovered from both galls and soil of the infested field. The population of *Pratylenchus thornei* obtained from both roots and soil samples, whereas, the population of *Xiphinema americanum* obtained from the soil samples.

To find out the population status of plant parasitic nematodes, 200 cc soil samples were processed by using Cobb’s Sieving and Decanting Technique (Cobb, 1918) followed by Baermann’s Funnel Assembly (Christie and Perry, 1951). Roots were stained in 0.1% acid fuchsin lactophenol solution at 80ºC for 2-3 minutes (McBeth *et al.,* 1941). Thereafter, soil extract and roots were examined under stereoscopic binocular microscope then the process of fixation, dehydration and the mounting of nematodes were performed according to Seinhorst (1959) and morphologically identified through binocular compound microscope. For the identification of species of root-knot nematode the perineal patterns were prepared as

Table 1. Details of sample collection and nematode infection symptoms in *Trigonella corniculata*

Village	GPS Location	No. of Samples collected	No. of samples infested	Soil type	Leaves colour	Plant vigour
Inana	27^{0}07’41.5"N73^{0}49’24.8"E	10	08	Sandy Loam	Pale yellow	Stunted in small patches
Mundawa	27^{0}01’34.7"N73^{0}48’31.8"E	10	09	Sandy Loam	Yellowish	Stunted in large patches
Kuchera	26^{0}59’14.89"N73^{0}58’15.89"E	10	07	Sandy Loam	Pale yellow	Stunted in small patches
Butati	26^{0}55’37.2"N73^{0}58’55.5"E	10	08	Sandy Loam	Pale yellow	Stunted in large patches
Deshwal	26^{0}50’17.3"N73^{0}52’58.4"E	10	06	Clay Loam	Pale yellow	Stunted in small patches
Total	5	50	38	-	-	-

described to (Taylor and Netscher, 1974) and the nematode species was identified (Eisenback *et al.,* 1981).

Identification of nematodes

Identification of root-knot nematode, *Meloidogyne incognita* confirmed through typical morphological measurements of juvenile, female, perineal pattern and male (Chitwood, 1949). The presence of *Pratylenchus thornei* was confirmed on the basis of morphometric characters of females similar to original description of Sher and Allen (1953)and *Xiphinema americanum* confirmed through description of females and males similar to description of (Lamberti and Golden, 1984).The set of parameters used to characterize nematode species such as a, b, c, c' and V % also were added in the morphometrics description (De Man,1876; De Man, 1880).The arithmetic mean, standard error of mean (SEM), standard deviation (SD) and coefficient of variance % (CV) for each measurement were computed for interpretation of findings.

RESULTS AND DISCUSSION

Population status and occurrence of nematodes infecting *Trigonella corniculata*

The plant and soil samples of *Trigonella corniculata* tested in the laboratory and results revealed that the crop was mostly infected with three major plant parasitic nematodes *viz.,Meloidogyne incognita, Pratylenchus thornei* and *Xiphinema americanum* (Figs. 1,2,3) and they are identified on the basis of morphological characters.The samples collected from 5 different locations and 10 samples were collected from each location.Results showed that 38 samples found infested with nematodes out of 50 and the maximum 90 percent

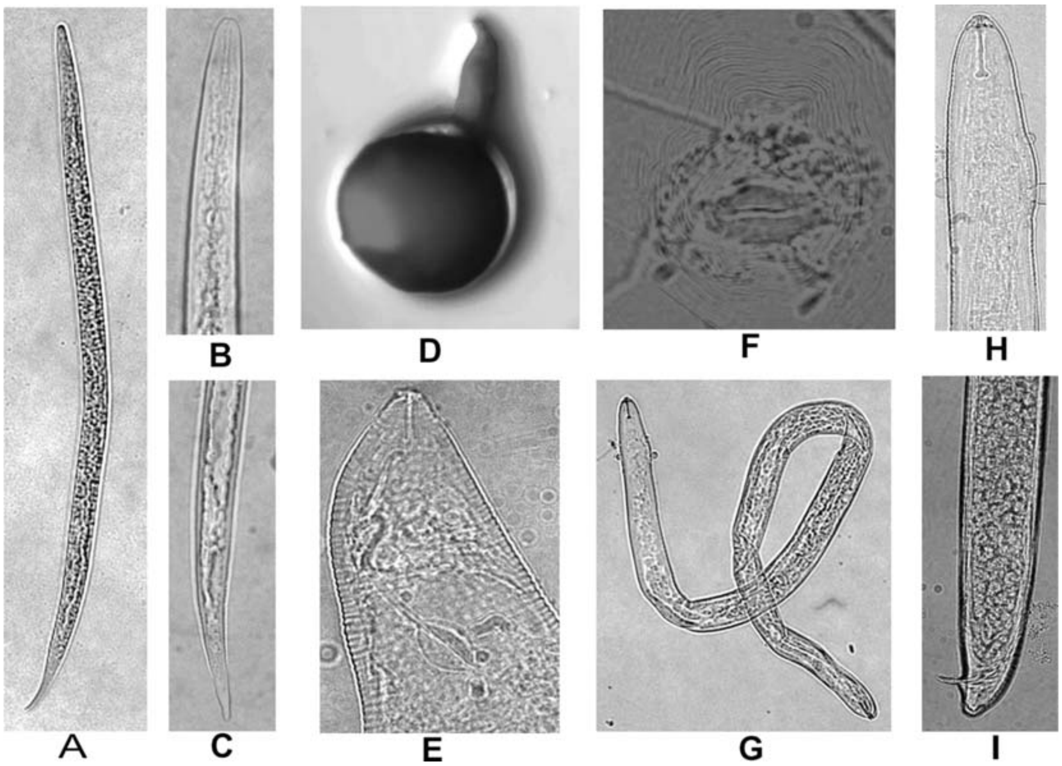

Fig.1.*Meloidogyne incognita*: (A) Second stage Juvenile; (B) J2 Head; (C) J2 Tail; (D) Mature Female; (E) Anterior portion of female; (F) Perennial pattern; (G) Male; (H) Male head region; (I) Male tail region

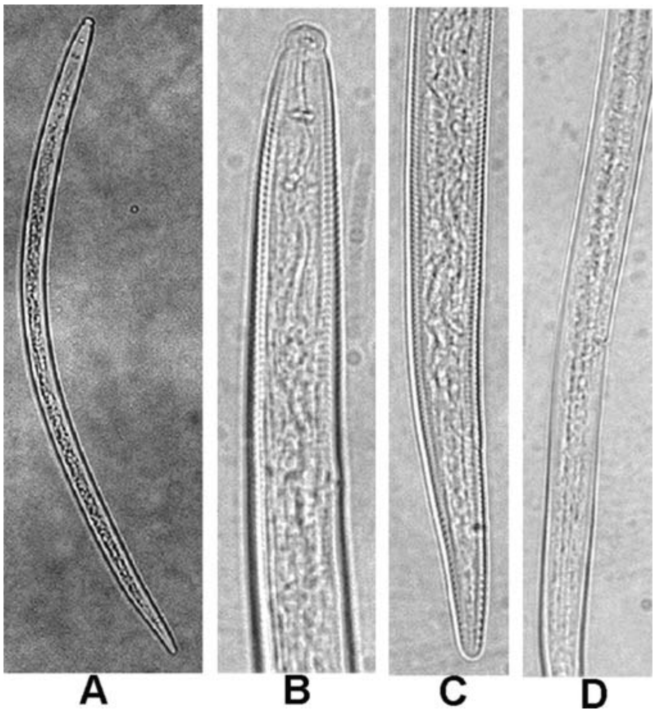

Fig. 2. ***Pratylenchus thornei:*** **(A) Mature female; (B)Anterior region; (C) Tail region; (D) Vulva**

occurrence was reported from Mundawa while the minimum 60 per cent occurrence was reported from Deshwal location.

The maximum 1066 nematodes per 200 cc soil recorded from Butati location. Whereas, the minimum 812 nematodes per 200 cc soil was observed from Kuchera location. During the survey it was the main conscious subject that all samples were infested with nematodes population above the ETL level and details are listed in Table 2.

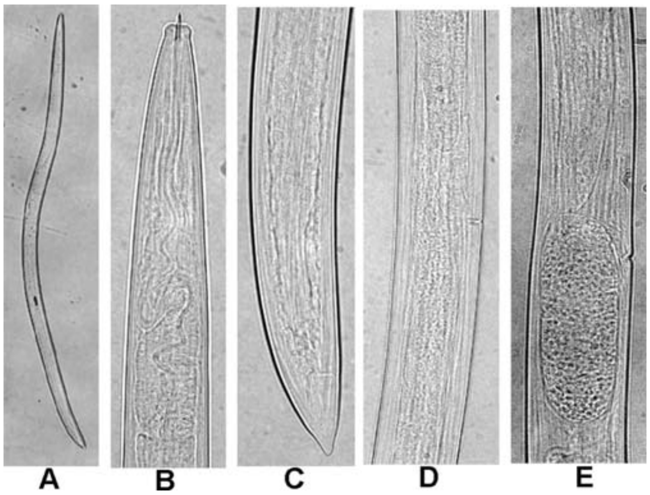

Fig. 3. ***Xiphinema americanum:*** **(A) Full body of female; (B) Anterior region; (C) Tail region; (D) Middle portion; (E) Vulva**

Morphological description of nematodes infecting *Trigonella corniculata*

Three major plant parasitic nematodes *Meloidogyne incognita, Pratylenchus thornei* and *Xiphinema americanum* were collected from the field of *Trigonella corniculata* (Table 2) and measured morphological characters for the characterization and described below.

Table 2. Population status and occurrence of nematodes infecting *Trigonella corniculata*

Location	No. of infested samples out of 10	Per cent Occurrence	Number of samples infested with nematode species			Average no. of nematodes/200 cc soil
			Meloidogyne incognita	*Pratylenchus thornei*	*Xiphinema americanum*	
Inana	08	80	08	06	08	892
Mundawa	09	90	07	08	09	936
Kuchera	07	70	05	06	07	812
Butati	08	80	04	08	08	1066
Deshwal	06	60	06	06	06	854
Total/Mean*	38/50	76*	30/38	34/38	38/38	912*

Morphological description of *Meloidogyne incognita* infecting *T. corniculata*

Juvenile

Vermiform long body; head slightly set off from body; large rounded knob; hyaline tail terminus and broad tail tip (Fig. 1). The measurements of 10 juvenile's body were recorded.

Themean body length=378.04±31.21(324.6-408.8)µm; maximum body width=14.67±0.72 (13.6-15.9)µm; a=25.85±2.78 (20.41-30.04); c=9.71±1.03 (8.34-11.80); c'=3.71±0.44 (3.10-4.22);stylet length= 13.10±0.67 (12.3-14.1)µm; tail length=39.13±3.75 (34.60-45.10) µm and anal body diameter=10.59±0.75(9.4-11.7) were observed and listed in Table 3 and illustrated in Fig. 1.

Female

The female is pear shaped white body; tail absent; anus and vulva terminal, long stylet and oesophagus with large muscular bulb. The measurements of 10 female's body were observed as the mean body length=690.32±44.66 (622.5-762.4)µm; maximum body width=587.99±21.09 (544.6-611.4)µm; a=1.17±0.09 (1.02-1.30); neck length=281.9±17.74 (256.5-305.2)µm; stylet length=16.17±0.96 (14.7-17.5)µm; length of median bulb=32.74±3.99(27.3-38.5)µm and width of median bulb =29.94±3.32(25.6-34.9)µm and listed in Table 4 and illustrated in Fig. 1.

Perineal pattern

Oval to round; high dorsal arc; lateral field weakly demarcated by forked striae; striae distinct and wavy and dorsal striae smooth.The measurements of 10 perineal patterns of females were observed. The LVS=22.34±1.25 (20.4-23.9)µm; IPD=24.94±2.17 (21.2-27.6)µm; AVS=19.31±1.98 (16.8-22.1)µm; 24.73±2.40(21.3-28.7)µm were recorded and listed in Table 5 and illustrated in Fig. 1.

Male

Male of the species is vermiform; head cap high and rounded; head slightly set off from body; stylet robust, stylet knob bold; spicules arcuate; short and rounded tail and bursa absent.

The measurements of 10 male's body were observed as the mean body length=1415.7±111.06 (1274.6-1589.1)µm; maximum body width=41.20±1.12 (39.2-

Table 3. Morphological measurements of Juvenile of *M. incognita* infecting *T. corniculata*

(n=10)

Character	Range	Mean	Standard deviation	Standard error of mean	Coefficient of variation (%)
L (µm)	324.60-408.80	378.04	31.21	9.86	8.25
Maximum Body width (µm)	13.60-15.90	14.67	0.72	0.23	4.96
a	20.41-30.04	25.85	2.78	0.87	10.74
c	8.34-11.80	9.71	1.03	0.32	10.61
c'	3.10-4.22	3.71	0.44	0.13	11.87
Stylet length (µm)	12.30-14.10	13.10	0.67	0.21	5.15
Tail length (µm)	34.60-45.10	39.13	3.75	1.18	9.58
Anal body diameter(µm)	9.40-11.70	10.59	0.75	0.24	7.15

Table 4. Morphological measurements of females of *M. incognita* infecting *T. corniculata*

(n=10)

Character	Range	Mean	Standard deviation	Standard error of mean	Coefficient of variation (%)
L (µm)	622.50-762.40	690.32	44.66	14.12	6.47
Maximum Body width (µm)	544.60-611.40	587.99	21.09	6.67	3.58
a	1.02-1.30	1.17	0.09	0.02	7.71
Neck length (µm)	256.50-305.20	281.90	17.74	5.61	6.29
Stylet length (µm)	14.70-17.50	16.17	0.96	0.31	5.97
Length of median bulb (µm)	27.30-38.50	32.74	3.99	1.26	12.20
Width of median bulb (µm)	25.60-34.90	29.94	3.32	1.05	11.09

Table 5. Morphological measurements of perineal pattern of *M. incognita* infecting *T. corniculata*

(n=10)

Character	Range	Mean	Standard deviation	Standard error of mean	Coefficient of variation (%)
LVS (µm)	20.40-23.90	22.34	1.25	0.39	5.61
IPD (µm)	21.20-27.60	24.94	2.17	0.68	8.71
AVS (µm)	16.80-22.10	19.31	1.98	0.62	10.25
ATT (µm)	21.30-28.70	24.73	2.40	0.76	9.72

LVS-length of vulval slit; IPD-interphasmid distance; AVS-anus to vulval slit; ATT-anus to tail terminus

42.6)µm; a=34.36±2.56 (31.89-37.65); stylet length=19.96±1.16 (18.3-21.5)µm; tail length=13.56±1.65 (11.2-15.8)µm andspicules length=27.64±1.32 (25.6-29.5)µm and listed in Table 6 and illustrated in fig. 1.

Morphological description of *Pratylenchus thornei* infecting *Trigonella corniculata*

The lesion nematode, *Pratylenchus thornei* dioeciously in nature, both males and females are separate but the reproduction may be sexual or parthenogenetic due to rare presence of males in this species. So in this study we described only female's morphological measurements.Female are vermiform; body gradually tapering posteriorly; continuous head with body; stylet strong with well developed knob; ventral overlapping of oesophagus, monodelphic-prodelphic and vulva posteriorly situated.

The morphological measurements of 10 female's body were recorded as the mean body length (L)=614.6±118.49 (456.2-774.6)µm;a=31.1±4.12 (25.9-36.2);b=6.8±0.91 (5.6-8.1);c=21.7±2.55 (18.4-25.2); V=75.11±2.07 (72.2-78.5);stylet length=16.81±1.14 (15.3-18.7)µm; maximum body width=19.6±1.54 (18.4-21.7)µm; oesophageal length=89.3±7.06 (77.3-97.6)µm; tail length=28.1±2.99 (24.7-32.8)µm and position of vulva from anterior end=462.1±92.5 (329.4-608.7)µm and listed in Table 7 and illustrated in Fig. 2.

Table 6. Morphological measurements of males of *M. incognita* infecting *T. corniculata*

(n=10)

Character	Range	Mean	Standard deviation	Standard error of mean	Coefficient of variation (%)
L (µm)	1274.60-1589.10	1415.70	111.06	35.12	7.84
Maximum Body width (µm)	39.20-42.60	41.20	1.12	0.35	2.71
a	31.89-37.65	34.36	2.56	0.81	7.46
Stylet length (µm)	18.30-21.50	19.96	1.16	0.36	5.81
Tail length (µm)	11.20-15.80	13.56	1.65	0.52	12.22
Spicules length (µm)	25.60-29.50	27.64	1.32	0.41	4.79

Table 7. Morphological measurements of females of *P. thornei* infecting *T. corniculata*

(n=10)

Character	Range	Mean	Standard deviation	Standard error of mean	Coefficient of variation (%)
L (µm)	456.2-774.6	614.60	118.49	37.47	19.28
a	25.9-36.2	31.10	4.12	1.30	13.26
b	5.60-8.10	6.80	0.91	0.28	13.35
c	18.40-25.20	21.70	2.55	0.80	11.71
V (%)	72.20-78.50	75.11	2.07	0.65	2.75
Stylet length (µm)	15.30-18.70	16.81	1.14	0.36	6.79
Maximum body width (µm)	18.40-21.70	19.60	1.54	0.48	7.88
Oesophageal length (µm)	77.30-97.60	89.30	7.06	2.23	7.90
Tail length (µm)	24.70-32.80	28.10	2.99	0.94	10.67
Position of vulva from anterior end (µm)	329.40-608.70	462.10	92.50	29.25	20.01

Morphological description of *Xiphinema americanum* infecting *Trigonella* corniculata

Dagger nematode, *Xiphinema americanum* belongs to order dorylaimida, the females having long body; vermiform; odontostylet with flanges and short tail. Male having filiform body; long spicules and bursa absent.

The morphological measurements of 10 female's body were recorded as the mean body length (L)=1613.52±119.97 (1422.51-1785.23) µm; a= 45.18±5.14 (34.86-52.51);b=6.53±0.75 (5.53-7.78);c=46.52±4.28 (38.23-52.66); c'=1.67±0.23 (1.40-2.09); V=50.34±1.26 (48.60-52.65);stylet length= 113.18±2.80 (109.10-117.50)µm; tail length=34.77±1.75 (32.50-37.20)µm; body diameter at lip region=9.98±0.53 (9.40-10.90)µm; body diameter at vulva=35.96±3.10 (31.60-40.80)µm; body diameter at anus=20.69±2.12 (17.30-23.72)µm and body diameter at beginning of J=8.67±0.78 (7.21-9.73)µm and listed in Table 8 and illustrated in Fig. 3.

Table 8. Morphological measurements of females of *X. americanum* infecting *T. corniculata*

(n=10)

Character	Range	Mean	Standard deviation	Standard error of mean	Coefficient of variation (%)
L (µm)	1422.51-1785.23	1613.52	119.97	37.93	7.43
a	34.86-52.51	45.18	5.14	1.62	11.39
b	5.53-7.78	6.53	0.75	0.23	11.5
c	38.23-52.66	46.52	4.28	1.35	9.21
c’	1.40-2.09	1.67	0.21	0.07	14.06
V (%)	48.60-52.65	50.34	1.26	0.40	2.52
Stylet length (µm)	109.10-117.50	113.18	2.80	0.88	2.47
Tail length (µm)	32.50-37.20	34.77	1.75	0.55	5.03
Body diameter at lip region (µm)	9.40-10.90	9.98	0.53	0.16	5.34
Body diameter at vulva (µm)	31.60-40.80	35.96	3.10	0.98	8.62
Body diameter at anus (µm)	17.30-23.72	20.69	2.12	0.67	10.24
Body diameter at beginning of J (µm)	7.21-9.73	8.67	0.78	0.24	9.09

The majot growing area of *Trigonella corniculata* is Nagaur and nearby districts of Rajasthan in India. In this region, soil is very poor in the term of organic matter and most of the soil is sandy and sandy loam and found favorable for survival of plant parasitic nematodes. In the investigation, it is found that *Trigonella corniculata* infested with three major plant parasitic nematodes *Meloidogyne incognita, Pratylenchus thornei* and *Xiphinema americanum,* this result agrees with the findings of Bohra (2008) collected soil samples from eight districts of Rajasthan state and found the wide variety of plant parasitic nematodes including *Meloidogyne* spp., *Hoplolaimus indicus, Helicotylenchus* sp.*, Rotylenchulus reniformis, Pratylenchus zeae, Pratylenchus thornei, and Xiphinema* spp. In all, 84 species have been identified belonging to 37 genera of 20 families of Orders, Tylenchida (43 spp.), Aphelenchida (1 sp.), Dorylaimida (35 spp.), Mononchida (3 spp.), Triplonchida (1 sp.) and Isolaimida (1 -1'.). 29 species out of 84 have been recorded for the first time from the State while, 14 species have been recorded for the first time from India. Besides, 12 species belonging to Order Tylenchida, Dorylaimida and Isolaimida have been found new to Science.

Ali and Sharma (2003) observed 20-30% yield losses in chickpea due to *Meloidogyne incognita* and *M. javanica* in Rajasthan and reported *Heterodera swarupi* first time on chickpea. Kant *et al.* (2017) conducted a survey in cumin crop of Ajmer district of Rajasthan, India. They found that there were three different species of nematodes associated with cumin root namely *Pratylenchus thornei*, *Hoplolaimus indicus* and *Meloidogyne incognita*.The nematode species *P. thornei* and *H. indicus* infection were found first time in cumin. Sharma and Trivedi (2005) reported cumin crop infected with *M. incognita* and *Fusarium* complex in Rajasthan. Similarly dill and fenugreek crops have been reported susceptible to *M. incognita* (Khan and Rizvi, 2013). Sultan *et al.* (2010) identified the root-knot, reniform, *Criconemoides insignis*, *Xiphinema americanum*, *Macrorhynchus mashhoodi* and

Psilenchus hilurus nematodes and reported first time from in medicinal, aromatic and spice plants in Punjab.

The accurate identification of different species of *Meloidogyne* is important for the management of nematodes (Cenis, 1993). Identification based on a combination of several methods such as morphological characteristics, morphometrics, host preferences biochemical and molecular techniques are essential methods for confirming the species (Eisenback *et al.,* 1981). Root-knot nematodes were characterized mainly based on morphological features and measurements of second stage juveniles, females, perineal patterns and males (Jepson, 1987; Carneiro and Cofcewicz, 2008).

The morphometrics data of the specimens of *M. incognita* recovered from the *Trigonella corniculata* were similar to the description of (Chitwood, 1949; Eisenback and Triantaphyllou, 1981). The characters of *M. incognita* were in agreements with the previous comparison study on stylet of females of *Meloidogyne* spp. (Jepson, 1983). The taxonomy of *Meloidogyne* with description of four species (Whitehead, 1968) also advocates the findings of research. (Dasgupta and Gaur, 1986) studied on root-knot species in India. Morphological and morphometrical characterization of *M. incognita* from different host of Punjab (Kaur and Attri, 2013) were recorded and the given characters were similar to the present description of *M. incognita*.

The description of the *Pratylenchus thornei* in the result was similar to the original description given by (Sher and Allen, 1953) and the characters of genus *Pratylenchus* first described by (Filipjev, 1936). The morphometrics of the specimens were strongly related to the key characters of *Pratylenchus thornei* described in a compendium (Handoo and Golden, 1989). The morphometrics (Loof, 1960) helps in precise identification and confirmation of the presence of *Pratylenchus thornei* in the samples.

The morphological characters and morphometrics of *Xiphinema americanum*i isolated from the field of *Trigonella corniculata* were similar to the original description of (Cobb, 1913). The descriptions of the specimen were also similar to the redescription of morphometric variations of *Xiphinema americanum* (Lamberti and Golden, 1984). The results were also similar to the study of Lamberti and Zacheo, 1979; Malik and Jairajpuri, 1983; Loof and Luc, 1990; Lamberti and Carone, 1991 and strongly indicates the presence of this species in the sample. Lamberti and Zacheo (1979) described *Xiphinema americanum* sensu lato with fifteen new species and Lamberti and Carone (1991) give a dichotomous key for the identification. Halbrendt and Brown (1994) described the inter and intraspecific variation in the population of *Xiphinema americanum.*

CONCLUSION

The present study revealed to the identification of plant parasitic nematodes infecting *Trigonella corniculata*. The morphometrics data recorded the presence of three major nematode species *Meloidogyne incognita, Pratylenchus thornei* and *Xiphinema americanum* associated with the crop in farmer’s field. They are able to cause directly adverse effect on the plant vigor and yield. Plant parasitic nematodes also provide favorable environment to the other plant pathogens through wounds and synergistically cause more losses. Due to lack of awareness about nematodes infection in this crop, farmers are facing many problems. Present study would help farmers to adopt the perfect management practices.

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Biocontrol Potential of the Entomopathogenic Nematodes, *Heterorhabditis bacteriophora* and *Steinernema feltiae* from North Western Himalayan Region on Fruit Fly, *Bacterocera zahadi* Mahmood (Diptera: Tephritidae)

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ABSTRACT: The two indigenous Entomopathogenic Nematodes (EPNs) strains, *Heterorhabditis bacteriophora* HR2 and *Steinernema feltiae* HR1, have been evaluated against distinct developmental stages of the fruit fly, *Bacterocera zahadi* Mahmood *i.e.* larvae and pupae. Native EPNs strains were isolated and laboratory bioassay studies were done to determine their infectivity against pumpkin fruit fly (Diptera: Tephritidae). The maggots and pupae of *B. zahadi* Mahmood were exposed to EPNs in Petri plates (9.0 cm diameter) lined with Whatman No.1 filter paper and in plastic cups (4.0 cm diameter) filled with a substrate consisting of a 1:1 mixture of 30-mesh sieved sand and potting soil, respectively, and their susceptibility to nematode infection was determined at various concentrations of 10, 20, 40, 80, and 160 IJs/cm2. The 10 larvae and pupae of the test insect were added and allowed to burrow into the substrate within 1 hour of IJs treatment. Both EPN species induced varying amounts of mortality in petri plates containing 10 larval insects, with maximums of 68.66 percent and 54.66 percent caused by *H. bacteriophora* and *S. feltiae,* respectively after seven days of exposure at 160 IJs /cm^2. After seven days of exposure time in plastic jars, various levels of mortality caused by *H. bacteriophora* and *S. feltiae* was documented, with maximums of 53.33 percent and 50.00 per cent caused by *H. bacteriophora* and caused by *S. feltiae,* respectively. Based on its higher virulence and better ability to penetrate the larval/pupal tissue, the results indicated that *H. bacteriophora* was found to be the most effective species and may have potential as a biocontrol agent of *B. zahadi*. Further field testing can be performed for incorporating these indigenous strains of EPNs in an integrated pest management programme for fruit fly management under the mid hills of Himachal Pradesh, India.

Keywords: Entomopathogenic nematodes Strains, *Heterorhabditis bacteriophora*, *Steinernema feltiae*, pumpkin fruit fly, *Bacterocera zahadi* Mahmood, biocontrol

The fruit flies from tephritidae family are one of the most important and key pests of fruits and vegetable crops around the world, which reduces the quality of ripe fruits (Weems & Heppner, 2014). At present, about 4352 species in 483 genera are known around the world while in India only 200 species in 71 genera and five subfamilies are known (Kapoor, 2022). Each year they have been reported to cause partial or complete damage to various fruits particularly mango, guava, citrus and vegetables like cucurbits etc. in India (Agarwal *et. al.,* 1987 and Kapoor, 2022). Fruit flies have been predicted to inflict up to 100% damage to certain vegetable crops in some situations (Dhillon *et. al*., 2005; Philippe *et al*., 2010). Fruit flies lowered fruit yield directly by laying eggs under the pulp of the fruit; neonates fed inside the fruits, rendering them unfit for human consumption. When neonates emerge, they feed inside the fruit, lowering its aesthetic value (also, oviposition holes give access opportunities for fungal penetration) (Santiago-Alvarez and Quesada-Moraga, 2007). Because of the loss of quality, the afflicted fruits become unfit for ingestion and so lose their market value. Fruit flies are currently controlled mostly with organophosphate insecticides. Farmers have even dipped contaminated crops into insecticide solutions in containers in certain extreme circumstances. Chemical pesticides have been demonstrated to be efficient against a variety of cucurbit pests, such as fruit flies and nematodes (Thakur, 2011), however they are considered environmentally unsound. Fruits Cucurbits (vegetable crops), hence chemical application will leave poisonous residues on the fruits. As a result, finding alternate control strategies is crucial. There is not a single species of fruit fly in India which has been successfully controlled. Fruit flies have a diverse range of natural adversaries, including parasitoids, carnivores, and entomopathogens. The fly larvae that develop in the soil are immune to parasitoids, but they are vulnerable to entomopathogens including entomopathogenic nematodes (EPNs) and fungi. Different fruit fly species including *Rhagoletis indifferens* Curran*, Bacterocera oleae* Gmelin*, Ceratitis rosa* Karsch*, Ceratitis capitata* Wiedemann*, Anastrepha serpentina* Wiedemann, *Anestrepha oblique* Macquart and *Rhagoletis pomonella* Walsh were found to be susceptible to various species/strains of both S*teinernem*a and *Heterorhabditis* nematodes (Malan & Manrakhan, 2009; Sirjani *et al.,* 2008, Heve *et al.,* 2017; Usman *et al*., 2020). EPNs from the Steinernematidae and Heterorhabditidae families are commercially available in various regions of the world to manage a variety of soil insect pests (Kaya and Gaugler, 1993; Toledo *et al*., 2006; Karagoz *et al*., 2009). Application of the entmopathogenic nematodes can be a good alternative to the convenional harmful insecticides as due to their mode of action i.e. biological control, resistence is unlikely to develop against these agents (Aatif *et al*., 2019). EPNs' biology, safety and ease of mass production and application with standard spray equipment make them as an excellent candidates for controlling soil insect pests that spend part of their life cycle in the soil (Gazit *et al*., 2000; Lindegren and Vail, 1986; Stark 1999; Kaya *et al*., 2006).

The use of EPNs to combat fruit flies is a viable alternative to chemical pesticides, especially for organic growers. The goal of this study was to determine the susceptibility of larval and pupal stages of the fruit fly *B. zahadi* to native EPN strains (HR1 and HR2) in laboratory conditions. Under laboratory conditions, the efficacy of local strains of entomopathogenic nematodes *Heterorhabditis bacteriophora* (HR2) and *Steinernema feltiae* (HR1) against last instar maggots (larva) and pupae of the fruit fly *Bactrocera zahadi* Mahmood was assessed.

MATERIAL AND METHODS

Sampling and rearing of fruit fly, *Bactrocera zahadi* Mahmood

The *B. zahadi* Mahmood (Identified based on Singh *et al.*, 2022) infested fruits were collected from the local farmers of the area (mid hills of Himachal Pradesh, India- Rajgarh population) during 2019 and 2020. The full grown maggots hopped from infested cucumber fruits and adult flies were reared under laboratory conditions in cages by providing the continuous supply with sugar, dry protein x, and freshwater. Clean fresh cucumbers were regularly introduced in the fruit fly cages to enhance oviposition and larval development. Third instar larvae/maggots and pupae were obtained for all experiments by removing them from cucumber fruits (last instar maggots) and sandy soil (pupae).

Entomopathogenic nematodes

In the present study, two indigenous strains of Entomopathogenic nematodes *Heterorhabditis bacteriophora* HR2 and *Steinernema feltiae* HR1, were directly used after culturing. The native strains were isolated from the undisturbed soils of fruit orchards from known locations of Rajgarh area of Sirmour district of Himachal Pradesh, India (1682 m above mean sea level with 30^{o} 532 15 N latitude and 77^{o} 162 07 E longitude with the mid-hill zone of western Himalayas Rajgarh, Sirmaur district, Himachal Pradesh, India). The isolation was performed by using the technique given by Bedding and Akhurst (1975).

Raising the culture of entomopathogenic nematodes

The isolated strains of EPNs were multiplied separately on larvae of rice moth, *Corcyra cephalonica,* last instar larvae of the greater wax moth, *Galleria mellonella* (Kasi *et al.,* 2021) and cultured in nematology laboratory as per method described by Singh (1990). The insect larvae were infected with nematodes in 9 cm dia. Petri plates lined with Whatman No.1 filter paper. Evenly 1ml of the IJ (infective juveniles) suspension containing about 200IJs/ml was distributed on the filter paper. Ten last instar larvae of the greater wax moth, *Corcyra cephalonica* and *Galleria mellonella* were added to the dish. The dish was covered with lid. The Petri Plates were placed inside a loosely sealed plastic bag (to conserve moisture) and kept in the incubator at 25 ±1°C temperature. After 5-7 days, cadavers were removed with signs of nematode infection to a White trap. The infective juveniles (IJs) were collected from white traps stored at 4 °C in distilled water for up to 14 days. The nematodes were acclimatized at room temperature for about 30 min before being used in the experiments.

Effect of indigenous strains of EPNs concentrations on third instar larvae and pupae of fruit fly

The bioassay studies were conducted by exposing maggots and pupae to the EPNs in plastic cups filled with a substrate consisting of 1:1 mixture of 30-mesh sieved sand and potting soil. The nematodes were applied to the sand/soil substrate in water using a pipette at the rate of 10, 20, 40, 80 and 160 IJs /cm^2. Within 1 h of IJs application, ten larvae/pupae of fruit fly, *Z. zahadi* Mahmood were added to each cup and allowed to burrow into the substrate. The cups were covered with a muslin cloth and kept on a laboratory table. Each set was replicated 5 times. The infected larvae were observed under a stereo zoom microscope for each concentration at 72 h exposure time. The data on maggot and pupal mortality was recorded after 3rd and 7th day of treatment.

Statistical analysis

The analysis for mortality data of fruit fly maggots and pupae was conducted by using completely randomized design and treatments were compared through CD. The corrected per cent mortality data obtained from different concentrations was subjected to probit analysis (Finney, 1971) to calculate LC50 (IJs/cm^2) values. The data obtained from the experiment was analyzed by using OPSTAT computer programme, CD was calculated for comparison of treatments. Concentration-mortality response data was conducted.

RESULTS AND DISCUSSION

Efficacy of *H. bacteriophora* against the last instar maggots of *B. zahadi* Mahmood

At 3 days after treatment

At 3 days after treatment, *H. bacteriophora* caused maggot mortality at a minimum dose of 20 IJs/cm^2 (Table 1). No mortality was recorded in control and at 10 IJs/cm^2 dose. Significantly maximum maggot mortality was recorded at highest nematode dose (160 IJs/cm^2) i.e. 43.33 per cent, followed by 35.33, 28.33 and 18.33 per cent at 80, 40 and 20 IJs/cm^2 doses, respectively. The maggot mortality increased with increase in EPNs dose.

At 7 days after treatment

The maggot mortality was further increased after 7 days of exposure (Table 1). Highest maggot mortality (68.66 %) was recorded at 160 IJs/cm^2 which was also found at par with maggot mortality at 80 IJs/cm^2 (56.66 %). Similarly, no significant difference in maggot mortality were recorded between 80 & 40 IJs/cm^2 and 40 & 20 IJs/cm^2. The maggot mortality in control was significantly lowest as compared to entomopathogenic nematode treated maggots of fruit fly.

Table 1. Efficacy of indigenous strains of entomopathogenic nematodes against last instar maggots of fruit fly, *Bacterocera zahadi* Mahmood

DOSE (IJs/cm^2)	Maggot mortality after days (%)*			
	H. bacteriophora		*S. feltiae*	
	3 DAT	7 DAT	3 DAT	7 DAT
0	00.00(0.67)**	3.00(6.26)	00.00(0.67)	3.33(6.59)
10	00.00(0.67)	21.33(27.46)	00.00(0.67)	21.33(27.49)
20	18.33(25.34)	35.33(36.44)	21.00(27.2)	35.33(36.44)
40	28.33(31.11)	43.33(41.16)	28.33(32.12)	41.00(39.8)
80	35.33(36.43)	56.66(48.83)	35.33(36.44)	48.33(44.03)
160	43.33(41.15)	68.66(55.97)	41.33(39.40)	54.66(47.69)
CD (P=0.05)	(4.16)	(7.75)	(3.7)	(8.8)

*Average of five replications; **Figures in parentheses are arc sine transformed values

Efficacy of *S. feltiae* against the last instar maggots of *B. zahadi* Mahmood

At 3 days after treatment

At 3 days after treatment, maggot mortality in different concentrations due to *S. feltiae* treatments ranged between 21.00 and 41.33 per cent (Table 1). No mortality was observed in control and at 10 IJs/cm^2. The highest (41.33%) maggot mortality was recorded at maximum dose i.e. 160 IJs/cm^2 which was also statistically at par with maggot mortality at 80 IJs/cm^2 (35.33 %). The maggot mortality increased with higher concentrations.

At 7 days after treatment

The maggot mortality was further increased after 7 days of exposure. The maggot mortality ranged between 21.33 and 54.66 per cent (Table 1). Maximum maggot mortality was recorded at 160 IJs/cm^2 (54.66 %), followed by mortality at 80 IJs/cm^2 (48.33%), 40 IJs/cm^2 (41.00 %), 20 IJs/cm^2 (35.33%) and 10 IJs/cm^2 (21.33 %) against 3.33 per cent in control. The maggot mortality at dose 160 IJs/cm^2 was found statistically at par with maggot mortality at doses 80 IJs/cm^2 (48.33%) and 20 (41.00 %) IJs/cm^2. Similarly, no significant differences in maggot mortality were recorded at doses 40 & 80 IJs/cm^2 and 20 &40 IJs/cm^2.

Efficacy of indigenous strain of entomopathogenic nematodes (HR2 and HR1) against the pupae of *B. zahadi* Mahmood

Efficacy of *H. bacteriophora* strain HR2 against the pupae of *B. zahadi* Mahmood

At 3 days after treatment

At 3 days after treatment, pupal mortality in different concentrations ranged between 13.33 and 43.33 per cent as presented in Table 2. Highest (43.33%) pupal mortality was recorded at 160 IJs/cm^2, followed by 36.66, 23.33 and 13.33 per cent at 80, 40 and 20 IJs/cm^2, respectively. No pupal mortality was observed in control and EPNs concentrations of 10 IJs/cm^2. Pupal mortality increased with higher concentrations and significant differences in pupal mortality were recorded at 80 and 60 IJs/cm^2 doses.

At 7 days after treatment

The pupal mortality due to nematodes was further increase at 7days after treatment which ranged between 3.3 and 53.33 per cent (Table 2). Maximum maggot mortality was recorded at 160 IJs/cm^2 (53.33 %), followed by mortality at 80 IJs/cm^2 (36.60 %), 40 IJs/cm^2 (26.60%), 20 IJs/cm^2 (16.60%) and 10 IJs/cm^2 (6.66 %) against 3.3 per cent in control. The pupal mortality at 160 IJs/cm^2 was also found statistically at par with pupal mortality at 80 IJs/cm^2 (36.6 %). Similarly, no significant differences in pupal mortality were observed between 80 & 40 and 40 & 20 IJs/cm^2 doses.

Efficacy of *S. feltiae* HR1 against the pupae of *B. zahadi* Mahmood

At 3 days after treatment

At 3 days after treatment, pupal mortality in different concentrations ranged between 6.6 and 36.66 per cent as shown in Table 2. No mortality was observed in control and at 10 IJs/cm^2 dose. Significantly highest (36.66%) pupal mortality was recorded at 160 IJs/cm^2. The pupal mortality at 40 IJs/cm^2 was also found at par with pupal mortality at 80 IJs/cm^2 and 20 IJs/cm^2 pupal mortality increased with increase in EPNs concentrations.

At 7 days after treatment

After 7 days of exposure, the pupal mortality ranged between 6.66 and 50.0 per cent (Table 2). The pupal mortality remained maximum at 160 IJs/cm^2 (50.0 %) which was also at par with mortality at 80 IJs/cm^2 (43.33 %) followed by 40 IJs/cm^2 (23.33 %), 20 IJs/cm^2 (13.33%) and 10 IJs/cm^2 (6.66 %) against 3.33 per cent in control. Similarly, pupal mortality at 20 and 10 IJs/cm^2 was at par with each other.

Concentration mortality response of last instar maggots and pupae of *Z. zahadi* Mahmood to indigenous strains HR2 and HR1

The data on mortality of last instar maggots and pupae of *B. zahadi* Mahmood caused by indigenous strains of entomopathogenic nematodes which was closely falling between 6.66-68.66 per cent was subjected to probit analysis to calculate the LC50 and LC90 values for *H. bacteriophora* HR2 and *S. feltiae* HR1 and log dose response curve is presented in Fig. 1-4.

The LC50 for *H. bacteriophora* against last instar maggots, was 178.01(IJs/cm^2) and 59.52 (IJs/cm^2) after 3 and 7 days of treatment, respectively. While it was 195.37 (IJs/cm^2) and 107.55 (IJs/cm^2) for *S.*

Table 2. Efficacy of indigenous strains of entomopathogenic nematodes against pupae of fruit fly, *Bacterocera zahadi*

DOSE (IJs/cm^2)	Pupal mortality after days (%)*			
	H. bacteriophora		*S. feltiae*	
	3 DAT	7 DAT	3 DAT	7 DAT
0	0.00 (5.73)**	3.30 (10.28)	0.00 (5.73)*	3.33 (5.73)
10	0.00 (5.73)	6.66 (14.82)	0.00 (5.73)	6.66 (14.82)
20	13.33 (22.01)	16.6 (24.63)	6.6 (14.82)	13.33 (22.00)
40	23.33 (29.47)	26.6 (31.64)	13.33 (22.01)	23.33 (29.46)
80	36.66 (37.82)	36.6 (37.82)	23.33 (29.47)	43.33 (41.73)
160	43.33 (41.73)	53.33 (47.5)	36.66 (37.82)	50.00 (45.56)
CD (P=0.05)	(5.53)	(9.80)	(7.58)	(8.63)

*Average of five replications; **Figures in parentheses are arc sine transformed values

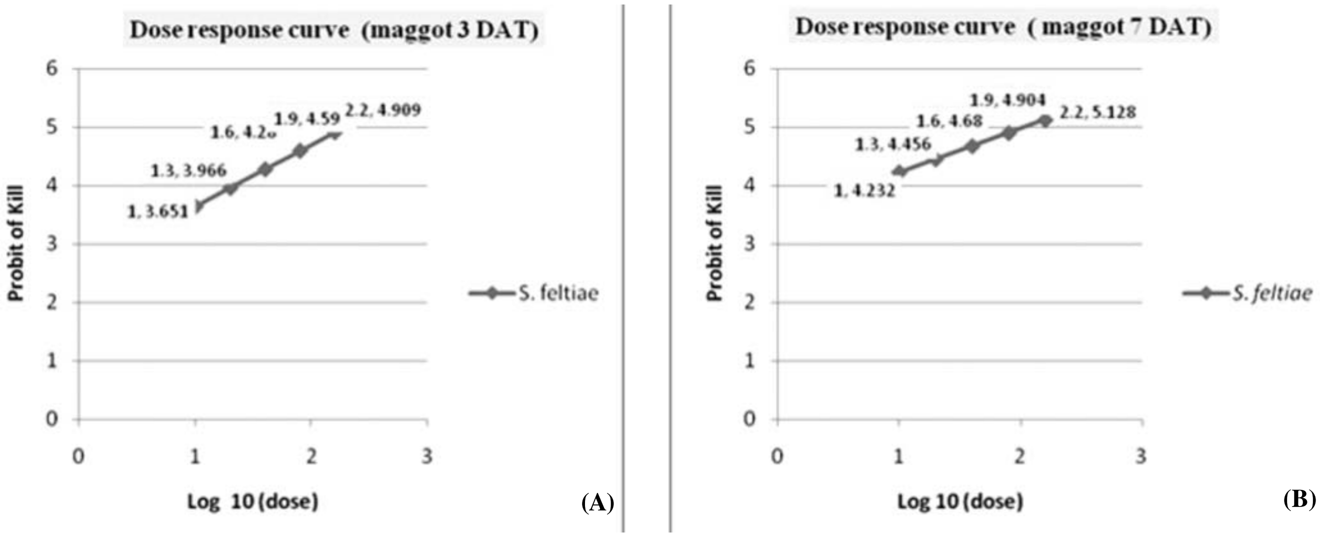


Fig. 1. Last instar maggot Log Dose Response Curve (A- 3 DAT, B- 7 DAT) (*S. feltiae)*

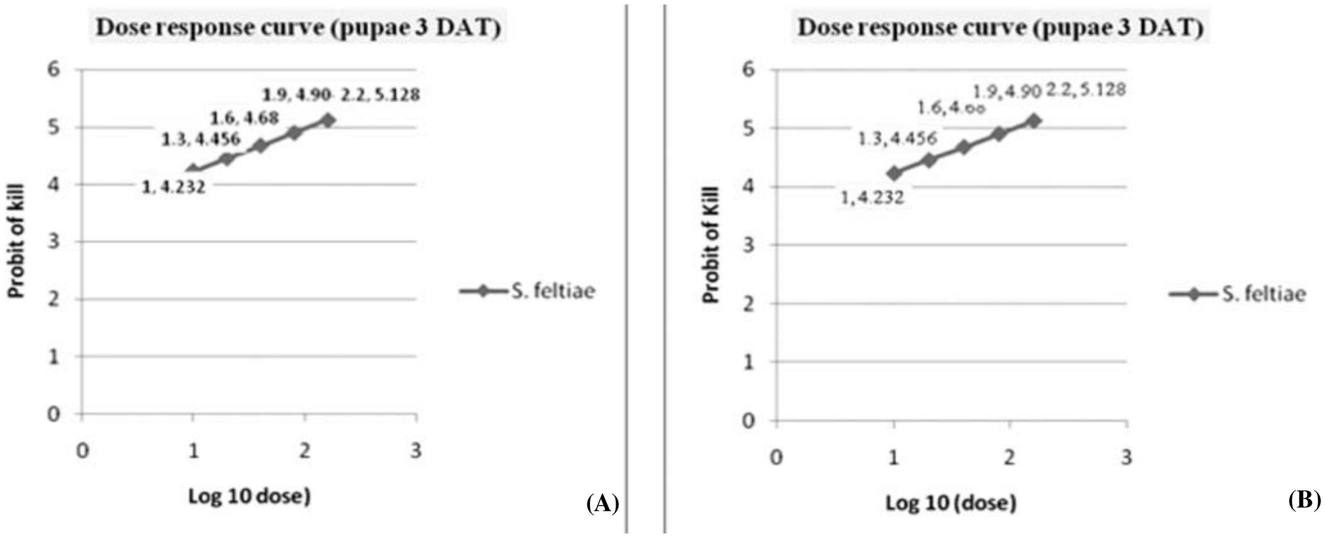


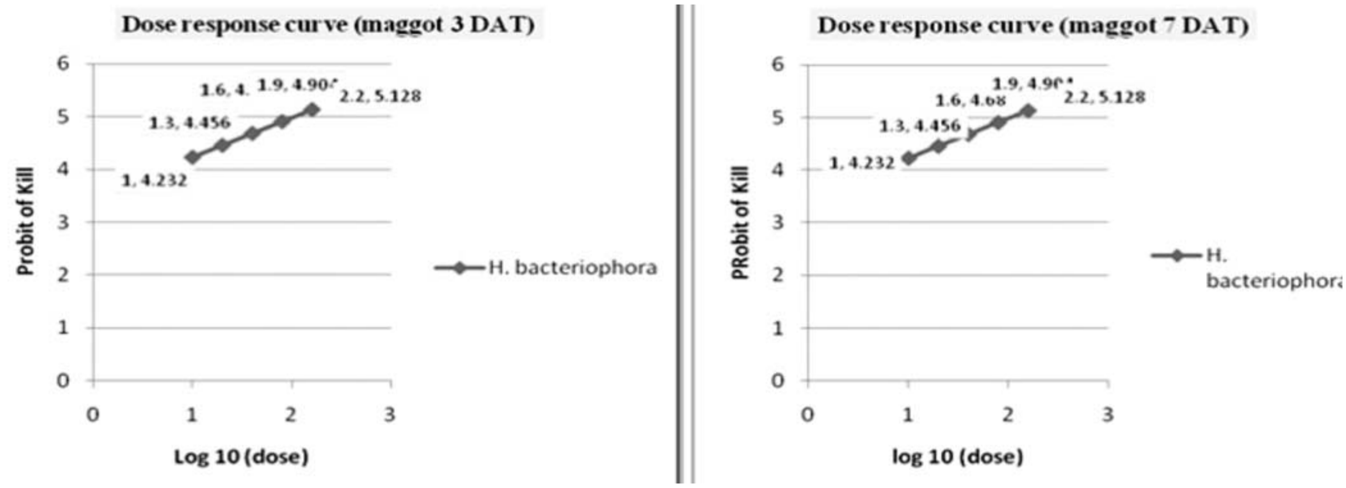
Fig. 2. Pupal Log Dose Response Curve (A- 3 DAT, B- 7 DAT) (*S. feltiae)*

feltiae against last instar maggots after 3 and 7 days of treatment, respectively (Table 3).

The LC50 for *H. bacteriophora* against pupae was 174.63 (IJs/cm^2) and 145.17 (IJs/cm^2) after 3 and 7 days of treatment, respectively. While, it was 286.85 (IJs/cm^2) and 143.60 (IJs/cm^2) against pupae after 3 and 7 days of treatment, respectively (Table 3).

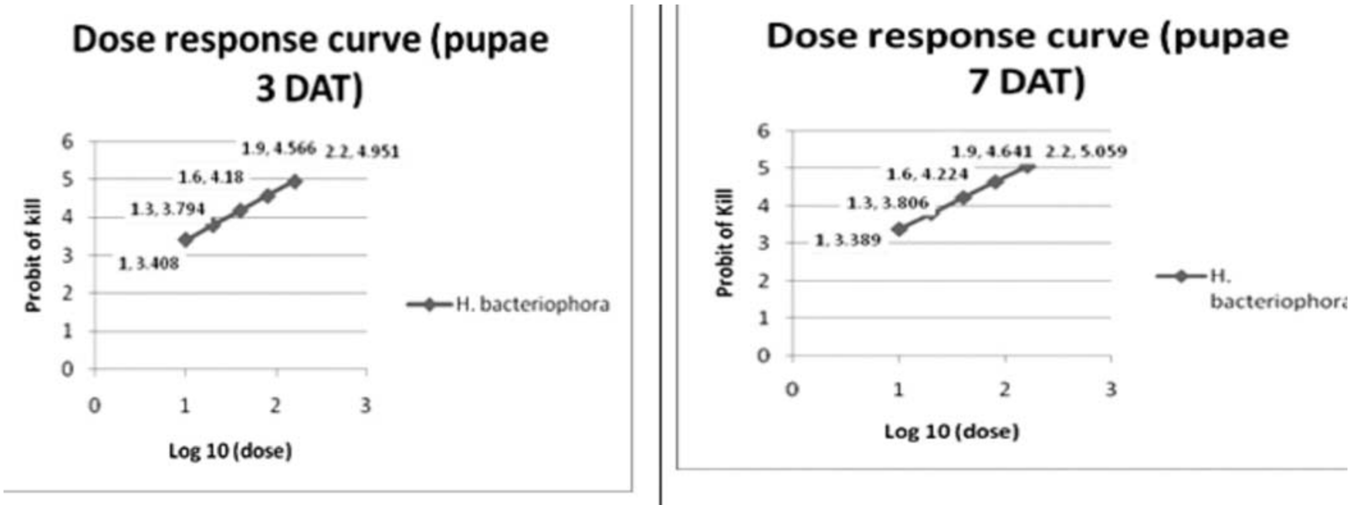
DISCUSSION

In the present study, the two strains of EPNs (HR2 and HR1) caused significant mortality at different exposure periods in laboratory conditions to both distinct developmental stages of fruit fly. *H. bacteriophora* caused slightly higher mortality than that of *S. feltiae* against maggots and pupae of test species (Table 1,2).



(A) (B)

Fig. 3. Last instar maggot Log Dose Response Curve (A- 3 DAT, B- 7 DAT) (*H. bacteriophora)*



(A) (B)

Fig. 4. Pupal Log Dose Response Curve (A- 3 DAT, B- 7 DAT) (*S. feltiae)*

Table 3. Lethal concentraions (LC50 and LC90) of the tested EPNs against *Bacterocera zahadi* Mahmood last instar maggots and pupae

Nematode spp.	Stage of Insect	Exposure time (DAT)	LC50 (IJs/cm^2)	LC90 (IJs/cm^2)	$\tau 2$	p-value
H. bacteriophora	Last instar maggot	3 DAT	178.01	1.28	7.817	0.001
	Last instar maggot	7 DAT	59.52	1.28	0.529	0.001
	Pupae	3 DAT	174.63	1.28	4.056	0.001
	Pupae	7 DAT	145.17	1.28	1.600	0.001
S. feltiae	Last instar maggot	3 DAT	195.37	1.28	10.120	0.001
	Last instar maggot	7 DAT	107.55	1.28	1.667	0.003
	Pupae	3 DAT	286.85	1.28	0.043	0.001
	Pupae	7 DAT	143.60	1.28	2.580	0.000

The authors concluded that the activity of IJs was correlated with EPNs species as well as concentration rate. Mexican strain of *S. feltiae* resulted in 6, 28 and 54 per cent maggot mortality in melon fruit fly (*D. cucurbitae*) at 15, 50 and 150 IJs/cm^2 doses (Lindergen and Vail, 1986). Our findings are in agreement to other studies that found pathogenicity of these two species against fruit flies. *H. bacteriophora* and *S. feltiae,* have caused 68.4 per cent mortality of maggots and 65.6 per cent mean pupal mortality in *B. zonata*, respectively. (Nouh and Hussien, 2004). Hussein *et al.* (2006) also observed 90 and 100 per cent mortality of *D. ciliates* maggots by *S. feltiae* at 500 and 1000 IJs/cm^2 after 3 and 7 days of treatment respectively. The maggot mortality of 80 and 90 per cent after 3 and 7 days of treatment in *C. capitata* with *H. bacteriophora* (SF1) has been observed (Malan and Manrakhan, 2009).

In unison with present results, various studies have reported maggot and pupal mortalities of fruit fly species. Sirjani *et al.* (2008) have reported that *H. bacteriophora* caused 10.2 and 27 per cent infection in third instar larvae of *B. olae* in olive fruit and soil, respectively at 25 IJ/cm^2. Dolinski and Souza (2011) reported highest mortalities i.e. 81.5 and 100 per cent maggots mortality in Mediterranean fruit fly by *H. bacteriophora* at 45 IJs/3 larvae and 105 IJs/3 larvae, respectively and also reported 80 per cent pupal mortality by *Heterorhabditis* spp. at 155 IJs/3 pupae. After one hour of application, 74 and 41.3 per cent maggot mortality in *C. cpitata* by *S. feltiae* and *H. bacteriophora*, respectively has been recorded. Djelouah *et al.* (2015) reported 88.1, 96.4 and 93.2 per cent mortalities of *B. zonata* at 200, 400 and 1600 IJs/ml concentrations, respectively. Hussein and Nouh (2014) reported, *H. bacteriophora* caused 68.4 and 62.8 per cent pupal mortality in Mediterranean fruit fly (*C. capitata*) and peach fruit fly (*B. zonata*), respectively at 25^{0}C. Aatif *et al.* (2019) reported 69.08 per cent pupal mortality of *B. dorsalis* at 200 IJs/ml concentration after 9 days of exposure. Maximum mortality of 81.8% and 73.64% caused by *H. bacteriophora* has been reported after 72 h of exposure by Usman *et. al.,* 2020 in *B. zonata* and *B. dorsalis* among ten species of EPNs.

CONCLUSIONS

Among the insect pests, fruit fly was the most destructive as it incurred damage to fruits rendering them unwholesome. Two indigenous strains of entomopathogenic nematodes (HR2 and HR1) belonging to two genera *Heterorhabditis* and *Steinernema* were used at different concentrations and their efficacy against the fruit fly was tested. Both the local strains of EPNs infected the maggots and pupae of fruit fly. The maggots were found more susceptible to nematode infection than pupae. The maximum average maggot mortality caused by *S. feltiae* and *H. bacteriophora* at seven days after treatment was 54.66 and 68.66 per cent, respectively. Whereas in case of pupae the maximum per cent mortality obtained was 50.0 and 53.33 with respective inoculation of *S. feltiae* and *H. bacteriophora*. The efficacy of *H. bacteriophora* against maggots and pupae of fruit fly, *B. zahadi* Mahmood was slightly better than *S. feltiae*. It can be concluded that both the local strains of EPNs infected the maggots and pupae of fruit fly. The maggots were more susceptible to nematode infection than pupae. The efficacy of *H. bacteriophora* was slightly better than that of *S. feltiae.* Future research should focus on applications of EPNs in open field and greenhouse conditions. Furthermore, infectivity of these EPNs in combination with other microbial control agents or other pest management should be explored so that these indigenous strains of EPNs could be incorporated in an integrated pest management program for fruit fly management.

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Bio-Management of Soil-Borne Pathogens Infecting Capsicum (*Capsicum annum* L.) under Protected Cultivation

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ABSTRACT: Repeated *in-vivo* trials (2019-20) were conducted to manage the concomitant infestation of soil-borne pathogens infecting capsicum (bell pepper) through the application of *Bacillus subtilis, Pseudomonas fluorescens* and *Trichoderma harzianum* under protected cultivation system. The initial mean population of soil-borne pathogens,*Meloidogyne incognita, Fusarium oxysporum* and *Rhizoctonia solani* was 1.87 J_2/g soil, 2.03×10^3 cfu/g soil, and 14.9 propagules/50 cc soil respectively. To manage these pathogens freshly prepared bio-agents were used for soil and drench applications. The combined application of (*T. harzianum* + *B. subtilis* + *P. fluorescens*) caused a significant ($P \leq 0.05$) reduction (up to 90%) with respect to nematode multiplication in soil and disease severity of all pathogens with significant ($P \leq 0.05$) enhancement in plant health (up to 54%) at the end of the subsequent second trial followed by chemical controlin comparison to untreated control. The eggs of *M. incognita* obtained from plant roots treated with all tested bio-agents together showed the least hatching (14.5%) compared to untreated control (90%) when subjected to an in-vitro bioassay test after the termination of each trial. All tested bio-agents showed that they are highly compatible with each other in the rhizosphere under protected cultivation of capsicum. Therefore, *T. harzianum, B. subtilis* and *P. fluorescens* are potential biological control agents in plant disease management to overcome serious threats of chemical pesticides under a protected cultivation system.

Keywords: Bio-management, capsicum, soil-borne pathogen, protected cultivation

The protected cultivation system (PCS) is a relatively new technology and is popular among farmers/growers globally and covering 405000 ha throughout the world (Reddy, 2016). However, this technology is still in its infancy stage in India and contributes only 7.4% of the global acreage. Among protected crops, capsicum performs better under the PCS in terms of better fruit quality, higher yield, economic feasibility, and ensuring year-round availability (Murthy *et al.* 2009), but optimum high temperature and high humid conditions under protected cultivation round the year make capsicum crops more vulnerable to soil-borne pathogens i.e. root-knot nematode, wilt and root rot fungus. These pathogens are agents that are involved in poor establishment of seedlings and the ultimate cause of death under a PCS and can cause up to 100% losses. Further, in the latter stages, seriously affect the yield (between 42-83%) and the quality of the capsicum crop (Lopez-Marin *et al.* 2013). These pathogens can cause serious damage to the host plant (Gajera *et al.* 2016) which gradually increased in the subsequent next season crop if kept unattended (Singh and Balodi 2021) by disrupting the vascular system, interface with physiological processes involved in water and nutrient uptake (Ayala-Donas *et al.* 2020).

To manage these pathogens, under PCS, farmers/ growers are applying uncounted rounds of chemical pesticides which are associated with not only contamination of soil phytotoxicity, increased toxicity content in produce and human health issues (Rajkumar *et al.* 2005; Sharma *et al.* 2008) but produce resistance in the target pest (Pramanik *et al.* 2020).The use of biocontrol agents (BCAs) is presented as an alternative to chemical pesticides, for the control of pests and/or pathogens. Research on the biological control of diseases through the use of BCAs has increased in the last few years not only because of their antagonistic action but also due to their potential to promote the growth of plants (Elnahal *et al.* 2022). Recently, we isolated and identified local strains of *Trichoderma harzianum* (Accession no. MT734519), *Bacillus subtilus* (Accession no. W008011) and *Pseudomonas fluorescens* (NCIPM/ PCPF/01) and established their bio-control potential and compatibility under in-vitro bioassay test (Singh *et al.* 2021). Therefore, two consequent in-vivo trials were conducted to control the concomitant infestation caused by *M. incognita*, together with *F. oxysporum* and *R. solani*on capsicum through strains of *T. harzianum*, *B. subtilus* and *P. fluorescens* under PCS which help to develop a bio-intensive IPM module for protected crops.

MATERIALS AND METHODS

The trials were conducted with 30 cm dia. pots, which were filled with 15 kg of infested soil from the infested field (experimental site) located at village-Jainpur, district-Sonipat (29.0678° N and 77.1277° E) (Haryana), India. The observations of both trials were recorded after 120 days of transplanting of capsicum seedlings. The transplanting of seedlings for the first trial was completed on 1st August 2019 and soon after the harvest of the first trial, the transplanting of seedlings for the second trial was completed on 2nd December 2019. The pots were kept in between the main capsicum crop in randomized design in strips in a manner that they get water and nutrients easily through the drip irrigation system. Each pot was transplanted with a twenty-five-day-old seedling of capsicum cv. Bachata, which has grown separately in pro trays containing sterilized coco-peat mixture. The soil was fertilized as per recommended crop-specific agronomical requirements of the capsicum crop.

The initial mean population of *M. incognita, F. oxysporum,* and *R. solani* was 1.87 J_2/g soil, 2.03×10^3cfu/ g soil, and 14.9 propagules/50cc soil respectively at the time of transplanting of capsicum seedlings in pots during first season trial. The experiments comprises of six treatments: T1- Control (untreated); T2- *T. harzianum* alone (2.0×10^6 cfu/g); T3- *B. subtilis* (1.0×10^8 cfu/g); T4- *P. fluorescens* (1.0×10^8 cfu/g); T5- T2 + T3 + T4 and T6- Fluensulfone (480 EC) (Nematicide) at 7.0 kg/ ha (0.7 g/pot) +Azoxystrobin 18.2% +Difenocanazole 11.4% SC (fungicide) at 1ml/litre as Control. The dose of bio-agents was calculated on the area (0.94 m^2) basis of pots at 10 kg and or litre/ha (i.e. 1.10g or ml/pot as the case may be) were used. The freshly prepared liquid formulation of bio-agents (*T. harzianum*-15 day old culture, *B. subtilis,* and *P. fluorescens*-5 day old culture) was used through drip irrigation and/or by drenching at 5 ml/l water. For soil application, well-decomposedfarmyard manure (FYM), neem cake and vermicompost were fortified with the first half dose of all bio-agents before amending the pots, and the rest half dose of *T. harzianum* was applied manually in three equal doses directly to the root system in soil by drenching, whereas *B. subtilis* and *P. fluorescens* was applied through drenching after 15 days of transplanting at 15 days interval. All treatments were repeated in the second season trial without disturbing the soil and replicated four times. All standard techniques for nematological and pathological studies were adopted (Singh and Balodi 2021). The observations were made on plant growth parameters (shoot length, shoot weight, and root weight), multiplication of nematode (number of galls and egg masses/root system, eggs/egg mass, and soil population of *M. incognita* J_2) and fungal pathogens (multiplication in soil and shoot and root disease).

The pure culture of all BCAs was obtained through repeated sub-culture technique and maintained at 4°C in a refrigerator. The spore load of *T. harzianum* was estimated in diluted samples using a hemocytometer and maintained at 2.0×10^6 cfu/g throughout the study period. The spore load of both *B.subtilis* and *P. fluorescens* was estimated in diluted samples using a hemocytometer and maintained at 1.0×10^8 cfu/ml throughout the study period.

Statistical analysis

The original data on plant growth parameters and nematode multiplication were square-root transformed to normalize the distribution. The data for two consecutive experiments on plant growth parameters and multiplication of *M. incognita, F. oxysporum,* and *R. solani* were analysed and subjected to ANOVA separately using SPSS ver.16. A test for homogeneity of variances was conducted for pooling the data since it showed a difference, the data were presented separately for both trials. Reproductive factor (R.F) was also calculated by dividing the final population (P_f) by the initial population (P_i) ($R.F=P_f/P_i$).

RESULTS AND DISCUSSION

Concomitant infestation of *M. incognita*, *F. oxysporum,* and *R. solani* had a suppressive effect and caused a significant ($P \leq 0.05$) reduction in shoot length and shoot weight compared to treated plants. This reduction was 20.8 and 17.3% respectively more in the subsequent second trial. The infected plants showed heavy galling on roots and the root weight was found to be increased up to 2% more in the second trial.The greatest recovery in shoot length (34.6%) and shoot weight (35.2%) was recorded in the treatment where the combined application of *T. harzianum* + *B. subtilis* + *P. fluorescens,* which was subsequently increased up to 54.3% and 54.1% respectively in the second season trial compared to control and differ significantly i.e. p=1.56 with chemically treated plants (44.4 and 36.1% respectively) (Table 1). This enhancement was partly due to the reduction in *M. incognita*, *F. oxysporum* and *R. solani* infestation and partly due to improved soil fertility by the addition of bio-agents fortified FYM, neem cake and vermicompost (Singh and Balodi, 2021). In addition, bio-agents are well documented and equipped with plant growth-promoting properties which helped to promote the overall health of the plant (Abhilash *et al.* 2016). During both trials, the increased root weight of plants under untreated check was due to the presence of numerous root-knot galls caused by *M incognita* which did not show any significant difference (p=0.21) with our best treatment (i.e. combined bio-agents). It seems that the tested bio-agents contribute significantly to increasing the biomass of capsicum plants.The authors recorded an increased root weight due to the root galls' formation on cucurbit crops in their earlier research (Singh 2019, Singh and Balodi 2021).

All BCAs caused a significant ($P \leq 0.05$) reduction in the multiplication of *M. incognita* compared to control (Table 1). The greatest reduction up to 94.5, 94.2 and 94% respectively in the number of galls, number of egg masses/root system and soil population of *M incognita* were recorded in chemically treated plants which were followed by 72.4, 80.2 and 84.7% respectively in the treatment where all bio-agents, *T. harzianum* + *B. subtilis* + *P. fluorescens* wereapplied together compared to the control during the first season trial. But during subsequent the second season trial,the reduction in thenumber of *M. incognita* juveniles extracted from the soil samples of chemically treated plants (96.4%) did not show any statistical difference ($P<0.05$) with combined bio-agents (*T. harzianum* + *B. subtilis* + *P. fluorescens*) treated plants (95.8%). The rate of multiplication or reproductive factor was the same i.e. 0.1 in both the treatments.The population of *M. incognita* was recorded 2.8 times more (524.5 J_2/100 cc soil) i.e. 64.3% higher at the end of the second trial compared to the initial population i.e. 187J_2/100 cc soil (Table-1) under control pots.Several researchers recommend that a possible means of increasingthe consistency and efficacy of BCAs was due to applying bio-agents in combinations (Sharma *et al.* 2008b; Singh and Mathur 2010; Abo-Elyousr *et al.* 2014; Singh 2019; Singh and Balodi 2021). The bio-agents used in the present study express different modes of action (Singh *et al.* 2021) which helped to reduce the multiplication and development of soil-borne pathogens throughout the experimentation.

Table 1: Effect of bio-control agents on plant growth parameters of capsicum and nematode multiplication under concomitant infestation caused by *Meloidogyne incognita, Fusarium oxysporum* and *Rhizoctonia solani* after 120 days of transplantation under protected cultivation system.

Treatments	Trial	Plant growth parameters			Root-knot nematode, *M. incognita* multiplication at harvest			
		Shoot length (cm)	**Shoot weight (g)**	**Root weight (g)**	**No. of galls/ root system**	**No. of egg mass/ root system**	**No. of eggs/ egg mass**	**No. of J_2/ 100 cc soil &RF**
Untreated check (control)	1	52.5 (7.31±0.17)^{c} **[0.0]***	57.8 (7.66±0.16)^{c} **[0.0]**	63.8 (8.03±0.23)^{a} **[0.0]***	36.3 (6.04±0.49)^{a} **[0.0]***	30.3 (5.59±0.13)^{a} **[0.0]**	187.3 (13.72±0.32)^{a} **[0.0]** **#{+38.2}**^{A}**{1.6}**	302.5 (17.41±0.32)^{a} **[0.0]**
	2	41.6 (6.50±0.15)^{c} **[0.0]** {-20.8}	47.9 (6.97±0.36)^{c} **[0.0]**{-17.3}	65.4 (8.10±0.35) **[0.0]**{+2.0}	46.0 (6.80±0.49)^{a} **[0.0]**{+21.2}	41.5 (6.46±0.52)^{a} **[0.0]**{+27.1}	188.0 (13.73±0.38)^{a} **[0.0]** {+0.4}	524.5 (22.89±0.68)^{a} **[0.0]** **(+42.3){+64.3}{2.8}**
Trichoderma harzianum alone	1	65.1 (8.13±0.13)^{b} **[+19.4]**	74.0 (8.65±0.15)^{b} **[+21.9]**	50.3 (7.16±0.10)^{b} **[-26.9]**	19.5 (4.50±0.28)^{b} **[-46.2]**	15.3 (3.91±0.56)^{b} **[-49.6]**	68.0 (8.27±0.43)^{b} **[-63.7]**	70.0 (8.42±0.13)^{c}**[-76.9]** **{-47.77}{0.5}**
	2	69.8 (8.76±0.17)^{b} **[+45.5]**{+14.0}	83.5 (9.18±0.25)^{b} **[+42.6]**{+10.9}	57.0 (7.42±0.27) **[-16.5]**{7.4}	16.8 (4.12±0.52)^{b} **[-63.6]** {-14.1}	11.8 (3.54±0.29)^{b} **[-71.7]** {-23.0}	47.8 (6.93±0.48)^{d} **[-74.6]** {-29.8}	46.5 (6.80±0.63)^{b} **[-91.1]** **(-33.6){-75.1}{0.6}**
Bacillus subtilis alone	1	63.0 (7.99±0.23)^{b} **[+16.7]**	70.7 (8.46±0.22)^{b} **[+18.2]**	50.3 (7.16±0.09)^{b} **[-26.9]**	20.5 (4.62±0.24)^{b} **[-43.4]**	14.8 (3.93±0.30)^{b} **[-44.4]**	85.5 (9.28±0.37)^{b} **[-54.3]**	77.5 (8.85±0.19)^{c}**[-74.4]** {-44.3}**{0.6}**
	2	70.0 (8.57±0.21)^{b} **[+43.1]** {+13.1}	81.0 (9.04±0.33)^{b} **[+40.8]**{+12.5}	57.3 (7.61±0.10) **[-12.3]**{+11.8}	13.8 (3.80±0.33)^{b} **[-70.1]** {-32.9}	10.8 (3.37±0.37)^{b} **[-74.1]** {-27.1}	69.5 (8.37±0.40)^{b} **[-63.0]** {-18.7}	42.3 (6.53±0.46)^{b}**[-88.5]** **(-45.5){-77.4} {0.4}**
Pseudomonas fluorescens alone	1	66.0 (8.18±0.20)^{b} **[+20.4]**	70.3 (8.44±0.21)^{b} **[+17.8]**	50.8 (7.19±0.06)^{b} **[-25.6]**	22.8 (4.80±0.49)^{b} **[-37.2]**	12.3 (3.62±0.23)^{b} **[-59.5]**	78.5 (8.85±0.61)^{b} **[-58.1]**	81.5 (9.07±0.29)^{c}**[-73.1]** **{-32.9}{0.7}**
	2	70.8 (8.49±0.27)^{b} **[+42.1]** {+7.4}	79.8 (8.69±0.33)^{b} **[+35.9]**{+11.7}	57.9 (7.51±0.34) **[-14.2]**{+9.0}	15.0 (3.92±0.47)^{b} **[-67.4]** {-34.1}	12.0 (3.58±0.27)^{b} **[-71.1]** {-2.0}	62.5 (7.95±0.34)^{b} **[-66.8]** {20.4}	51.0 (7.19±0.35)^{b}**[-90.3]** **(-37.4){-72.7}{0.5}**
T. harzianum + *B. subtilis* + *P. fluorescens*	1	80.3 (9.01±0.16)^{a} **[+34.6]**	89.1 (9.48±0.28)^{a} **[+35.2]**	60.3 (7.82±0.15)^{a} **[-5.8]**	10.0 (3.23±0.43)^{c} **[-72.4]**	6.0 (2.61±0.25)^{c} **[-80.2]**	42.0 (6.50±0.52)^{c} **[-77.6]**	46.3 (6.85±0.32)^{d}**[-84.7]** **{-84.7}{0.3}**
	2	83.8 (9.55±0.21)^{a} **[+54.3]** {11.1}	99.0 (10.27±0.13)^{a} **[+54.1]**{+9.5}	68.5 (8.49±0.37) **[+10.0]**{+15.7}	4.3 (2.25±0.24)^{c} **[-90.8]** {-57.5}	3.0 (1.90±0.35)^{c} **[-92.8]** {-50.0}	35.3 (6.00±0.31)^{d} **[-81.3]** {-16.1}	22.0 (4.70±0.54)^{c}**[-95.8]** **(-52.4){-88.2}{0.1}**
Chemical pesticides (N+F)	1	71.5 (8.50±0.27)^{ab} **[+26.6]**	69.0 (8.36±0.25)^{b} **[+16.3]**	51.3 (7.23±0.11)^{b} **[-24.4]**	2.0 (1.61±0.37)^{d} **[-94.5]**	1.8 (1.64±0.14)^{d} **[-94.2]**	173.5 (13.21±0.15)^{a} **[-7.3]**	18.0 (4.23±0.60)^{e}**[-94.0]** **{-84.9}{0.2}**
	2	71.5 (8.66±0.32)^{b} **[+44.4]** {+3.7}	85.5 (8.71±0.27)^{b} **[+36.1]**{+18.6}	58.5 (7.67±0.35) **[-14.4]**{+12.0}	10.8 (3.39±0.28)^{b} **[-76.6]** {-81.4}	4.0 (2.21±0.21)^{d} **[-90.4]** {+56.3}	171.5 (13.13±0.21)^{a} **[-8.8]** {-1.2}	19.0 (4.41±0.45)^{c}**[-96.4]** **(+5.3){-89.8}{0.1}**
CD ($P \leq 0.05$)	1	0.58	0.55	0.41	1.03	0.96	1.28	0.97
	2	0.66	0.9	NS	1.18	0.91	0.96	1.69

Note: Figures presented in parentheses ( ) are square root transformed value ± Standard Error; Figures presented in parentheses **[ ]** and bold are percent increase (+) or decrease (-) over untreated check (control); Figures presented in parentheses { } are percent increase (+) or decrease (-) over first trial; Means in each column with different superscript letters differ significantly (P<0.05); Observations were made after 120 days of transplantation. Figures presented in parentheses **( )** and bold are per cent increase or decrease over first trial; #Figures presented in parentheses **{ }** and bold are percent increase (+) or decrease (-) over initial population (i.e. 187 J_2/100 cc soil); Chemical pesticides (N+F) - Fluensulfone (480 EC) (Nematicide) + Azoxystrobin 18.2% + Difenocanazole 11.4% SC (fungicide); ^{A}Figures presented in parentheses **{ }** and bold are reproductive factor (R.F. = P_f/P_i) where R. F.-Reproductive factor, P_f -Final population and P_i - initial population.

During the first trial, data presented in Table 2,the greatest reduction in shoot disease severity (100%) and root disease severity (88.8%) caused by *F. oxysporum* and root disease severity (92%) caused by *R. solani* were documented in chemically treated plants which were followed by the treatment where all three bio-agents were applied together i.e. 83.3, 84.8, and 76% respectively. Further, shoot disease severity caused by *F. oxysporum* increased by 23.5% whereas root disease severity caused by *F. oxysporum* and *R. solani* increased by 5.3 and 13.9% respectively during the second subsequent trial as compared to the first trial. The application of *T. harzianum* + *B. subtilis* + *P. fluorescens* (together) performed better in reducing the fungal diseases in comparison to chemical control and caused a 98.2, 95 and 94.7% reduction in shoot disease severity caused by *F. oxysporum* and root disease severity caused by *F. oxysporum* and *R. solani* respectively. It is evident from the data that both chemicals and BCAs were equally effective to check all the pathogens significantly and preventing the entry of any of the pathogens into the plant system and confirming the findings of Singh and Balodi(2021) on cucumber under PCS.

Similar results were obtained in the reduction of soil population of both fungi in terms of cfu/g and no. of propagules/50 cc soil. Both, chemical control and application of combined bio-agents treatment did not show any significant difference (P=0.15) during the first trial. However, during the subsequent second season trial, the apparent greatest reduction in a number of colony/propagules was recorded in combined bio-agents treated soil (90.9%) followed by 84.1% under chemical control but did not differ statistically (P=0.38).

The success of bio-agent(s) is dependent upon its establishment in the rhizosphere and ability to colonise the pathogen for which they require energy to grow and colonise in the soil prior to parasitization and/or predation (Singh and Mathur 2010; Singh *et al.* 2021). Our findings recommend the fortification of FYM, neem cake and vermicompost with bio-agents before application in the soil, which not only helps to provide energy for survival and proliferation of *T. harzianum, B. subtilis* and *P. fluorescens* but also added nutrients to the soil and antagonise pathogens, resulting in good plant health throughout the present investigation.

During the subsequent second trial, the positive effect of bio-agents was equal to or even better in comparison to chemical pesticides, in reducing multiplication and the disease severity caused by *M. incognita*, *F. oxysporum* and *R. solani*. The result suggests that bio-agents may persist in the soil for years which could be an advantage over pesticides and as suggested by Lorena and Javiera (2020), if BCAs remain in the rhizosphere they are in the first line of defence against the attack of the soil-borne pathogens.Agradual increase in the number of colony forming units was detected after both trials compared to the initial population which showed the successful establishment of the bio-agents in all treatments, however, during the first trial, the number of colony-forming units was significantly greater with individual application of *P. fluorescens* (2.5 x 10^3) compared to other bio-agents application. A substantial proliferation in colony-forming unit counts was recorded up to the end of the second trial over initial population counts. The multiplication of all bio-agents, in terms of colony-forming units (range from 3.1 x 10^3 to 4 x 10^3), did not differ statistically (P>0.05) at the end of the second trial (Table 2).

After the termination of each trial, eggs were collected from the roots and subjected to a bio-assay test. All tested bio-agents caused various degrees of egg inhibition during both trials. All bio-gents showed a gradual decrease in egg hatching compared to the first trial. The significantly (P>0.05) greatest inhibition in egg hatching was recorded with the eggs obtained from the roots of plants where all bio-agents were applied together i.e. 75 and 83.9%, in the first and subsequent second trial respectively

Table 2: Effect of bio-control agents on multiplication and shoot and root disease severity caused by *F. oxysporum* (FO) and *R. solani* (RS) on capsicum under concomitant infestation caused by *Meloidogyne incognita, Fusarium oxysporum* and *Rhizoctonia solani* after 120 days of transplantation under protected cultivation system.

Treatment	Trial	Shoot and root disease severity			Multiplication of *F. oxysporum* and *R. solani* in soil				Multiplication of bio-agents x 10^3	
		F. oxysporum (Shoot)	*F. oxysporum* (Root)	*R. solani* (Root)	*F.oxysporum* (cfu/g) x 10^3 Initial	*F.oxysporum* (cfu/g) x 10^3 Final	*R. solani* (propagules/50cc soil) Initial	*R. solani* (propagules/50cc soil) Final	Initial	Final
Untreated check (control)	1	2.6 (1.90±0.06) ^{a} **[0.0]**	3.6Â (58.84±6.34)^{a} **[0.0]** {71.3}	3.1 (52.49±4.07)^{a} **[0.0]** {62.5}	1.9 (1.71±0.04)*	3.8 (1.65±0.07)^{a} **[0.0]# (+50.0)**	14.0 (3.86±0.12)	19.5 (4.51±0.24)^{a} **[0.0] (+28.2)**	0.0 (1.00±0.00)^{c}	0.0 (1.00±0.00)^{d} **[0.0]#**
	2	3.4 (2.09±0.06)^{a} **[0.0]***** **(23.5)**	3.8 (61.56±4.75)^{a} **[0.0]** **(+5.3)**{76.3}	3.6 (57.82±3.28)^{a} **[0.0]** **(13.9)**{71.3}		4.9 (2.42±0.06)^{a} **[0.0]** **(+61.3)**		22.0 (4.78±0.26)^{a} **[0.0]** **(+36.4)**		0.0 (1.00±0.00)^{c} **[0.0]**
Trichoderma harzianum alone	1	0.6 (1.23±0.13)^{b} **[-80.9]**	1.1 (28.13±2.24)^{b} **[-68.3]** {22.5}	0.9 (21.91±8.45)^{b} **[-70.0]** {18.8}	2.0 (1.72±0.06)	1.5 (1.12±0.08)^{b} **[-62.1]**	13.0 (3.73±0.20)	19.5 (4.51±0.24)^{a} **[0.0] (+28.2)**	0.3 (1.19±0.09)^{b}	1.9 (1.70±0.02)^{c} **[+83.2](84.2)**
	2	0.4 (1.16±0.10)^{bc} **[-88.8]**	0.6 (18.88±3.89)^{b} **[-84.8]** {11.5}	0.5 (18.60±2.09)^{b} **[-85.1]** {10.5}		1.0 (1.39±0.06)^{b} **[-79.6]**		22.0 (4.78±0.26)^{a} **[0.0] (+36.4)**		3.3 (2.07±0.05)^{a} **[+90.3](90.9)**
Bacillus subtilis alone	1	1.1 (1.40±0.16)^{b} **[-59.7]**	1.4 (31.50±2.09)^{b} **[-61.2]**{27.5}	1.7 (32.25±3.83)^{b} **[-46.0]** {33.8}	1.9 (1.70±0.03)	1.5 (1.36±0.07)^{b} **[-60.8]**	17.3 (4.26±0.15)	9.0 (3.15±0.15)^{b} **[-53.8]**	0.6 (1.33±0.08)^{a}	2.2 (1.78±0.03)^{b} **[+73.6](72.7)**
	2	0.6 (1.23±0.13)^{c} **[-83.4]**	0.8 (22.90±3.75)^{b} **[-79.0]** {16.0}	1.6 (33.93±1.50)^{b} **[-56.2]** {31.3}		1.1 (1.45±0.04)^{b} **[-77.3]**		7.5 (2.91±0.11)^{b} **[-65.9]**		3.6 (2.14±0.05)^{a} **[+84.0](83.3)**
Pseudomonas fluorescens alone	1	0.8 (1.30±0.19)^{b} **[-69.2]**	1.6 (33.81±2.71)^{b} **[-56.2]**{31.3}	1.2 (28.33±5.18)^{b} **[-62.0]** {23.6}	2.1 (1.75±0.08)	1.6 (1.32±0.04)^{b} **[-58.8]**	15.3 (4.02±0.21)	11.5 (3.52±0.17)^{b} **[-41.0]**	0.7 (1.37±0.08)^{a}	2.5 (1.86±0.03)^{a} **[+72.7](72.0)**
	2	0.3 (1.10±0.10)^{bc} **[-92.6]**	0.5 (18.05±2.29)^{b} **[-86.9]** {10.0}	0.9 (24.28±5.71)^{b} **[-73.6]**{18.8}		1.2 (1.49±0.08)^{c} **[-74.7]**		8.0 (2.99±0.15)^{b} **[-63.6]**		4.0 (2.24±0.01)^{a} **[+83.3](82.5)**
T. harzianum + *B. subtilis* + *P. fluorescens*	1	0.4 (1.18±0.11)^{c} **[-83.3]**	0.5 (19.01±1.34)^{b} **[-84.8]** {10.8}	0.8 (22.41±2.73)^{b} **[-76.0]** {15.0}	1.9 (1.70±0.07)	0.7 (1.59±0.05)^{c} **[-81.7]**	13.8 (3.83±0.16)	5.5 (2.54±0.13)^{c} **[-71.8]**	0.5 (1.30±0.13)^{b} 0.7 (1.40±0.08)^{a} 0.9 (1.45±0.10)^{a}	3.1 (2.01±0.02)^{a} **[+82.4](83.9)** 4.0 (2.23±0.05)^{a} **[+81.8](82.5)**
	2	0.1 (1.03±0.03)^{d} **[-98.2]**	0.2 (7.84±4.66)^{c} **[95.0]** {3.8}	0.2 (7.84±4.66)^{c} **[-94.7]** {3.8}		0.5 (1.20±0.05)^{d} **[-90.7]**		2.0 (1.72±0.12)^{c} **[-90.9]**		3.7 (2.16±0.07)^{a} **[+76.9](75.7)**
Chemical pesticides (N+F)	1	0.0 (1.00±0.00)^{b} **[100.0]**	0.6 (14.14±8.20)^{b} **[-84.3]** {11.3}	0.3 (9.21±5.32)^{b} **[-92.0]** {5.0}	2.4 (1.83±0.06)	0.5 (1.00±0.0)^{d} **[-86.9]**	16.0 (4.10±0.26)	4.8 (2.39±0.14)^{c} **[-75.6]**	0.0 (1.00±0.00)^{c}	0.0 **[0.0]** (1.00±0.00)^{d}
	2	0.4 (1.15±0.15)^{b} **[-88.8]**	0.4 (12.33±7.16)^{b} **[-88.5]** {8.8}	0.4 (12.44±4.35)^{b} **[-91.3]** {6.3}		0.3 (1.14±0.07)^{d} **[-93.3]**		3.5 (2.10±0.15)^{c} **[-84.1]**		0.0 **[0.0]** (1.00±0.00)^{c}
CD at 0.05	1	0.39	13.44	16.98	NS	0.22	NS	0.55	0.20	0.13
	2	0.28	13.88	11.50		0.18		0.53		0.12

Note: Figures presented in parentheses ( ) are square root transformed value ± Standard Error; Figures presented in parentheses [ ] and bold are percent increase (+) or decrease (-) over untreated check (control); Figures presented in parentheses ( ) and bold are percent increase (+) or decrease (-) over first trial; Means in each column with different superscript letters differ significantly ($P<0.05$); Figures presented in parentheses { } are original values in percentage; Chemical pesticides (N+F) - Fluensulfone (480 EC) (Nematicide) + Azoxystrobin 18.2% + Difenocanazole 11.4% SC (fungicide).

compared to the untreated check which was 34.1% more in the subsequent second trial compared to the first trial. The eggs obtained from the chemically treated plant roots egg masses did not differ statistically (P=4.33) from control where only 5% inhibition of egg hatching was recorded (Fig.1).

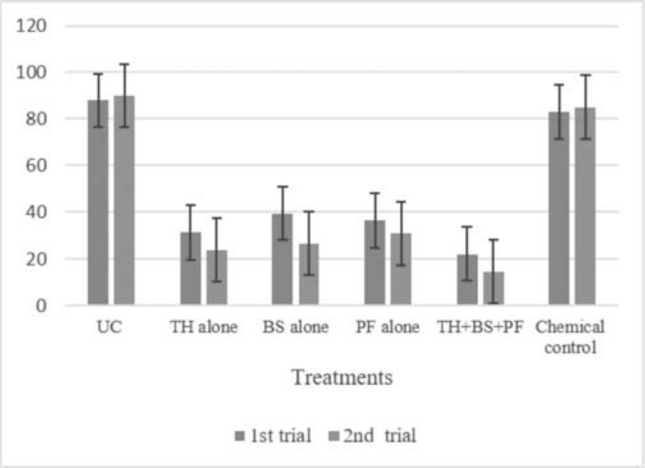

Fig. 1. Per cent egg hatching of *M. incognita* at the end of the trials

This article witnessed a significant reduction in all soil-borne pathogens and reduced disease caused by them under concomitant infestation through the use of combined application of BCAs, without showing any significant difference (P<0.05) with the performance of chemical pesticides. It is also notable that the damage caused by insect pests on capsicum plants was not traceable as compared to the control, which may be due to the fact that bio-agents keep away important insect pests from capsicum plants, however, some cutting and chewing insect pests (whitefly, thrips etc.) were recorded in the main capsicum crop under PCS. Findings of the present investigation have provided pieces of evidence that bio-agents have the potential to control soil-borne pathogens of capsicum and may help to replace pesticides to an extent by providing an environmentally friendly opportunity to promote sustainable agriculture under a protected cultivation system.

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Devising Integrated Nematode Management Technology against *Meloidogyne incognita* in Okra

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ABSTRACT: Root knot nematode, *Meloidogyne incognita* is sedentary endoparasite and plays important role in determining the yield of plants. Expression of phytotoxicity effect along with low efficiency and high input cost of chemical nematicides makes the farmers opt for other nematode management options. An experiment was conducted to evaluate the efficacy of different biocontrol agents (*Bacillus firmus, Bacillus subtilis, Trichoderma asperellum, Purpureocillium lilacinum, Rhizophagus intraradices*) and oil cake (Neem cake) and chemical nematicides (Fluensulfone and Fluopyrum). Plant growth parameters along with final nematode population density in soil and roots were recorded. The experimental results revealed that combined application of *B. firmus, B. subtilis, T. asperellum, P. lilacinum, R. intraradices* along with neem cake @1g/pot significantly reduced the nematode population and increased the plant growth characters than other treatments.

Keywords: Okra, *Meloidogyne incognita*, integrated nematode management, biocontrol agents, organic amendments.

Okra (*Abelmoschus esculentus* L. Moench) belonging to the family Malvaceae is an important vegetable crop cultivated across the tropical, subtropical and temperate countries (Singh, 2012). India stands second in harvested area, first in production, and fifteenth in productivity of okra across the world.

Plant parasitic nematodes plays important role in limiting the productivity of crops (Sharma *et al.,* 2022). Annual crop losses due to nematodes across the world accounts for about 100 billion Indian rupees and in India for about 242.1 billion Indian rupees with 14.6% yield loss in open field conditions and nearly 60% yield loss under protected cultivation (Gowda *et al.,* 2019). Among the different plant parasitic nematodes root knot nematode, *Meloidogyne* spp. are regarded to be the most serious polyphagous sedentary endoparasites invading over 5500 species of crops worldwide and are considered one among three important nematode genera which are placed under most serious pests in world (Kayani *et al.,* 2013).

In India, okra is infested by southern root knot nematode, *M. incognita*. They are obligate sedentary endoparasites of roots which are polyphagous in nature invading about 5500 species of host plants across the world (Singh *et al.,* 1993). Symptoms of okra crops infested with root knot nematode, *M. incognita* includes reduced plant growth in patches, yellowing of leaves, reduced fruit yield and poor emergence of seedlings d in first few weeks after sowing. As the result of nematode feeding hypertrophy and hyperplasia occurs in root cells leading to the formation of root galls. During final stages the root rots and predisposes for soil borne pathogens. The yield loss on okra due to *Meloidogyne* spp. in India accounts for about 27 percent (Gowda *et al.*, 2019).

Biological control agents have the capacity to supress the nematode population by exhibiting different mode of action such as competition for host nutrient, parasitism, antibiosis, lytic enzyme secretions. They also promote the plant growth by triggering the hormonal synthesis and inducing systemic resistance through production of production of jasmonic acid, salicylic acid and strigolactone which plays key role in promoting plant defence mechanisms. The commercial liquid and talc formulations of biocontrol agents such as *Bacillus subtilis, B. firmus, Purpureocillium lilacinum, Trichoderma asperellum* and others are proved to be effective against nematodes (Sharma *et al.,* 2008). The usage of chemical nematicides is kept as a major discourse topic by environmental scientists for its toxic residue accumulation in soil and host plants. Hence, we are in urge to formulate the environmentally benign management strategy against *M. incognita* (Patil *et al.,* 2021). Hence present was undertaken to develop an environmentally benign management strategy against root knot nematode, *M. incognita*.

MATERIALS AND METHODS

The liquid formulation of *Bacillus firmus, P. lilacinum* isolate TNAUPL1 and liquid formulation of *B. subtilis, T. asperellum* and vermiculite formulation of *Rhizophagus intraradices* were obtained from Department of Nematology, Department of Plant Pathology and Department of Microbiology, TNAU, Coimbatore respectively.

The pot culture experiments were conducted under glass house conditions at Department of Nematology, TNAU, Coimbatore to formulate the Integrated nematode management technology in okra. The experiments were carried out with eleven treatments and three replications in a Completely Randomized Design (CRD) in five kg earthen pots filled with sterilized pot mixture. Three seeds of okra variety CO-H4 were sown in each pot and thinned to one seedling/pot after 14 days after sowing. Freshly hatched juveniles were inoculated at 1 $J_2.g^{-1}$ of soil at 21 days after sowing. The experiments were terminated at 60 days after sowing by taking observations on shoot and root architectural parameters such as shoot length, root length, shoot weight, root weight and nematode population in soil and roots. The eleven treatments of the experiment were, T_1-Soil application of neem cake at 5g/ plant, T_2-Soil application of *B. firmus* at 5g /pot, T_3-Soil application of *B. subtilis* at 5g /pot , T_4-Soil application of *P. lilacinum* at 5g /pot, T_5-Soil application of *T. asperullum* at 5g /pot, T_6-Soil application of *R. intraradices* at 5g/pot, T_7-Soil application of fluensulfone at 1g/ pot, T_8-Soil application of fluopyram at 0.1ml/pot, T_9-Soil application with granule formulation of carbofuran 3G at 3g/pot, T_{10}-Combined soil application of neem cake at 1g/plant followed by *B. firmus, B. subtilis, T. asperellum*, *R. intraradices* at 1g each/pot, T_{11} – Untreated control

The data sets from various experiments of the present study were subjected to statistical analysis using IRRISTAT software version 91-1 developed by International Rice Research Institute, Philippines. The means of experiments were compared using Duncan's Multiple Range Test (DMRT) (Gomez & Gomez, 1984).

RESULTS

The perusal of data for the experiments on plant growth parameters and nematode population exhibited significant difference among the treatments compared to untreated control.

Plant growth parameters

There was significant increase in plant shoot and root architectural parameters due to application of different treatments (Table 1b).

i. Root length

Among all the treatments, combined application of *B. firmus, B. subtilis, T. asperellum* and *R. intraradices* along with neem cake @ 1g each/pot showed significant increase in root length with 82.3% which was followed by *B. firmus*@ 5g /pot, *P. lilacinus* @ 5g /pot, *B. subtilis* @ 5g /pot, *T. asperullum* @ 5g /pot, *R. intraradices* @ 5g /pot with 76.9, 69.1, 64.7% respectively.

Application of organic amendment, neem cake @5g/ pot and chemical fluopyram @0.1ml/pot were found statistically on par with 16.8% and 26 % increase over control.

Lowest root length was observed in plants treated with fluensulfone @ 1g /pot which exhibited 7.5% increase over control.

ii. Shoot length

Among all the treatments, highest shoot length data was observed in combined application of neem cake with *B. firmus, B. subtilis, T. asperellum* and *R. intraradices* @ 1g each/pot which recorded 50% increase over control. *B. firmus*@ 5g /pot *and P. lilacinus* @ 5g / potwas found to increase the shoot length by 76.9% and 69.1% over control.

Application of different biological control agents showed significant results ranging from 47.14 % to 76.9% increase over control. The chemical application with fluensulfone @ 1g /pot and *T. asperellum* @ 5g / potproved to be statistically on par by showing 15.17 and 11.1% (19 and 18 cm) increase in shoot length over control.

The lowest shoot length was recorded in plants treated with carbofuran 3G @ 3g /pot showing 8.5% increase over control which was followed by untreated control (16 cm).

iii. Fresh weight

The highest fresh weight was recorded in plants treated with combined application of neem cake with *B. firmus, B. subtilis, T. asperellum* and *R. intraradices* @ 1g each /pot which showcased 85.4% (25.5g) over control (3.7g).

Among the biocontrol agents *B. firmus*@ 5g /pot showed 67.8% (11.5g) increase over control. Application of *T. asperellum* @ 5g /pot and *P. lilacinus* @ 5g / potshowed 64.7 and 61.8 % increase over check by exhibiting 10.5g and 9.7g respectively. *B. subtilis* @ 5g /potand *R. intraradices* @ 5g /pot were found to be statistically on par with each other.

Lowest record on fresh weight among the treatments were observed in fluopyram @ 0.1 ml /pot and fluensulfone @ 1g /potwhich were statistically on par with each other by showing 37.2 and 33.9 % increase over control respectively. The untreated check plants exhibited the least number in fresh weight by showing 3.7g.

iv. Shoot weight

Application of *B. firmus*@ 5g /pot and combined application of neem cake with *B. firmus, B. subtilis, T. asperellum* and *R. intraradices* @ 1g each /potwas found effective in increasing shoot weight with 36.5% (21.7g) and 43.69% (22.2g) over control.

Results of plants treated with *B. subtilis* @ 5g /pot, *P. lilacinus* @ 5g /pot, *T. asperellum* @ 5g /pot were found to be on par with each other (19.7, 19.54, 20.1g) which recorded 42.3, 35.5, 37.8% increase over control respectively. The chemical application with fluopyram @ 0.1 ml/pot and fluensulfone @ 1g /pot showed 29.7 and 26.5% (17 and 17.8g) respectively.

Lowest shoot weight was observed in plants treated with neem cake @ 5g /pot (16.7g, 25.14%) followed by untreated control plants (12.5g).

v. Root weight

Combined application of neem cake with *B. firmus, B. subtilis, T. asperellum* and *R. intraradices*@ 1g each /pot showed 47.28% (12.9g) increase over control followed by *B. firmus* @ 5g /pot exhibiting 33.3% (10.2g) of root weight.

P. lilacinus @ 5g /potand *R. intraradices* @ 5g /pot was found to be on par with each other. Application of different biocontrol agents showed significant difference ranging from 25.5 to 33.3 % (8.9 to 10.9g) increase over control. Chemical application with fluopyram @ 0.1mlg /pot and fluensulfone @ 1g /pot was found to be on par with each other 20.9 and 19.4% (9.3, 8.4g).

Lowest root weight was recorded in plants treated with neem cake @ 5g /pot showing 18% (8.33g). The untreated control plants recorded the least root weight of 6.8g.

Nematode population

There was significant reduction in root knot nematode population was observed due to application of different treatments (Table. 1b).

i. Root knot index

Root knotindex was calculated to evaluate the nature of resistance in okra plants after application of treatments. Among all treatments, combined application of neem cake with *B. firmus, B. subtilis, T. asperellum* and *R. intraradices* @ 1g each /pot, *B. firmus*@ 5g /pot and *P. lilacinus* @ 5g /pot, fluensulfone @ 1g /pot, fluopyram @ 1g /pot showed lesser root knot index of 2 which was concluded to be in resistant category.

The application of *B. subtilis* @ 5g /pot, *T. asperellum* @ 5g /pot, *R. intraradices* @ 5g /pot, carbofuran @ 3g /pot and neem cake @ 5g /pot exhibited index (3) which was concluded to be in moderately resistant category.

The untreated control plants showed an index (4) which was found to be susceptible to root knot nematodes.

ii. Soil population

Application of different treatments significantly reduced the soil nematode population. The effective treatment was combined application of the of neem cake with *B. firmus, B. subtilis, T. asperellum* and *R. intraradices* @ 1g each /pot which showed 62.3% reduction over control. It was followed by *B. firmus*@ 5g /pot and *P. lilacinus* @ 5g /pot with 56.7 and 55.9% reduction over control respectively.

The effect of other biocontrol agents such as *B. subtilis* @ 5g /pot, *T. asperellum* @ 5g /pot and *R. intraradices* @ 5g /pot on soil population differed from 45.4 to 50.48%. The organic amendment neem cake @ 5g /pot also showed drastic decrease in soil nematode population with 48.08% reduction over control.

The chemicals fluensulfone @ 5g /pot and fluopyram @ 0.1ml /pot was found to be statistically on par with each other and reduced the population by 56.4 and 56.6 % reduction over control.

The highest number of soil population was observed in plants treated with *B. subtilis* @ 5g /pot and *T. asperellum* @ 5g /pot with 114.2 and 109.7 per 200cc of soil which was followed by untreated control exhibiting over 209.2 nematodes per 200cc of soil.

iii. Number of females

Combined application of neem cake with *B. firmus, B. subtilis, T. asperellum* and *R. intraradices* @ 1g each /pot drastically reduced the female population in roots up to 61.4%.

Among different biocontrol organisms, application of *B. firmus*@ 5g /pot showed 54.6% reduction in female population which was followed by *R. intraradices* @ 5g /pot, *P. lilacinus* @ 5g /pot showed on par reaction with 51.8 and 49.4% decrease over control.

Chemical application also exhibited better results in suppressing the female population in roots where fluensulfone @ 1g /pot and fluopyram @ 0.1ml /pot found statistically on par with 52.7 and 51.1% reduction over control. Highest number of females was found plants treated with neem cake @ 5g /pot with 37.7 females per 5g of roots which was followed by control plants showing 58.6 females per5gof roots.

Table 1. Evaluation of integrated nematode management technology for *M. incognita* on okra plant growth parameters

Treatments (Soil application)	**Root length**	**Shoot length**	**Fresh weight**	**Shoot weight**	**Root weight**
Neem cake @ 5g/ pot	8.9^{ghi}(16.8)	21.8^{d}(26.6)	4.5^{h}(17.7)	16.7^{e}(25.14)	8.33^{ef}(18)
Bacillus firmus@ 5g/pot	31.96^{b}(76.9)	27^{b}(40.74)	11.5^{b}(67.8)	21.7^{a}(36.54)	10.2^{b}(33.3)
Bacillus subtilis @ 5g/pot	21^{d}(64.76)	19.5^{d}(17.9)	8^{e}(53.7)	19.7^{b}(42.39)	8.9^{cd}(23.5)
Purpureocillium lilacinus @ 5g/pot	24^{c}(69.1)	24^{c}(33.3)	9.7^{d}(61.8)	19.4^{b}(35.56)	9.2^{c}(26.08)
Trichoderma asperullum @ 5g/pot	14^{e}(47.14)	19^{fg}(15.7)	10.5^{c}(64.7)	20.1^{b}(37.8)	8.4^{ef}(19.04)
Rhizophagus intraradices @ 5g/pot	15^{e}(50.6)	23^{c}(30.4)	7.7^{e}(51.9)	18.3^{c}(31.6)	9.3^{c}(25.5)
Fluensulfone @ 1g/pot	8^{hi}(7.5)	18^{gh}(11.1)	5.6^{g}(33.9)	17.02^{de}(26.5)	8.4^{ef}(19.04)
Fluopyram @ 0.1ml/pot	10^{g}(26)	20.4^{e}(21.56)	5.9^{fg}(37.2)	17.82^{cd}(29.7)	8.6^{de}(20.9)
Carbofuran3G@ 3g/pot	12.5^{f}(68.1)	17.5^{h}(8.5)	6.3^{f}(41.2)	16.8^{e}(25.5)	8.1^{f}(16.04)
Neem cake + *B. subtilis* + *B. firmus* + *T. asperellum*+ *R. intraradices* @1g each/pot.	42^{a}(82.3)	32^{a}(50)	25.5^{a}(85.49)	22.2^{a}(43.69)	12.9^{a}(47.28)
Untreated Control	7.4^{i}	16^{i}	3.7^{i}	12.5^{f}	6.8^{g}
SE(d)	0.53	0.48	0.20	0.39	0.23
CD (0.05)	1.1	0.98	0.43	0.82	0.48

*Values are mean of three replications, in column means followed by common letter are not significant at 5% level by DMRT. Figures in parentheses are per cent increase (+) over control.

Table 2. Evaluation of integrated nematode management technology for *M. incognita* nematode population on okra

Treatments (Soil application)	**No. of females (5 g roots)**	**No. of egg mass (5 g root)**	**Root knot index**	**Soil population (250cc soil)**
Neem cake @ 5g/ pot	37.7^{g}(35.6)	19^{g}(50.7)	3	108.6^{d}(48.08)
Bacillus firmus@ 5g/pot	26.6^{b}(54.6)	11.6^{b}(75.9)	2	90.4^{b}(56.78)
Bacillus subtilis @ 5g/pot	33.3^{e}(43.1)	17.3^{fe}(55.1)	3	114.2^{e}(45.4)
Purpureocillium lilacinus @ 5g/pot	29.6^{d}(49.4)	9.3^{b}(69.9)	2	92.2^{b}(55.9)
Trichoderma asperullum @ 5g/pot	35^{f}(40.27)	18^{f}(53.3)	3	109.7^{d}(47.5)
Rhizophagus intraradices @ 5g/pot	28.2^{dc}(51.8)	11.7^{c}(69.6)	3	104^{cd}(50.28)
Fluensulfone @ 1g/pot	27.7^{cb}(52.7)	12.4^{c}(67.8)	2	91.2^{b}(56.4)
Fluopyram @ 0.1ml/pot	28.6^{dc}(51.1)	13.2^{d}(65.8)	2	90.7^{b}(56.6)
Carbofuran3G@ 3g/pot	32.3^{e}(44.8)	16.6^{e}(56.2)	3	98.7^{c}(52.9)
Combined application of Neem cake + *B. subtilis* + *B. firmus* + *T. asperullum* + *R. intraradices*@1g each/pot.	22.6^{a}(61.4)	8.3^{a}(78.4)	2	78.8^{a}(62.3)
Untreated Control	58.6^{h}	38.6^{h}	4	209.2^{h}
SE(d)	0.69	0.404	-	2.7
CD (0.05)	1.44	0.843	-	5.6

*Values are mean of three replications, in column means followed by common letter are not significant at 5% level by DMRT. Figures in parentheses are per cent decrease (-) over control.

iv. Number of egg mass

Among different treatments, combined application of neem cake with *B. firmus,*

B. subtilis, T. asperellum and *R. intraradices* @ 1g each /pot showcased lower number of egg mass per 5gof roots with 78.4% reduction over control. It was followed by application of

B. firmus @ 5g /pot with 75.9% reduction over control. *P. lilacinus* @ 5g /pot, *R. intraradices* @ 5g / pot was found to be statistically on par with each other which showed and 69.9 and 69.6% reduction over control respectively. *B. subtilis* @ 5g /pot, *T. asperellum* @ 5g /pot exhibited 55.1 and 53.3% reduction over control.

Fluensulfone @ 1g /pot and fluopyram @ 0.1 ml /pot showed significant decrease in egg mass with 67.8 and 65.8% reduction over control. Highest number of egg mass was observed in plants treated with neem cake @ 5g /pot with 50.7% reduction over control which was followed by untreated control plants exhibiting 38.6 egg mass per 5g of roots.

DISCUSSION

In the present study, among different treatments tested, combined application of neem cake with *B. firmus, B. subtilis, T. asperellum, R. intraradices* @1g each/ pot had dominating effect over nematode population and plant growth promotion when compared to untreated control. Similarly, Mahalik and Sahoo (2019) found that combined application of jatropha oil cake and neem cake along with seed treatment of *T. viride, P. fluorescens* and soil application of *P. lilacinu*s increased the plant growth and lowered nematode populations over check. They concluded that treatments which had neem cake and biocontrol agents as promising integrated module which recorded the highest BC ratio (1:2.12.).

The present findings is in line with findings of Mishra *et al.* (2018). Combined application of neem cake prior to planting followed by application of *T. viride* expressed lowest nematode reproduction factor and population with highest plant growth parameters. The findings also stated that neem cake was effective at different concentration. Integrated nematode management approach was underlined in findings of Das and Sinha (2005). The combined application of *P. lilacinum*, carbosulfan, poultry manure showed maximum plant growth parameters and significantly reduced the nematode population in kharif seasons.

The present findings were found in line with Goswami *et al.* (2006) where bioagents *viz*, *T. viride, P. lilacinus* either alone or in combination with mustard oil cake and furadan not only promoted the plant growth but also reduced the nematode population in tomato ecosystem. Similarly, Ashraf and Khan (2010) reported that *M. javanica* on brinjal was effectively managed by combined application of *P. lilacinum* with groundnut cake followed by neem cake with *Cladosporium oxysporum* were also proved to be effective in management of *M. javanica* in brinjal.

From the present study we observed that *B. firmus* @ 5g each/pot and *P. lilacinus* @ 5g each/pot performed well when applied without combining other treatments. They significantly increased the plant growth parameters and suppressed the nematode population. The results were in line with findings of Mendoza *et al.* (2008) where they concluded that *B. firmus* reduced the nematode population and affected the egg hatching and juvenile motility. Terefe *et al.* (2009) reported that single dose of *B. firmus* suppressed the nematode population in tomato for entire season with enhanced plant growth parameters in pot culture and field studies. Application of *B. firmus* reduced the nematode population and promoted plant growth. Ghahremani et al. (2020) stated that *B. firmus* I-1582 had the potential to suppress the nematode population, number of galls and root knot index and degraded the eggshell of nematodes and also promoted the plant growth by inducing systemic resistance in plants.

Okra seeds treated with *P. lilacinum* reduced the nematode population under glasshouse conditions (Dhawan *et al.,* 2004). Similarly, (Kannan & Veeravel, 2012)reported that okra seeds when treated with *P. lilacinum* documented maximum shoot length, shoot weight, root length and minimum nematode population when compared to individual treatments.

The present findings states that the bacterial biocontrol organisms such as *B. firmus, B. subtilis* was found to be compatible with each other and with fungal biocontrol organism *T. asperellum. P. lilacinum* was found to be incompatible with bacterial biocontrol agents. *B. firmus* and *B. subtilis* was compatible with each other and also with *T. asperullum.*

The neem cake amended medium was used to check the growth of different biocontrol agents. The results indicated that *B. firmus, B. subtilis, T. viride* was found to have good growth. The present findings were in correlation with Zope *et al.* (2019). The neem cake was found to be an excellent carrier material for dispersing the biocontrol agents. *T. viride* was found to have better shelf life 200 days and 35.7 x 10^7 CFU per gram and also inhibited the growth of pathogens under *in-vitro* conditions. The results of Bagwan (2010) also quotes that the biocontrol organisms were found to be compatible with neem cake at different concentrations. The present findings were in correlation with the above findings.

From the present study we conclude that the integration of different components, neem cake with *B. firmus, B. subtilis, T. asperullum* and *Rhizophagus intraradices* @1g each/pot prevents the nematode damage in okra. Though the chemicals also performed well under glasshouse conditions, the environmental hazards should be kept in mind to promote the plant growth promoting microbiomes near rhizosphere regions. Further investigations of the above-mentioned treatments under field conditions are required to develop an effective integrated nematode management strategy to suppress the *M. incognita* population in okra ecosystems.

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Determination of Defence Enzyme Induction in Chilli in Response to Root-Knot Nematode, *Meloidogyne incognita* upon Oil Cake Application

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ABSTRACT: An experiment was conducted with naturally infested soil of root-knot nematode 2 J2s/g soil to assess the induction of defence enzymes peroxidase (PO), polyphenol oxidase (PPO), Phenylalanine ammonia lyase (PAL) and superoxide dismutase (SOD) by oil cakes against *M. incognita* in a susceptible variety of chilli. Application of oil cakes castor, mahua, karanj and mustard were added to soil each @ 2.5 and 5.0 q/ha and neem cake @ 5.0 q/ha as a standard check. The role of selected oil-cakes in enzymatic activity was assayed in chilli roots infested with the root-knot nematode *M. incognita.* Application of oil-cakes increased the level of PO, PPO, PAL and SOD in chilli roots. Among all the treatments application of mustard cake @ 5.0 q/ha was found to be the best treatments to enhance enzymatic activity. However, standard check Neem cake @ 5.0 q/ha was found the best treatment to increase the enzyme activity against *M. incognita* in chilli roots. The enzyme activity showed gradual increase from 7 DAI to 28 DAI in plant roots treated with oil-cakes as compared to untreated ones. The lowest enzyme activity in among enzymes was recorded at 60 DAI in untreated control plant roots. Among enzymes the PO was found highest in chilli roots during different time of observations followed by SOD, while PPO and PAL were observed in low quantity.Among all the treatments application of mustard cake @ 5.0 q/hawas found the best treatments to improve plant growth character.As regard to nematode reproduction, mustard cake @ 5.0 q/ha soil proved best treatment in reducing number of galls per plant (20.33), number of egg masses per plant (14.50), number of eggs per egg mass (87.33), nematode population/200cc soil (203.50) and total nematode population of *M. incognita* (2283.83) in chilli.

Key words: Oil-cakes, defence enzymes, root-knot nematode, chilli

Chilli (*Capsicum frutecens*) belongs to family Solanaceae. Chilli is grown for its pungent fruits, which are used both green and ripe red form to add pungency and taste to the food. The conditions required for growing these crops also suites to many pathogens. Hence the chilli production is tremendously reduced by pest and diseases which are considered as major biological constraints to low productivity. The diseases include fungal, bacterial, viral, nematodes andinsect pests. Nematode is a most dangerous enemy for plants because it causes infection on plant root which was clearly not visible. Among plant parasitic nematodes, root-knot nematode has been considered as severe constraint in vegetable production. These are most commonly occurring and causing major economic damage to agricultural vegetable production around the world (Anwar *et al.*, 2007; Williamson and Hussey, 1996). Sasser and Freckman (1987) recorded 12.2 % loss on worldwide basis on chilli crop by plant parasitic nematodes. A national loss due to this nematode pest in chilli was worked out to be 12.85 per cent and in monetary terms has been worked out to the tune of 210 million rupees (Jain *et al.*, 2007). Several control strategies, such as host plant resistance, rotation with non-host crops, sanitation, destruction of residual crop roots and discriminating use of nematicides, have been reported to effectively keep the root knot nematode population below damaging threshold level (Barker and Koenning, 1998).

In order to obtain effective control, nematicides are often applied at higher doses, which may be costlier, uneconomical and phyto-toxic and may cause residue problems which may create ecological disturbance in the nature. Therefore, out of several methods tasted from time to time, the use of nematode resistant varieties remains the most viable option.

Plant resistance is one of the eco-friendly options for the management of nematode diseases. A series of biochemical and physical reactions occur in plants in response to root-knot nematode infection. Plants synthesize certain compounds that are toxic to root-knot nematode. Resistance is usually associated with hypersensitive reaction (HR), a rapid and localized cell death in the infected plant in response to nematode attack. Reactive oxygen species (ROS) play an important role in plant defence and during pathogen attack, levels of ROS detoxifying enzymes like peroxidase (PO) and catalase (CAT) are often suppressed in resistant plants (Klessing *et al.,* 2000). As a result, plants produce more ROS and accumulation of these components leads to HR in plant cells. For example, hydrogen peroxide plays a major role in triggering HR in incompatible interactions. Antioxidant enzymes such as PO, PAL, PPO, SOD, POD and CAT are considered to be the main protective enzymes engaged in the removal of free radicals and activated oxygen species (Devi *et al.,* 2000; Pankaj *et al.,* 2013; Chawla *et al.,* 2013; Chandrawat *et al.,* 2018; Chandrawat *et al.,* 2020a; Chandrawat *et al.,* 2020b). While, oxidative enzymes such as PO, PAL, PPO, SOD and CAT are reported to be involved in the mechanism of disease resistance.

So far substantial work has been done on various aspects of *M. incognita* on chilli. However, there is not much information available on determining the role of oil-cakes on defence enzymes activity in plants against root-knot nematode, *M. incognita*. Keeping this in view, the present investigations were undertaken to assess the potential of oil cakes in defence enzymes against root-knot nematode.

MATERIALS AND METHODS

The experiment was laid out in Department of Nematology, RCA, Udaipur. Utmost care was taken right from sowing to till harvest of experiment for proper growth and development of plants.

I. Preparation and maintenance of pure culture of *M. incognita*

Egg masses, collected from the *M. incognita* infected roots were kept in distilled water in watch glasses at laboratory temperature for hatching. Freshly hatched 2nd stage juveniles were then inoculated on one month old chilli plants already grown and maintained in 6" sized earthen clay pots filled with steam sterilized soil to obtained adequate pure population of *M. incognita* on the plants and in soil to carry out further experiments.

II. Disinfection and Filling of pots

6" size earthen pots were washed, cleaned and disinfected before use by rinsing them with 4 per cent formalin solution. Pots were filled with 1 kg infested soil. Oil cakes castor, mahua, karanj and mustard were added to soil each @ 2.5 and 5.0 q/ha. Each treatment was replicated three times. Untreated and standard check (neem cake @ 4g/kg soil) was also maintained for comparison.

Uniform size seedlings were transplanted in pots, one healthy plant in each pot was maintained and others were uprooted carefully without disturbing the one to be maintained.Care was taken right from sowing till harvest of experiments.

III. Harvest

Assessment of the induction of defence enzymes PO, PPO, PAL and SOD by oil-cakes against *M. incognita* in a susceptible variety of chilli was done on every 7days interval after transplanting (7, 14, 21, 28) and 60 days after transplanting. The experiments were harvested 60 days after transplanting, while harvesting, the care was taken to avoid losses of both roots and nematodes in adhering soil. Observation on enzyme analysis and various growth parameters *viz*., fresh root and shoot weight, shoot and root length were recorded without delay whereas for studying nematode infestation, the plant tissues were stained in 0.1% acid fuchsin in lacto phenol at 80ºC for 2-3 minutes (McBeth *et al.,* 1941). Then after gentle wash, roots were kept in clear lacto phenol for 24 hrs and then examined under microscope for nematode infection and observation of number of galls/plant, number of egg masses/plant and number of eggs/egg mass were recorded. Final soil population/100 cc soil and total population were also calculated.

IV. Estimation of soil population:

The soil samples (100 cc) collected from the experimental pots were processed using Cobb's sieving and decanting technique (Cobb, 1918) followed by Baermann's funnel technique (Christie and Perry, 1951). After 24 hrs the suspension was drawn in a beaker from the funnel and kept for some time to allow the nematode to settle down. The excess water was gently poured out of the beaker without disturbing the nematodes already settled at bottom. The volume of suspension was made to 100 ml and then after thoroughly bubbling 10 ml of suspension was drawn with the help of a pipette and poured over a counting dish for counting. Population count was done under a stereoscopic binocular microscope.

V. ENZYME ANALYSIS

1. Determination of peroxidase (PO) enzymes in chilli roots

The method proposed by Hammerschmidt *et al*., (1982) was adopted for assaying the activity of peroxidase (EC 1.11.1.7). A 20% homogenate was prepared in 0.1M phosphate buffer (pH 7.0) from the root parts of the plant. The homogenate was centrifuged at 16,000 rpm at 4°C for 15 min and the supernatant was used for the assay. The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1 per cent H_2O_2. The reaction mixture was incubated at room temperature (28 ± 2 °C). The changes in absorbance at 420 nm were recorded at 30s intervals for 3 min. The enzyme activity was expressed as changes in the absorbance min^{-1} mg^{-1} protein

2. Determination of polyphenol oxidase (PPO) enzymes in chilli roots

Polyphenol oxidase(EC 1.10.3.1) activity was estimated simultaneously by the method of Mayer *et al.,* (1965). The enzyme extract was prepared by homogenizing 0.5 g of plant tissue in 2.0 ml of the extraction medium 0.1M sodium phosphate buffer (pH 6.5). The homogenate was centrifuged at 16,000 rpm for 15 min at 4 °C and the supernatant was used for the assay. The reaction mixture consisted of 200 ìl of the enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5). To start the reaction 200 ìl of 0.01 M catechol was added and the activity was expressed as changes in absorbance at 495 nm min^{-1} mg^{-1} protein.

3. Determination of phenylalanine ammonia lyase (PAL) enzymes in chilli roots

The activity of phenylalanine ammonia lyase (EC 4.3.1.5) was measured following the method of Dickerson *et al*., (1984). Root samples (1g) were homogenized in 3 ml of ice cold 0.1 M sodium borate buffer, pH 7.0 containing 1.4 mM of 2-mercaptoethanol and 0.1 g of insoluble polyvinyl pyrrolidine. The extract was filtered through cheese cloth and the filtrate was centrifuged at 16,000 rpm for 15 min. The supernatant was used as enzyme source.PAL activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nM. A sample containing 0.4 ml of enzyme extract was incubated with 0.5 ml of 0.1M borate buffer, pH 8.8

and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30°C. The amount of trans-cinnamic acid synthesized was calculated using its extinction coefficient of 9630 m-1. Enzyme activity was expressed as nmol trans-cinnamic acid min^{-1} mg^{-1} protein.

4. Determination of super oxide dismutase (SOD) enzymes in chilli roots

SOD was assayed according to the method of Beauchamp and Fridovich(1971). The activity of SOD (EC 1.15.1.1) was assayed by measuring its ability to inhibit the photochemical reduction of NBT. The roots (0.5g) were homogenized with 3.0ml of potassium phosphate buffer, centrifuged at 2000 rpm for 10 minutes and the supernatants were used for the assay.The 3 ml reaction mixture contained 50 mM phosphate buffer pH 7.8, 13 mM methionine, 75 μM NBT, 2 μM riboflavin, 1.0 mM EDTA and 20 μl enzyme extract. Riboflavin was added last and the reaction was initiated by placing the tubes 30 cm below 15 W fluorescent lamps. The reaction was started by switching on the light and was allowed to run for 10 min. Switching off the light stopped the reac-tion and the tubes were covered with black cloth. Non-illuminated tubes served as control. The absorbance at 560 nm was read. One unit of SOD is the amount of extracts that gives 50% inhibition the rate of NBT reduction.

RESULTS AND DISCUSSION:

Determination of defence enzymes

Results on accumulation of PO, PPO, PAL and SOD revealed that all oil-cakes significantly increased the enzyme activity as compared to untreated check. The highest activity was recorded as with Mustard cake at 28 DAI followed by Castor cake and Mahua cake @ 5q/ha. However, Neem cake @ 5q/ha was observedsuperior over all treatments while, minimum was observed in untreated control plant at 60 DAI (Table 1).

Plant growth characters

Data revealed that all oil-cakes significantly increased the plant growth parameters as compared to untreated check. The maximum plant growth parameters were recorded with Mustard cake followed by Castor cake and Mahua cake @ 5 q/ha. However, neem cake was found superior over other treatments while,untreated check was observed inferior (Table 2).

Nematode reproduction

Data presented in table-3 showed that the reproduction of *M. incognita* on chilli reduced significantly as compared to untreated check when oil-cakes was used as soil application. Among different oil-cakes, minimum nematode reproduction was observed with Mustard cake @ 5 q/ha followed by Castor cake and Mahua cake @ 5 q/ha. However, minimum nematode reproduction per plant was observed with Neem cake @ 5 q/ha.

The role of selected oil-cakes in enzymatic activity was assayed in chilli roots infested with the root- knot nematode *M. incognita.* Application of oil-cakes increased the level of PO, PPO, PAL and SOD in chilli roots.

Among all the treatments application of mustard cake @ 5.0 q/ha was found to be the best treatments to enhance enzymatic activity. However, standard check Neem cake @ 5.0 q/ha was found the best treatment to increase the enzyme activity against *M. incognita* in chilli roots. The enzyme activity showed gradual increase from 7 DAI onwards to 28 DAI in plant roots treatedwith oil-cakes as compared to untreated ones. The lowest enzyme activity in among enzymes was recorded at 60 DAI in untreated control plant roots.Among enzymes the PO was found highest in chilli roots during different time of observations followed by SOD, while PPO and PAL were observed in low quantity. Among all the treatments application of mustard cake @ 5.0 q/hawas found the

Table 1: Effect of oil-cakes on PO, PPO, PAL and SOD activity in chilli roots infected with *M. incognita.*

	Specific activity of Enzymes (umol/min/gm)																			
DAI	**7**	**14**	**21**	**28**	**60**	**7**	**14**	**21**	**28**	**60**	**7**	**14**	**21**	**28**	**60**	**7**	**14**	**21**	**28**	**60**
Treatments	**PO**					**PPO**					**PAL**					**SOD**				
T 1	20.639	30.084	38.881	45.845	5.272	0.219	0.410	0.547	0.638	0.184	0.120	0.172	0.261	0.336	0.091	1.83	2.86	3.17	3.49	0.97
T 2	28.449	40.122	51.428	64.567	15.874	0.288	0.456	0.593	0.691	0.263	0.138	0.210	0.295	0.368	0.118	2.14	3.38	3.79	3.91	1.16
T 3	18.468	25.996	37.330	44.943	5.018	0.212	0.406	0.539	0.637	0.180	0.119	0.169	0.257	0.333	0.089	1.76	2.78	3.08	3.32	0.94
T 4	27.152	39.389	50.216	62.650	14.689	0.279	0.449	0.588	0.684	0.259	0.137	0.207	0.291	0.365	0.116	2.08	3.29	3.66	3.81	1.14
T 5	15.225	25.460	33.580	42.857	4.172	0.205	0.399	0.535	0.630	0.172	0.118	0.165	0.254	0.329	0.086	1.69	2.56	2.98	3.14	0.91
T 6	25.178	37.669	49.454	60.479	13.843	0.272	0.445	0.577	0.676	0.254	0.135	0.203	0.288	0.365	0.115	1.99	3.15	3.58	3.72	1.13
T 7	20.639	31.804	40.178	47.932	7.528	0.227	0.414	0.552	0.642	0.192	0.124	0.176	0.266	0.339	0.096	1.86	2.99	3.32	3.58	1.01
T 8	29.520	41.842	52.669	66.541	16.578	0.296	0.464	0.606	0.700	0.266	0.141	0.213	0.297	0.373	0.121	2.19	3.46	3.85	4.04	1.19
T 9	37.612	56.701	75.225	77.227	22.218	0.310	0.478	0.664	0.798	0.311	0.154	0.222	0.318	0.391	0.139	2.21	3.58	3.99	4.11	1.21
T 10	6.400	11.672	22.866	23.740	4.821	0.127	0.173	0.201	0.139	0.085	0.072	0.087	0.095	0.072	0.040	1.18	1.28	1.63	1.76	0.27
SEm±	0.004	0.004	0.004	0.004	0.004	0.004	0.087	0.114	0.132	0.046	0.026	0.038	0.055	0.069	0.021	0.404	0.625	0.705	0.744	0.212
CD at 5%	0.012	0.013	0.012	0.013	0.012	0.012	0.255	0.337	0.390	0.134	0.077	0.113	0.162	0.203	0.062	1.191	1.845	2.079	2.194	0.625
CV	0.03	0.02	0.02	0.01	0.06	2.85	1.75	0.85	0.83	1.95	4.61	3.59	2.69	1.92	5.65	0.35	0.20	0.19	0.17	0.35

Note:(1) Data are average value of three replications. (2) Initial inoculums level 2 J_2/g soil.

Table 2: Effect of oil-cakes on plant growth characters of chilli infected with *M. incognita*

Treatments	Shoot length (cm)			Shoot weight (g)			Root length (cm)			Root weight (g)		
	Ist Year	IInd Year	Pooled	Ist Year	IInd Year	Pooled	Ist Year	IInd Year	Pooled	Ist Year	IInd Year	Pooled
T 1 Castor cake @ 2.5q/ha.	13.90	14.10	14.00	4.91	4.80	4.86	25.53	26.10	25.82	19.27	19.48	19.38
T 2 Castor cake @ 5q/ha.	17.93	18.57	18.25	7.08	7.25	7.17	32.50	32.87	32.68	25.73	26.07	25.90
T 3 Mahua cake @ 2.5q/ha.	12.82	13.07	12.94	4.33	4.50	4.42	23.47	23.13	23.30	18.17	18.38	18.28
T 4 Mahua cake @ 5q/ha.	17.07	17.55	17.31	6.74	6.63	6.69	31.40	31.23	31.32	24.15	24.48	24.32
T 5 Karanj cake @ 2.5q/ha.	11.85	11.62	11.73	4.05	4.20	4.12	22.17	22.38	22.28	17.65	17.83	17.74
T 6 Karanj cake @ 5q/ha.	16.13	16.38	16.26	6.05	6.20	6.13	29.97	30.32	30.14	22.27	21.93	22.10
T 7 Mustard cake @ 2.5q/ha.	14.53	14.78	14.66	5.37	5.53	5.45	27.80	28.07	27.93	19.93	20.27	20.10
T 8 Mustard cake @ 5q/ha.	18.97	19.15	19.06	7.93	8.02	7.98	34.83	35.10	34.97	27.23	27.45	27.34
T 9 Neem cake (Standard check) @ 5q/ha.	20.13	20.55	20.34	8.48	8.63	8.56	36.37	36.67	36.52	29.10	29.50	29.30
T 10 Control	6.07	6.28	6.18	3.13	3.28	3.21	15.03	14.85	14.94	7.70	7.57	7.63
SEm±	0.441	0.452	0.439	0.24	0.284	0.26	0.31	0.319	0.29	0.40	0.366	0.36
CD at 5%	1.300	1.335	1.294	0.702	0.838	0.762	0.927	0.942	0.864	1.176	1.080	1.075
CV	5.110	5.153	5.040	7.09	8.33	7.64	1.95	1.97	1.81	3.27	2.98	2.98

Note: (1) Data are average value of three replications; (2) Initial inoculums level 2 J_2/g soil.

Table 3: Effect of oil-cakes on nematode reproduction of chilli infected with *M. incognita*

Treatments	No. of galls/ plant			No. of egg masses / plant			No. of eggs and larvae / egg mass			Larval population/ 200cc soil			Total population		
	Ist	IInd	Pooled	Ist	IInd	Pooled	Ist	IInd	Pooled	Ist	IInd	Pooled	Ist	IInd	Pooled
T 1	30.00	30.33	30.17	23.33	22.67	23.00	120.33	119.00	119.67	283.67	288.33	286.00	4225.67	4139.67	4182.67
T 2	21.67	21.33	21.50	15.67	15.00	15.33	94.00	90.67	92.33	209.33	211.67	210.50	2522.67	2417.67	2470.17
T 3	31.33	30.67	31.00	25.00	24.33	24.67	131.00	128.33	129.67	302.67	298.67	300.67	4788.67	4619.33	4704.00
T 4	23.00	22.00	22.50	17.00	16.33	16.67	99.00	96.33	97.67	218.33	215.33	216.83	2775.00	2650.00	2712.50
T 5	33.00	33.67	33.33	27.33	27.67	27.50	145.00	147.00	146.00	350.33	342.33	346.33	5714.33	5778.00	5746.17
T 6	23.67	22.67	23.17	19.67	18.00	18.83	104.33	102.00	103.17	234.00	230.33	232.17	3222.33	2985.67	3104.00
T 7	28.67	28.00	28.33	21.33	21.67	21.50	112.00	114.67	113.33	268.33	274.33	271.33	3730.00	3856.00	3793.00
T 8	20.33	20.33	20.33	14.33	14.67	14.50	86.67	88.00	87.33	201.33	205.67	203.50	2249.33	2318.33	2283.83
T 9	18.67	18.00	18.33	12.00	11.00	11.50	81.00	77.67	79.33	191.67	190.00	190.83	1932.67	1806.00	1869.33
T 10	58.33	60.67	59.50	36.33	39.00	37.67	209.33	215.67	212.50	765.67	775.33	770.50	11438.33	12293.67	11866.00
SEm±	0.79	0.97	0.78	0.94	0.67	0.63	2.09	1.88	1.62	1.67	3.91	2.32	180.26	145.97	115.12
CD at 5%	2.33	2.87	2.30	2.78	1.97	1.85	6.17	5.53	4.77	4.91	11.53	6.85	531.77	430.60	339.61
CV	4.74	5.86	4.69	7.70	5.50	5.16	3.06	2.76	2.37	0.95	2.23	1.33	7.33	5.90	4.67

Note:(1) Data are average value of three replications; (2) Initial inoculums level 2 J_2/g soil best treatments to improve plant growth character, reducing number of galls per plant, number of egg masses per plant, number of eggs per egg mass, nematode population/200cc soil and total nematode population of *M. incognita* in chilli. However, standard check neem cake @ 5.0 q/ha was found the best treatment to improving plant growth characters as well as in reducing nematode population over other treatment. Hossam *et al.* (2012) revealed that all treatments [organic amendments, composts (1, 2, 3), neem, poultry, inorganic fertilizers and nematicide (nemacur 10 G)] reduced number of galls, nematode reproduction, fecundity and increased antioxidant substances over control in chilli. Bhuvaneshwari and Paul (2012) observed that fruit extract of neem induces defense response through enhanced activities of phenylalanine ammonia-lyase, tyrosine ammonia-lyase, polyphenol oxidase, peroxidase in chilli. Mukherjee *et al.* (2012) showed induction of PAL activity with SA in chilli plants increased at 40 days after inoculation with *M. incognita* and the highest activity of PAL was observed at four days after inoculation with nematodes. Plant products significantly enhanced growth of maize and reduced nematode infection over check.However, neem leaves powders at 4 q/ha was found to be the most effective in improving growth ofmaize and reducing infection of *Heterodera zeae* (Meena *et al.*, 2015). Goel and Paul (2014) evaluated that neem extract significantly reduced disease severity in the treated plants by inducing activities of PO and Lipoxygenase. Xiao, Z. (2016) found vermin-compost could significantly suppress root pests via modulating soil properties as well as plant defences, particularly for the susceptible plant. Application of mustard and neem cake @ 5.0 q/ha was found best to enhance enzymatic activity as well as in improving plant growth characters and reducing nematode population over other treatmentin tomato (Chandrawat *et al.* 2020). Efficacy of different organic amendments (@5g / kg soil) were evaluated against root-knot wilt disease complex developed by *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici* in whichneem cake was found significantly superior to increase plant growth parameter, reduce nematode population and wilt disease incidence followed by castor cake (Meena *et al.*, 2021).

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Occurrence of Plant Nematodes in Rice IPM Fields of Rice-Wheat Cropping System in Districts Rohtak (Haryana) and Gautam Buddh Nagar (Uttar Pradesh)

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ABSTRACT: Rice (*Oryza sativa* L.) is the most widely consumed food for over half of the world's human population, especially in India. Rice production is steady decline due to several factors that could be responsible for the low production. The one of major constraints in rice production is diseases caused by plant nematodes. A study was conducted to know the level of infestation of plant nematodes in rice IPM fields of rice-wheat cropping systems in district Gautam Buddh Nagar (Uttar Pradesh) and district Rohtak (Haryana). *M. graminicola* and *A. besseyi* were the most frequent and dominant plant nematodes in the Districts Rohtak and Gautam Buddh Nagar, respectively. The information generated from the present study may be useful from the management point of view in the area.

Keywords: Plant nematodes, IPM, Occurrence, diversity, Rice

Rice (*Oryza sativa* L.) is the most widely consumed food for over half of the world's human population, especially in Asia and Africa. It is used as a staple food for the majority of the world's population, predominantly in Asia where more than 90% of world rice is grown and consumed. India is one of the major producers of rice in the world. This is the source of fiber, vitamins B1(thiamin) and B6, magnesium, phosphorus, selenium, and manganese. Rice production is steady decline due to a number of factors that could be responsible for the low production.

The one of major constraints in rice production is diseases caused by plant parasitic nematodes (PPNs) are major bottlenecks to crop productivity in the high input intensive cropping systems such as the rice-wheat cropping systems (Dangal *et al.*, 2009; Sharma *et al.*, 2002). About 200 plant nematode species have been reported to be associated with rice (Prot, 1994). Coyne and Plowright (2000) considered that nematodes are important pests in the rapidly changing production system of rice. There may be rice yield loss in every plant nematodes infested field. Hence there is an urgent need for practical nematode management options for the farmers. Thus, this study was conducted to know the level of infestation of plant nematodes in rice IPM fields of rice-wheat cropping systems in district Gautam Buddh Nagar (Uttar Pradesh) and district Rohtak (Haryana).

MATERIALS AND METHODS

Soil samples collection site

Soil samples were collected around the root zone of rice crops cultivated under IPM validation in the farmer's participatory mode program in villages Bambawad (n= 11) of District Gautam Buddh Nagar (Uttar Pradesh) and village Nidana (n= 10), District Rohtak (Haryana) during Kharif session 2021. Within the collection site, soil samples were collected at a depth of 10-20 cm using a hand trowel, each sample containing a composite from 3-5 random subsamples. These samples were mixed to make a composite sample and from this 100 cc of soil was taken for further processing. The hand trowel was sterilized with 70% ethanol before leaving the sampling site. Samples were placed in polyethylene bags to minimize dehydration, tag a label providing all necessary information, and transported into the laboratory.

Isolation of nematodes from soil

Soil samples were processed for plant nematode extraction by the combination of the Baermann funnel technique (Baermann, 1917) and Cobb's sieving and decanting method (Cobb, 1918).

Identification of nematodes

Nematodes were fixed and processed to dehydration as per the method described by Seinhorst (1959) and prepared permanent slides for identification. Identification up to the generic level was done using the taxonomic key described by Siddiqi (2000). The interactive diagnostic key to plant parasitic nematodes from the Nematology laboratory, University of Nebraska Lincoin, which is an available website (http://nematode.unl.edu/knozlistbutt.htm) was also used as an aid in the identification.

Counting of nematodes

Nematodes were collected and fixed in hot TAF (Triethelene Amine Formaline) and stored for population analysis. The population of nematodes in each sample was counted five times with the help of Syracuse counting dish under the stereoscopic zoom microscope and the mean value was worked out.

Analysis nematode population

Absolute frequency (AF) and density (D) of plant nematodes were analyzed as described by Norton (1978).

RESULTS AND DISCUSSION

Total five plant nematodes viz., stunt nematode *Tylenchorhynchus mashhoodi* (Siddiqi and Basir, 1959), rice root-knot nematode *Meloidogyne graminicola* (Golden and Birchfield, 1965), lance nematode *Hoplolaimus indicus* (Sher, 1963), rice leaf nematode *Aphlenchoides besseyi* (Christie, 1942) and rice root nematode *Hirschmanniella oryzae* (Van Breda de Haan, 1902) Luc and Goodey, 1964 have been encountered during present study. *A. besseyi*, *T. mashhoodi* and *H. oryzae* were encountered in Gautam Buddh Nagar districts whereas *M. graminicola, H. indicus* and *T. mashhoodi* found from Rohtak districts.

Maximum number of plant nematode (2 genera/location) contained in three locations of districts Gautam Buddh Nagar, followed by one plant nematode genera/location were found in 4 and 6 location of Gautam Buddh Nagar and Rohtak districts, respectively. Whereas neither plant nematode was found in four location each in Gautam Buddh Nagar and Rohtak districts (Fig. 1).

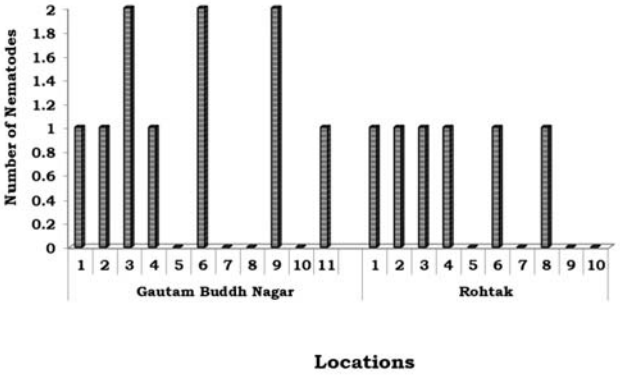

Fig. 1. Location wise plant nematode population associated with rice

Data revealed that plant nematode incidence was more in Gautam Buddh Nagar compared to Rohtak district. Among the plant nematodes, *A. besseyi* had the highest absolute frequency (60%), followed by *T. mashhoodi*, whereas *H. oryzae* (20%) had the lowest absolute frequency in the Gautam Buddh Nagar district (Fig. 2). *T. mashhoodi* was the maximum absolute frequency (30%), followed by *M. graminicola* (20%), whereas *H. indicus* (10%) had less absolute frequency in the Rohtak district (Fig. 3).

The result showed that plant nematodes abundance was more in Rohtak district in comparison to district Gautam Buddh Nagar. *A. besseyi* (1.54) were more abundant with the highest density, followed by *H. oryzae* (0.72), whereas *T. mashhoodi* (0.36) was less abundant with least density in Gautam Buddh Nagar district (Fig. 4). *M. graminicola* (12.8) was the maximum density, followed by *T. mashhoodi* (1.2), whereas *H. indicus* (0.9) was less abundant with the least density in the Rohtak district (Fig. 5).

Nematode's frequency, density, and diversity would vary depending on ecological and edaphic factors (Pervez and Rao, 2020a,b) because of coinciding together in different environments (Bongers and Bongers, 1998). Nematodes may form the most significant group for community indicator analysis because more information

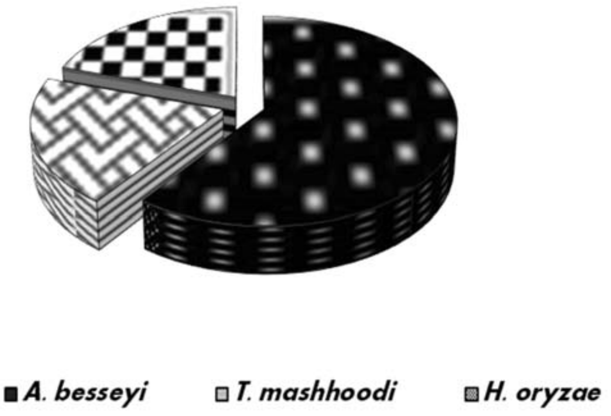

Fig. 2. Frequency (%) of plant nematodes in IPM rice based cropping system in district Gautam Buddh Nagar (n=11)

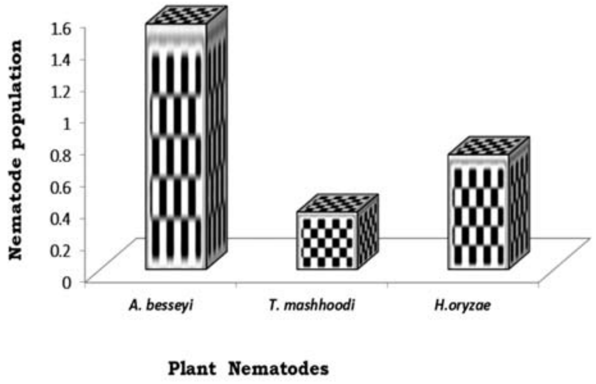

Fig. 4. Density of plant nematodes in IPM rice based cropping system in district Gautam Buddh Nagar (n=11)

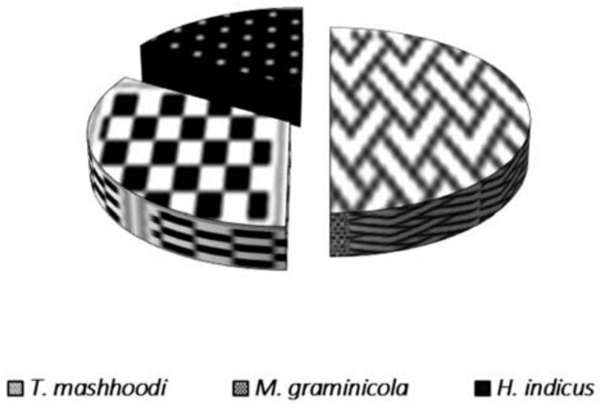

Fig. 3. Frequency (%) of plant nematodes in IPM rice based cropping system in district Rohtak (n=10)

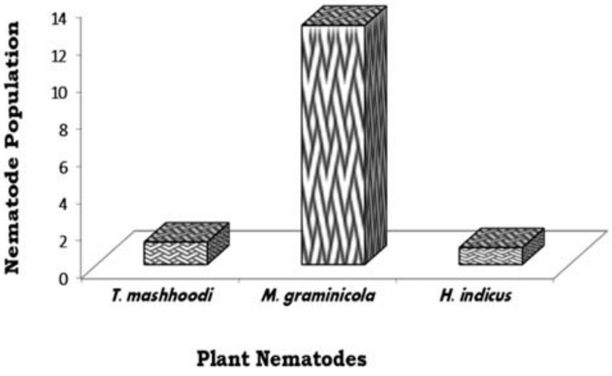

Fig. 5. Density of plant nematodes in IPM rice based cropping system in district Rohtak (n=10)

exists on their taxonomy and macrofauna (Pervez, 2006; Bilgrami and Pervez, 2000).

Nematode-caused diseases are generally not of sudden epidemic type but rather of the slow decline in yields, spreading very gradually in extent year after year. Changing the scenario of regular cultivation of rice on an extensive basis helps in the building up of nematode communities. Nematode problems are therefore likely to increase year after year. Because of the paucity of information on the distribution and population dynamics of nematodes associated with rice, the present investigation was undertaken in important rice-growing areas where the crop is being intensively cultivated to pinpoint the distribution of plant nematodes and also their level of infestation.

It is concluded that *M. graminicola* and *A. besseyi* were the most frequent and dominant plant nematodes in the Districts Rohtak and Gautam Buddh Nagar, respectively. The information generated from the present study may use from the management point of view in the area.

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Potential of Native *Pseudomonas* Isolates for the Biocontrol of Root-Knot Nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood in Tomato

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ABSTRACT: Antagonistic effect of native *Pseudomonas* isolates was assessed *in vitro* against *Meloidogyne incognita* in terms of juvenile mortality. Potential isolates from *in vitro* study were further tested in tomato (cv. PKM-1) under pot culture and open field conditions to determine the ability of isolates to inhibit gall formation and nematode egg hatching. Out of 21 native *Pseudomonas* isolates tested, Pfb9 isolate resulted in highest *in vitro* mortality of *M. incognita* juveniles. This isolate, when evaluated against *M. incognita* in tomato under pot culture and open field conditions, was recorded to reduce nematode population both in soil and root as well as improved growth characteristics of tomato.

Key words: Bio management, defense enzymes, juvenile mortality, plant growth promotion, *Pseudomonas* isolates, root knot nematodes, tomato

Tomato, *Lycopersicon esculentum* Mill. is one of the widely grown vegetable crop, accounting for about 14 % of global vegetable production. It covers the area of about 789.15 ha in India, with the production of 19759.32 MT (Horticultural statistics at a glance, 2018). It contains high concentrations of lycopene, as well as reported as a rich source of minerals and vitamins (Giovannucci, 1999).

Among several nematodes infesting solanaceous vegetables, damage caused due to root knot nematodes, *Meloidogyne* spp. is a major obstacle to the production of these vegetable crops growing in tropical and subtropical regions of the world. Considering the possible detrimental effect of chemicals in the environment and restriction in the use of chemical based products, researchers prompted for an alternative ecofriendly management strategy against the nematodes.

Plant growth promoting rhizobacteria (PGPR) are the most effective agents for the control of plant parasitic nematodes. Metabolites emanating from these bacteria have the ability to reduce the population of root knot nematodes (Devapiyanga *et al.*, 2012; Jonathan *et al.,* 2012). *Pseudomonas fluorescens* is one among the most studied rhizobacteria with highest correlation to soil suppressiveness. Through the synthesis of enzymes, antibiotics and siderophores, this bacterium helps in the suppression of nematodes. Colonization of plant roots by this bacterium alters the production of root exudates, which reduces the attraction of nematodes towards the roots (Siddiqui and Shaukat, 2002). Production of 2,4 Diacetylphloroglucinol (2,4 DAPG) and hydrogen cyanide (HCN) by *Pseudomonas* spp. are often associated with the nematode suppression in tomato, which occurs as an outcome of interaction of the bacteria with host plants. In light of the above findings, present study has been conducted to assess the comparative efficacy of native isolates of *Pseudomonas* spp. against *M. incognita* infesting tomato, as well as their potential in enhancing the biomass of tomato plants.

MATERIALS AND METHODS

Maintenance of pure culture of root knot nematode, *Meloidogyne incognita*

Pure culture of *M. incognita* was maintained in tomato (cv. PKM-1) grown under pot culture condition (28± 2°C) in the research farm, Adhiparasakthi Agricultural College (APAC), Kalavai, Ranipet District, Tamil Nadu, India.

Isolation of *Pseudomonas* spp.

Soil samples were randomly collected from the rhizosphere of various horticultural crops grown in Ranipet and Tiruvannamalai District, TN. *Pseudomonas* isolation agar was used to isolate *Pseudomonas* spp. *P. fluorescens*, Pf1 was obtained from the culture collection centre of Plant Pathology, Tamil Nadu Agricultural University, Tamil Nadu, India.

In vitro efficacy of *Pseudomonas* isolates against *M. incognita* juveniles

The 21 *Pseudomonas* isolates collected from the soil samples were biochemically characterized (Jayaprakashvel *et al.*, 2010) as well as studied for their *in vitro* efficacy against *M. incognita.*

For the *in vitro* studies against nematodes, about 100 second stage juveniles of *M. incognita* were added to the Petri plates (5 cm diameter) containing 2 ml of cell free culture filtrate of different bacterial suspension taken at different concentrations (25, 50 and 100%). Each treatment was replicated 4 times in completely randomized design (CRD). Petri plates were incubated at room temperature (28 ± 2ºc) and observations on dead juveniles were recorded at 24, 48 and 72 h interval.

Preparation of liquid formulation of *Pseudomonas* isolates

Effective 2 native *Pseudomonas* isolates from *in vitro* studies along with Pf 1 was taken for the preparation of liquid formulation. For developing the same, one litre of nutrient broth was prepared in combination with glycerol (10 mM) and starch (2 %) (Meena *et al.*, 2014). One ml of log phase bacterial cultures of different isolates was inoculated individually into the broth and incubated at room temperature. Nutrient broth without bacteria served as control.

Evaluation of liquid formulation of *Pseudomonas* isolates against *M. incognita* infesting tomato under pot culture condition

About 4 weeks old tomato seedlings (cv. PKM 1) were transplanted into the mud pots (15cm dia × 30 cm height) filled with one kg of sterilized soil (1 sand:1 soil:1 FYM). After the establishment of seedlings in the pots, second stage juveniles of *M. incognita* (1 J_2 / g soil) were inoculated around the root zone by removing the top soil layer. Three days after inoculation of nematodes, liquid formulation of the respective *Pseudomonas* isolates (10^8cfu/ml) were inoculated at the rate of 0.05µl along with 2 ml water to the rhizosphere region of the plant. Carbofuran 3G (1kg ai / ha) was applied as chemical check. Plants inoculated with nematode alone served as untreated control. Treatments were arranged in CRD with 4 replicates per treatment. Plants were watered regularly to the field capacity. Thirty days after nematode inoculation, plants were uprooted carefully and assessed for their growth parameters, defense enzymes and nematode population.

Estimation of defense enzymes induced due to the application of *Pseudomonas* isolates in tomato roots infested with *M. incognita*

Tomato root samples (1 g) were collected from 1 to 5 days post inoculation of *Pseudomonas* isolates and homogenised with one ml of 0.1 M phosphate buffer (pH 7.0) at 4°C in a pre chilled pestle and mortar. The homogenates were centrifuged at 20,000 rpm for 15 minutes at 4°C and the supernatant collected served as enzyme source for the assessment of the activity of peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (Meena *et al*., 2012).

Evaluation of liquid formulation of *Pseudomonas* isolates against *Meloidogyne incognita* infestation in tomato under open field conditions

This study was conducted in the farmers field (heavily infested with nematodes) of Ranipet District, Tamil Nadu, India located at 307 m above mean sea level at 10°49'10.12"N latitude and 77°52.66'E longitude, and has a tropical climate. Two season field trials (season 1: 2016-2017; season 2: 2017-2018) were performed in tomato cv. PKM 1. About 4 weeks old tomato plants (cv. PKM 1) were transplanted in the field at a distance of 60 x 60 cm. After the establishment of seedlings in the field, bacterial (Pfb1, Pfb9 and Pf1) suspensions (10^8cfu/ml) were applied at 500 ml / ha to the rhizosphere. Carbofuran (1kg a.i /ha) was applied as chemical check. All the treatments were laid out in randomized complete block design with 4 replicates per treatment. The treatments were given twice at 30 days interval on 30 and 60 days after transplanting the crop. About 30 days after final application of the bioagents, soil and root sample were randomly collected to assess the nematode population. Around 5 plants samples and 5 soil samples (250 cc soil /sample) from each replication were collected and pooled to assess the plant growth parameters and nematode population. Tomato yield was recorded in different treatment plots and expressed in t/ha.

Data analysis

Data analysis were carried according to the methods of Gomez and Gomez (1984) using SAS Software Version 9.3 (SAS, 2011). Data were analysed on absolute values using ANOVA for *in vitro*, pot culture and field studies. The means were analysed by Least Square Difference (LSD) and separated using Tukey's HSD test at *P*d" 0.001.

RESULTS

Characterization of *Pseudomonas* isolates

Biochemical characterization of 21 native *Pseudomonas* isolates revealed that Pfb 9 and Pfb 1 reported positive to all the 4 biochemical tests, Pft 11 recorded positive to 3 biochemical tests, while other isolates exhibited least number of positive reactions to the biochemical studies. Pf 1 exhibited positive reaction to all the biochemical studies (Table 1).

In vitro effect of *Pseudomonas* isolates against *M. incognita* juveniles

Cell free culture filtrate of all the tested bacteria enhanced the mortality of *M. incognita* juveniles (Table 2). Among the different isolates tested, Pfb9 (100 %) recorded 85.3± 0.95 % juvenile mortality at an exposure period of 72 h, followed by Pf1 (84.8 ± 1.10 %) and Pfb1 (83.8±1.60 %) at the same concentration and exposure period, while Pfc10 recorded least juvenile mortality (47.8±3.11 %).

Pfb9 and Pfb1 that reported positive to all the biochemical tests exhibited maximum control of nematode juveniles *in vitro,* which confirmed that the production of secondary metabolites / enzymes plays a significant role in the biocontrol mechanisms.

Effect of liquid formulation of *Pseudomonas* isolates on *M. incognita* population and growth of tomato plants under pot culture

Under pot culture experiment, all the *Pseudomonas* isolates enhanced growth characteristics of the plants and reduced nematode severity (Table 4). Among the

Table 1. Biochemical characterization of *Pseudomonas* isolates

S. No.	Isolates	Methyl red test	Starch hydrolysis test	Hydrogen cyanide production	Siderophore production
1	Pfb9	+	+	+	+
2	Pf1	+	+	+	+
3	Pfb1	+	+	+	+
4	Pfb3	-	+	+	-
5	Pfb10	+	-	+	-
6	Pfb12	+	-	-	+
7	Pfp3	-	-	+	+
8	Pfp4	+	+	-	-
9	Pfp5	-	+	-	+
10	Pfp6	-	+	+	-
11	Pfp8	+	+	-	-
12	Pfj5	+	-	+	-
13	Pfj8	+	-	+	-
14	Pfc6	+	-	-	+
15	Pfc10	-	+	-	-
16	pft4	-	-	-	+
17	Pft9	-	+	+	-
18	Pft11	+	-	+	+
19	Pfm17	-	-	+	-
20	Pfm5	-	+	+	-
21	Pfg8	+	+	-	-
22	Pfg11	-	+	+	-

different treatments, least number of galls (29.0±1.47), root and soil population of nematodes (60.3±1.70 females /g root; 2713 ± 100.09 eggs /g root; 183.3±3.32 juveniles /200 cc soil) has been recorded in Pfb1 treatment. It was followed by Pfb9 (29.3±2.43 galls /root; 61.3±1.25 females /g root; 2761.8± 122.41 eggs /g root; 191.3±5.28 juveniles /200 cc soil). Pf1 recorded as the next best treatment in reducing root and soil population of nematodes (30.0±1.35 galls / g root; 67.8±3.19 females /g root; 3018.8± 69.17 eggs /g root; 210.5±3.51 juveniles /200 cc soil), while the maximum population of nematodes was recorded in untreated control (94.3±5.42 galls/g root; 127.5±6.02 females /g root; 5766.5±115.87 eggs /g root; 619.8±34.61 juveniles /200 cc soil).

Similarly, plant growth characteristics increased significantly in all the bioagents treated plants over untreated plants. Pfb1 recorded the maximum fresh and dry weights of shoot (23.8±0.85 g and 4.5±0.28 g) and root (4.9±0.26 g and 1.3±0.06 g) respectively among all the treatments, followed by Pf 1 (fresh and dry weights of shoot (22.8±0.85 g; 4.3±0.25 g) and root (4.7±0.17g; 1.2±0.02)). Untreated control recorded least plant growth characteristics (fresh and dry weights of shoot (16.8±0.85 g; 3.0±0.40 g) and root (3.4±0.52 g; 0.9±0.03g)).

Effect of liquid formulation of *Pseudomonas* isolates in the induction of defense enzymes in tomato

All the bacterial isolates enhanced the defense enzymes activities significantly over time upon root

Table 2. ***In vitro* effect of *Pseudomonas* isolates on *Meloidogyne incognita* juveniles**

S.No.	Treatments	Percent Mortality of juveniles[#] (Average ± SE)								
		25% concentration			50% concentration			100% concentration		
		24h	48h	72h	24h	48h	72h	24h	48h	72h
1.	Pfb9	5.8±0.25	12.3±0.75	14.3±1.31	16.5±0.86	31.3±0.47	33.5±1.19	48.0±2.16	73.0±2.27	85.3±0.95
2.	Pf1	6.3±0.75	12.8±1.18	15.0±0.40	19.3±2.01	32.8±1.37	36.5±3.20	49.3±1.03	75.3±1.49	84.8±1.10
3.	Pfb1	6.0±0.40	12.3±0.62	14.5±0.64	19.0±1.95	32.0±1.82	34.8±1.37	48.3±1.10	75.3±3.32	83.8±1.60
4.	Pfb3	4.8±0.25	11.8±1.18	13.3±0.75	15.3±1.70	23.8±1.70	31.8±0.47	37.8±1.31	53.8±4.28	59.5±4.13
5.	Pfb10	4.8±0.48	12.0±1.08	13.3±0.75	15.8±1.65	24.8±1.25	31.3±0.94	42.5±1.75	47.5±3.17	53.8±1.25
6.	Pfb12	4.5±0.29	11.5±0.28	14.0±1.22	14.0±1.87	23.5±1.50	29.8±0.75	38.0±1.22	56.5±1.84	57.0±0.91
7.	Pfp3	3.3±0.75	11.5±1.32	13.0±0.40	13.0±1.29	20.8±2.35	29.8±0.75	38.3±2.56	59.5±2.21	60.0±2.34
8.	Pfp4	3.3±0.48	9.8±0.85	14.0±0.70	16.0±0.70	20.8±2.56	27.8±1.03	38.0±3.24	63.3±2.95	63.3±1.10
9	Pfp5	4.5±0.86	11.0±0.70	12.3±1.43	15.3±0.47	18.5±1.25	27.5±1.32	38.3±2.25	55.3±1.70	55.3±1.93
10.	Pfp6	4.0±0.70	10.0±0.70	10.0±1.68	13.5±0.64	19.8±1.88	27.3±1.95	37.3±1.75	55.8±1.25	56.5±1.55
11.	Pfp8	4.0±0.82	10.3±0.85	11.5±0.28	14.0±1.22	21.0±2.38	25.5±2.21	37.5±1.93	53.5±0.86	53.8±0.62
12.	Pfj5	3.5±0.65	8.8±0.47	8.8±0.62	12.5±0.95	20.8±1.31	25.0±1.47	35.0±3.53	52.3±0.62	53.5±0.86
13.	Pfj8	4.3±0.48	10.0±1.68	10.3±0.94	15.0±2.27	21.3±2.01	25.5±1.04	34.5±2.84	56.0±2.55	56.5±0.86
14.	Pfc6	3.5±0.50	9.0±1.80	10.8±0.62	15.0±0.70	20.8±2.78	26.3±2.95	39.5±2.02	51.8±3.59	55.0±1.68
15.	Pfc10	4.8±0.25	9.8±1.10	11.0±0.70	15.3±0.47	19.3±2.32	27.5±2.63	37.8±1.43	47.5±2.36	47.8±3.11
16.	pft4	3.5±0.50	9.8±0.25	9.8±0.25	15.8±1.31	17.8±2.28	27.3±1.49	38.5±2.10	44.5±1.55	52.5±3.12
17.	Pft9	4.3±0.25	9.8±1.43	12.8±0.75	15.3±0.85	20.3±2.39	26.3±1.70	36.8±4.40	56.8±1.10	57.0±0.91
18.	Pft11	4.0±0.91	9.5±0.95	11.3±0.94	14.3±1.60	22.3±2.81	27.0±1.58	38.5±2.90	58.0±2.48	65.5±1.44
19.	Pfm17	3.3±0.48	9.5±1.04	9.5±0.86	13.8±1.25	18.3±1.88	26.5±1.19	36.0±2.48	45.0±1.29	53.0±1.22
20.	Pfm5	4.3±0.75	9.3±1.37	11.5±1.44	14.8±1.18	19.5±1.70	25.8±3.09	40.3±1.88	54.3±3.96	58.5±3.30
21.	Pfg8	3.3±0.75	9.3±0.75	10.3±1.03	12.0±1.08	18.3±1.25	24.3±2.05	38.3±1.65	55.0±3.02	55.3±2.56
22.	Pfg11	3.8±0.25	10.0±1.08	10.3±1.31	9.5±0.86	17.0±2.48	28.3±2.05	39.3±3.11	54.0±2.30	55.3±2.25
23.	KB	2.8±0.48	3.0±0.57	3.5±0.28	4.8±0.47	4.8±0.25	5.0±0.57	5.3±0.47	5.5±0.28	6.0±0.70
24.	D. water	0.3±0.25	1.0±0.57	1.8±0.28	0.3±0.25	1.0±0.57	1.8±0.85	0.3±0.25	1.0±0.57	1.8±0.85
	df	23,72	23,72	23,72	23,72	23,72	23,72	23,72	23,72	23,72
	F value	4.500	7.368	11.545	10.105	13.179	19.387	23.821	51.573	94.180
	P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[#] Mean of four replications, data in interaction analyzed with least squares means (LSD) and means separated with a standard error of the mean at *P*d”0.01 as determined by Tukey’s HSD test.

Table 3. Effect of *Pseudomonas* isolates on growth of tomato and population of *Meloidogyne incognita* under pot culture condition

Treatments	Application rate	Plant growth parameters# (Average ± SE)					Nematode population# (Average ± SE)			
	(per pot)	Shoot length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight(g)	No. of galls/ g root	No. of females/ g root	No. of eggs/ g root	Juvenile population/ 200 cc soil
Pfb9(10^8cfu/ml)	0.05µl	31.5±0.64	19.0±1.08	3.8±0.25	4.7±0.41	1.3±0.04	29.3±2.43	61.3±1.25	2761.8±122.41	191.3±5.28
Pf1 (10^8cfu/ml)	0.05µl	32.3±1.10	22.8±0.85	4.3±0.25	4.7±0.17	1.2±0.02	30.0±1.35	67.8±3.19	3018.8±69.17	210.5±3.51
Pfb1 (10^8cfu/ml)	0.05µl	30.5±1.75	23.8±0.85	4.5±0.28	4.9±0.26	1.3±0.06	29.0±1.47	60.3±1.70	2713.3±100.09	183.3±3.32
Carbofuran	33 kg	25.3±1.10	18.5±1.55	3.5±0.28	4.7±0.15	1.4±0.06	29.3±2.56	61.3±1.93	2743.5±78.29	192.0±12.02
Untreated Control	-	24.5±1.04	16.8±0.85	3.0±0.40	3.4±0.52	0.9±0.03	94.3±5.42	127.5±6.02	5766.5±115.87	619.8±34.61
df		4, 15	4, 15	4, 15	4, 15	4,15	4,15	4, 15	4, 15	4,15
F value		9.439	7.652	3.886	3.148	12.773	91.801	77.806	178.730	130.180
P value		<0.001	<0.001	0.0232	0.0457	<0.001	<0.001	<0.001	<0.001	<0.001

#Mean of four replications, data in interaction analyzed with least squares means (LSD) and means separated with a standard error of the mean at *P*d”0.01 as determined by Tukey's HSD test.

colonization. Highest per oxidase (PO) activity was recorded in Pfb9 treatment (1.38min^{-1} g^{-1} root) followed by Pfb1(1.14min^{-1} g^{-1} root), while in Pf1, the value tends to be 0.78 min^{-1} g^{-1} root on 4th day of bacterization (Fig 1). Activity of polyphenol oxidase (PPO) followed the same trend as that of PO, where maximum activity of PPO has been recorded on 4th day after bacterization. Pfb9 recorded the maximum PPO activity (1.487min^{-1}g^{-1} root), followed by Pfb 1 (1.297min^{-1}g^{-1}root) and Pf 1 (1.097 min^{-1}g^{-1}root) (Fig 2). Similarly, application of Pfb9 induced maximum production of phenylalanine ammonia lyase (PAL) activity (0.306 min^{-1} g^{-1} root) on 4th day post bacterization followed by Pfb1 (0.304 min^{-1} g^{-1} root) and Pf1 (0.304 min^{-1} g^{-1} root) (Fig 3). Carbofuran treatment did not recorded any fluctuation in PO, PPO and PAL activity during the study period, while untreated control recorded the lowest defence enzymes activities *viz.*, PO (0.103 min^{-1}g^{-1} root), PPO (0.170min^{-1}g^{-1}root) and PAL (0.123 min^{-1} g^{-1} root) which has been recorded as the average of 5 days reading.

Effect of liquid formulation of *Pseudomonas* isolates on *M. incognita* population and yield of tomato under open field conditions

Under open field condition, all the bioagents treated plots recorded enhanced vigour over chemical treated and control plots (Table 5). In season 1, Pfb9 treated plants recorded least gall count (35.25±1.31galls/g root), soil (326.25±3.40 juveniles/200 cc soil) and root (102.0±3.74 females/g root; 5137.75±40.01 eggs/g root) nematode population. It was followed by Pf1, where the plants recorded about 36.75±1.10 galls/g root; 365.25±3.35 juveniles/200 cc soil; 107.75±4.90 females/g root and 5756.25±17.33 eggs/g root. Highest number of galls (120.0±1.95/g root), soil (978.0±19.23 juveniles/200 cc soil) and root population (160.0±4.74 females/g root; 8572.0±67.13 eggs/g root) was obtained in untreated control.

Similarly, plants that received Pfb9 treatment recorded maximum yield (32.70±0.30 t/ha) followed by

Table 4. Effect of *Pseudomonas* isolates on the population of *Meloidogyne incognita* and yield of tomato under field condition

Treatments	Application rate	Nematode population[*] (Average ± SE)				Yield[#] (t/ha)
	(per ha)	No. of juveniles/ 200 cc soil	No. of galls/ g root	No. of females/ g root	No. of eggs/ g root	
Season 1						
Pfb9 (10^8cfu/ml)	500 ml	326.25±3.40	35.25±1.31	102.00±3.74	5137.75±40.01	32.70±0.30
Pf1 (10^8cfu/ml)	500 ml	365.25±3.35	36.75±1.10	107.75±4.90	5756.25±17.33	31.63±0.37
Pfb1 (10^8cfu/ml)	500 ml	372.75±3.90	34.50±1.19	105.75±1.25	5620.75±78.59	31.03±0.28
Carbofuran 3G	1kg a.i.	365.00±7.59	34.25±0.25	105.25±5.23	5686.75±38.85	31.05±0.35
Untreated Control	-	978.00±19.23	120.00±1.95	160.00±4.74	8572.00±67.13	29.70±0.20
df		4,12	4,12	4,12	4,12	4,12
F value		834.13	966.432	40.585	755.086	18.941
P value		<0.001	<0.001	<0.001	<0.001	<0.001
Season 2						
Pfb9 (10^8cfu/ml)	500 ml	342.75±6.15	34.25±1.10	99.50±2.17	5101.25±41.43	32.48±0.86
Pf1 (10^8cfu/ml)	500 ml	354.75±11.36	35.25±2.17	105.75±4.51	5731.25±30.59	32.20±0.57
Pfb1 (10^8cfu/ml)	500 ml	370.25±12.07	34.00±2.48	105.25±5.80	5544.75±23.95	31.73±0.21
Carbofuran 3G	1kg a.i.	354.00±4.20	34.75±1.37	113.75±3.96	5621.25±61.74	30.58±0.16
Untreated Control	-	1082.50±30.84	117.00±1.47	157.50±3.79	8664.50±49.35	29.83±0.13
df		4,12	4,12	4,12	4,12	4,12
F value		429.190	355.985	26.203	1,522.532	6.212
P value		<0.001	<0.001	<0.001	<0.001	0.006

[*] Mean of four replications, data in interaction analyzed with least squares means (LSD) and means separated with a standard error of the mean at *P*d”0.01 as determined by Tukey's HSD test.

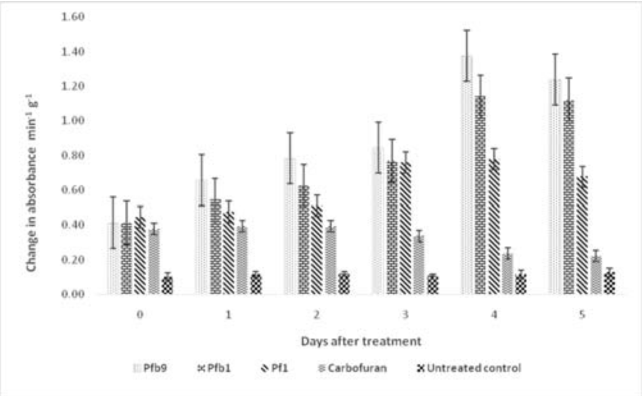

Fig. 1. Induction of PO activity due to the application of *Pseudomonas* isolates in tomato roots infested with *M. incognita*

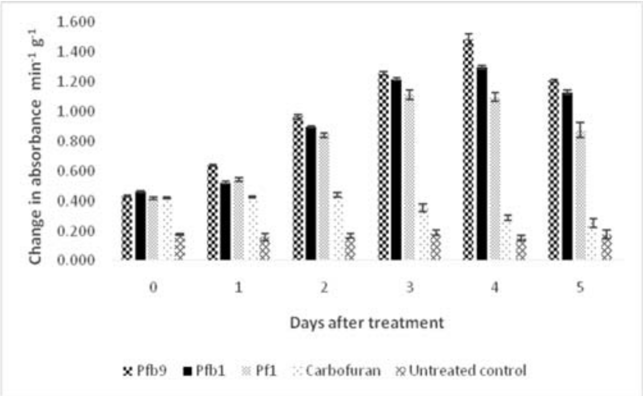

Fig. 2. Induction of PPO activity due to the application of *Pseudomonas* isolates in tomato roots infested with *M. incognita*

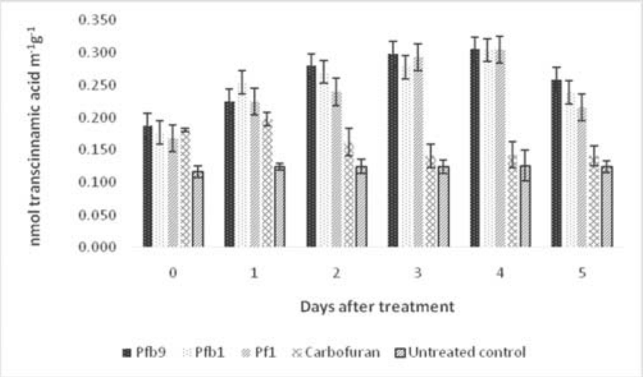

Fig. 3. Induction of PAL activity due to the application of *Pseudomonas* isolates in tomato roots infested with *M. incognita*

Pf1 treatment (31.63±0.37 t/ha). Even though, application of carbofuran reduced root galls to 34.25±0.25/g root, yield parameters recorded with carbofuran treated plants was 31.05± 0.35 t/ha, which was recorded to be lesser than biocontrol treatments. Least yield parameter was recorded in untreated control plot (29.70±0.20 t/ha).

In season 2, Pfb9 treatment recorded least gall count (34.25±1.10 galls/g root), soil (342.75±6.15 juveniles / 200 cc soil) and root (99.50±2.17 females/g root; 5101.25±41.43 eggs/g root) nematode population over other treatments. It was followed by Pf1, where the plants recorded 35.25±2.17galls/g root; 105.75±4.51 females/g root; 5731.25±30.59 eggs/g root and 354.75±11.36 juveniles /200 cc soil. Highest number of galls (117.0±1.47/g root), soil (1082.50±30.84 juveniles/ 200 cc soil) and root population (157.50±3.79 females/g root; 8664.50±49.35 eggs/g root) was obtained in untreated control. Pfb9 increased the yield of tomato to 32.48±0.86 t/ha, followed by Pf1, where the yield has been reported as 32.20±0.57 t/ha. Carbofuran recorded the yield of 30.58±0.16 t/ha and the least yield parameter was recorded in untreated control plot (29.83±0.13 t/ha).

Results of pooled data of two season confirmed the efficacy of Pfb9 in reducing the root galls (34.75±1.20 / g root), soil (334.50±4.77 juveniles / 200 cc soil) and root population of nematodes (100.75±2.95 females /g root; 5119.50±40.72 eggs /g root) and increasing yield of the crop (32.59±0.58 t/ha) over other treatments.

DISCUSSION

In the present study, a total of 78 bacterial isolates were collected from various horticultural crops grown in different districts of Tamil Nadu and biochemically characterized to determine the basic characters of the bacteria. Ramyabharathi *et al*. (2014) identified and characterized rhizobacterial isolates from maize and cut flowers using phenotypic and biochemical studies.

In vitro mortality of *M. incognita* juveniles with the cell free culture filtrates of native bacterial isolates was demonstrated in the current study. It has been evident from the study that the mortality rate is directly proportional to concentration of the culture filtrate and the length of exposure period as recorded by Kavitha *et al*. (2010) and Meena *et al*. (2012). Nematicidal properties of the bacteria are due to the presence of several antibiotics, lytic enzymes and volatile compounds in their culture filtrates (Meena *et al.,* 2013; Devi and Bora, 2019). Among the several compounds, 2,4 diacetylphloroglucinol, hydrogen cyanide, phenazine and pyocyanine plays a significant role in the reduction of nematode juveniles (Cezairliyan *et al*., 2013; Meena *et al.,* 2014).

Application of *P. fluorescens* through soil drenching resulted in significant reduction in nematode population in soil and roots, as shown in the present study. This finding was in consistent with that of Kempester *et al*. (2001) who found that using *P. fluorescens* strains P29 and P80 as soil drench decreased *Heterodera trifolii* fecundity and increased the proportion of distorted females in white clover (*Trifolium repens*). Induced systemic resistance (ISR) is the major mechanism associated with the application of *Pseudomonas* isolates which brought about substantial increase in nematode fecundity rate (Siddiqui and Shaukat, 2005; Meena *et al.,* 2012). ISR induced by *Pseudomonas* spp. increases the activity of defense enzymes such as peroxidase, polyphenoloxidase and phenylalanine ammonia lyase and phenol which are essential in preventing nematode entry into the roots.

Besides reducing nematode population, application of *Pseudomonas* spp. recorded enhanced growth and yield parameters of tomato in the present study. Even though the distinct mechanism by which rhizobacteria promote the plant growth is not clearly known, several mechanisms have been identified to contribute for plant growth promotion, including the production of phytohormones, promotion of uptake of mineral nutrients and suppression of deleterious organisms. Generally, nematodes wreak on the vascular system of plants cutting off their supply of water and nutrients (Bird, 1959). Application of *P. fluorescens* in the rhizosphere helps to prevent the nematode development into the root system and thus help in the progressive growth of the plants (Jonathan *et al*., 2012). Meena *et al*. (2012) observed an indicative increase of plant growth of tomato and remarkable reduction of nematode population in the combined application of *P. fluorescens* (Pf 128) and *Bacillus subtilis* (Bbv 57).

Bioagents treated plots had greater vigour than chemical check and control plots in the current study, which supported the previous studies that demonstrated the impact of rhizobacteria against *M. incognita* in tomato through the attributes of induction of systemic resistance, competition, production of toxic secondary metabolites and cell wall lytic enzymes.

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Response of Tomato Genotypes to Root-Knot Nematode (*Meloidogyne incognita*) infection under Laboratory and Protected Cultivation

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ABSTRACT: The root-knot nematode, *Meloidogyne incognita* is the most severe problem in tomato crop grown under protected cultivation. Finding and use of resistance against *M. incognita* in plant breeding program could be most valuable to control nematode incidence. The host response of 113 tomato genotypes were studied at three temperatures, 28 ± 2°C, 30 ± 2°C and 32 ± 2°C in Pluronic gel. The results revealed that change in temperature had a significant effect on the gall formation and juvenile penetration. Further, in pot screening under polyhouse condition out of 99 genotypes, 16 genotypes were moderately resistant, 81 susceptible and 9 highly susceptible. The response of moderately resistant genotypes obtained from pots screening was evaluated under naturally infested polyhouse beds with *M. incognita* population density of 5-6 J2s/cc soil, resulting in resistance breakage with only 5 genotypes out of 12 moderately resistant with reduced nematode incidence. Data obtained from this study revealed that temperature and soil nematode population density had a significant effect on resistance of tomato genotypes. The moderately resistant genotypes could be used to develop resistant cultivars.

Keywords: Tomato genotypes, resistance, temperature, *Meloidogyne incognita,* pluronic gel, penetration, protected cultivation

Tomato (*Solanum lycopersicum* L.) is a major vegetable crop grown all around the world and is a good source of vitamins, minerals, antioxidants, organic acids, calcium, and niacin (Mourvaki *et al.*,2005; Olaniyi *et al.*, 2010). Annually, India produces about 16.38 MT of tomato, which is very low as compared to the developed countries due to vulnerability of the crop to several fungal, viral, bacterial, and nematode diseases (Horna *et al.*, 2006). Plant parasitic nematodes cause 21.3 % crop losses amounting to INR 102,039.79 million (1.58 billion USD) annually. The losses in 19 horticultural crops were assessed at INR 50,224.98 million and in tomato alone causing INR 6035.2 million (Kumar *et al.*, 2020). Globally root-knot nematodes (RKNs) cause significant loss to agricultural productivity, reducing crop yields by 12-14 % and causing a huge economic decline amounting to more than $125 billion annually (Lu *et al.*, 2017; Postnikova *et al.*, 2015). Due to congenial environment condition under protected cultivation nematode multiply faster and attracts secondary infestation by fungal and bacterial pathogens causing disease complexes leading to enhanced crop losses. (Sharma *et al.*, 2008).

Development and cultivation of resistant cultivars is one of the available strategies to manage RKNs, which is non-chemical, most economical and environmentally safe and has been prioritized over chemical, biological, other cultural, and regulatory control components (Dharani *et al.*, 2019). Although, some novel synthetic chemicals have recently been put forward as nematicides, including fluopyram (Faske and Hurd, 2015), fluensulfone (Kearn*et al.*, 2014), tioxazafen (Slomczynska*et al.*, 2014), and fluazaindolizine (Lahm *et al.*, 2017), efficient management of the plant parasitic nematodes is still challenging. Intrinsic phyotoxic result of fluensulfone is also a matter of worry (Giannakou and Panopoulou, 2019). Continuous use of these synthetic chemical nematicides, often at higher than recommended rates lead to biomagnification, and environment deterioration due to their toxicity (Rao *et al.*, 2015). In this situation, avoiding the use of chemical nematicides, breeding for resistant crop cultivars and other cultural methods can limit the damage toll and less harmful to environment and other non-target organisms including human beings. Alternatively, integrated nematode management approaches which involve a combination of cultural, chemical, and biological methods could more efficiently regulate nematode populations (Sikora *et al.*, 2005). An important tool and key factor to the success of such control strategies is the careful selection and use of cultivars that suppress nematode populations and subsequently prevent yield losses of tomato production (Molinari, 2011). Thus, a comprehensive study was planned to evaluate and find the tomato genotypes resistant to root-knot nematode (*M. incognita*).

MATERIALS AND METHODS

Tomato genotypes

One hundred and seven tomato genotypes together with six susceptible commercial tomato cultivars (Table 1) were included in this study. Seeds were collected from the ICAR-NBPGR (National Bureau of Plant Genetic Resources), Regional station, Hyderabad, India,and ICAR-IARI-Centre for Protected Cultivation Technology (CPCT), New Delhi, Delhi, India.

Nematode culturing

The *M. incognita* population used in the study was originally collected from the heavily infected tomato plants at ICAR-IARI-CPCT, New Delhi, India. The identification of the species was done morphologically based on perineal patterns of mature females picked from galled roots of tomato (Jepson, 1987).The infected roots were washed and egg masses were removed with sterile forceps and kept for hatching using the modified Baermann method. Second stage juveniles (J_2s) were collected in the Petri plate containing water after 24 h. From infested soil samples, the juveniles were extracted by using Cobb's decanting and sieving technique (Cobb, 1918). Further, egg masses of uniform size were collected from the galled roots and inoculated (one egg mass/pot) into the root zones of susceptible Pusa Purple Long variety of brinjal and tomato cv. NS 4266. Pots were maintained in green house and growth chambers at 25-30 ºC with a photoperiod of 12 h. For laboratory and pot experiments, egg masses from heavily galled roots were handpicked and transferred to vial containing 0.5 % (v/v) sodium hypochlorite (NaOCl) and shaken for 3 min. The egg mass suspension was then passed through a series of filters with pore sizes of 74, 45 and 25 µm. Eggs that were retained on the 25 µm filter were collected with sterile distilled water (Hussey, 1973) and allowed to hatch in modified Baermann setup at 28 °C to get freshly hatched second stage juveniles (J_2s) which were used for subsequent experiments (Viglierchio and Schmitt, 1983).

Screening of tomato genotypes for *M. incognita* resistance in Pluronic gel

In vitro bioassay was conducted on Pluronic gel, a tri-block copolymer of poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) (Lippens *et al.*, 2013). Excellent gel transparency allows better monitoring of the plant-parasitic nematode infection process around the host radicular tissues under a microscope (Devaraja *et al.*, 2022). To make a 23% gel, 2 g of Pluronic F-127 powder (PF-127; Sigma-Aldrich) was added to 80 mL distilled water and allowed to dissolve with stirring for 24 h at 4°C. The dissolved gel was refrigerated at 4°C and aliquots were dispensed for the experiments (Wang *et al.*, 2009).

Seeds of 113 tomato genotypes were surface sterilized with 70% ethanol before soaking overnight in distilled water and put to germinate in a sterile Petri dish containing wet filter paper in a biological oxygen demand (BOD) incubator at 28±2°C. Seeds with well-developed radicle and plumule were used for bioassay in Pluronic gel medium. PF-127 (25-30 mL) was poured into each Petri dish (90 mm) and five germinated seeds were placed at equal distance on the medium. Approximately 10 J_2s of freshly hatched *M. incognita* were inoculated at the root tip of each seedling and allowed to set at room temperature. The covered Petri plates were placed in BOD incubator at 28 ± 2 °C, 30 ± 2 °C and 32 ± 2 °C (photoperiod 12 h). Five plants/genotype/plate was evaluated and repeated twice. Plantlets were harvested at 7 days post inoculation (dpi) from the gel by placing the Petri dishes briefly over an ice bath. The number of galls/ root was counted. Further, the roots were stained with acid fuchsin and destained using plain lactophenol (Southey, 1986; Byrd *et al*., 1983). The stained roots were observed under compound microscope and the number of juveniles inside the roots recorded.

Screening of tomato genotypes for *M. incognita* resistance in pots

After the initial screening of 113 genotypes in the Pluronic gel medium, pot experiment was conducted to know the response of only 99 genotypes out of 113 due to poor germination and availability of seed material. 21 days old tomato seedlings of each genotypes raised with standard nursery preparations protocols by using sterilized mixture, cocopeat: vermiculite: perlite (3:1:1) were transplanted into plastic pots (6 inches) containing steam sterilized soil. After one-week, nematodes were inoculated at 2 J_2s/cc of soil in the rhizosphere of each plant (Campos and Campos, 2005). Four replicated pots for each genotypes were arranged in completely randomized design. During the polyhouse experiments all agronomic practices like irrigation by drip at 3-4 days interval, weeding was done thrice throughout the crop period, nutrient management (N:P:K :: 19:19:19 at 3g/L, through fertigation at 2 months interval) and training of tomato plants after attaining particular stage was done. The average temperature during the pot and field experiments under protected cultivation was 32 ± 2 °C and the crop season was Kharif-Rabi.

Screening of tomato genotypes for *M. incognita* resistance in naturally infested field plots

Twelve out of sixteen tomato genotypes recorded as moderately resistant to *M. incognita* in pot experiment were selected to investigate their response against *M. incognita* under naturally infested polyhouse beds.Beds were selected based on the initial nematode population density (5-6 J_2s/cc soil) of *M. incognita* present in the soil were assessed using Cobb's decanting and sieving technique (Cobb, 1918). Each beds were made into different blocks of 10 m^2 size to allocate the different treatments comprising of genotypes (21 days old seedlings) in a randomized complete block design with five replications. The planting distance of 60 cm x 60 cm was maintained in each block having 14 plants in an area of 10 m^2. Each block had 2 rows 7 plants in each row. Distance between each blocks was 0.5 m.

In both the pot and field experiments the observations on number of galls/root system, galling index, number of egg masses/root system, nematode reproduction factor [$RF=P_f/P_i$, P_f=final nematode population and P_i=initial nematode population in soil], grouping of genotypes according to the level of their resistance or susceptibility at the time of harvest was done. The whole root system was rated for galling and egg mass presence on a 0 to 5 where, 0 = no gall or egg masses, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 = >100 galls or egg masses per root system scale (Taylor and Sasser, 1978).

Experimental designs and statistical analysis

The experiments were carried out using completely randomized and randomized block designs in controlled pot and soil beds, respectively under polyhouse conditions. The experimental data obtained from the field experiment was statistically analyzed by using Web Agri Stat Package (WASP) version 2.0 (at 5 %.). Multivariate neighbour joining cluster analysis was performed by PAST version 4.03 software.

RESULTS AND DISCUSSION

Screening of tomato genotypes for *M. incognita* resistance in Pluronic gel

A total of 113 genotypes were evaluated for gall formation and juvenile penetration at different temperatures (28±2 °C, 30±2 °C and 32±2 °C) in PF-127 medium. The susceptible control NS-4266 showed highest in gall formation and juvenile penetration across all temperatures when compared to all tomato genotypes (Table 1). Out of 113 genotypes screened, data in the Table. 1 revealed that there was no gall formation in 8 genotypes at 28±2 °C (EC-5, EC-4, EC-36, EC-3, EC-67, EC-57, EC-60 and G-43) followed by 9 genotypes at 30±2 °C (EC-5, EC-4, G-40, G-60, G-26, EC-67, EC-57, EC-59 and EC-60) and 12 genotypes at 32±2 °C (EC-5, EC-4, EC-36, EC-3, EC-67, EC-57, EC-59, EC-56, EC-60, G-20, G-26 and G-40). Maximum gall formation was recorded in the susceptible tomato cv. NS-4266 (4.60 galls/root) at 28±2 °C.Similarly with respect to juvenile penetration study (Table 1) there was least penetration (0-8 J_2s/gall) recorded in 7 genotypes at 28±2 °C (EC-5, EC-4, EC-36, EC-3, EC-57, EC-60 and G-43) followed by 11 genotypes at 30±2 °C (G-60, G-40, G-26, EC-5, G-19, G-32, G-53, EC-59, EC-56, EC-60 and G-21) and 18 genotypes at 32±2 °C (EC-3, G-60, G-40, G-26, G-16, EC-5, EC-67, EC-60, EC-59, EC-45, EC-4, EC-57, EC-63, EC-56, EC-20, G-12, EC-36 and EC-62). Maximum juvenile penetration was recorded in the susceptible tomato cv. Pusa cherry (3.80 J_2s/gall) at 28±2 °C.The data obtained in this study clearly showed that the abiotic factor, temperature is one of the significant factor to be considered while screening the tomato genotypes to *M. incognita* resistance. There was a significant variation in the gall formation and juvenile penetration across all genotypes tested at different temperatures. Out of 113 genotypes screened, 8 genotypes (28±2 °C), 9 genotypes (30±2 °C) and 12 genotypes (32±2 °C) were recorded no gall formation. Similarly, in penetration study, there was least J_2s penetration (0-8 J_2s/gall) observed in 7 genotypes (28±2 °C), 11 genotypes (30±2 °C) and 18 genotypes (32±2 °C). This variation in gall formation and J_2s penetration clearly indicates that the temperature is one of the most important influential factor in response to *M. incognita* infection in tomato plants. This method of screening using PF-127 medium can be named as early detection method which is simple, and the susceptible genotypes can be easily identified within a week after inoculation of infective juvenile's (J_2s). This method can only be used to separate the genotypes based on the gall formation prior conventional pot screening as well as polyhouse screening in the infected soil.

There are two types of resistance mechanisms for RKN in plants have been reported, at pre-infection stage due to presence of toxic or antagonistic chemicals in root tissue, which avoid the penetration of root knot nematodes into the roots (Haynes and Jones, 1976; Bendezu and Starr, 2003) while in post-infection, nematodes enter into the roots but fail to develop further which is often allied with an early hypersensitive reaction owing to the death of the cell in root tissue around the nematode. This mechanism avoids the formation of a feeding site leading to resistance (Dropkin, 1969; Williamson, 1999).Ornat*et al*. (2001) studied the reproductive potential of *M. arenaria,M. incognita* and *M. javanica* on resistant tomato cultivars and found that *M. javanica* populations had a higher reproduction rate on resistant tomato cultivars than those of *M. incognita* and *M. arenaria*. The use of host plant resistance is well-thought-out to be the most imperative practically viable and economically acceptable key to combat nematode incidence (Atkinson *et al*., 2003). There was a loss of efficacy of the resistance trait due to variations in the soil and surrounding environment temperature is one of the major limiting factor of using nematode-resistant genotypes (Devran*et al*., 2010; Newton *et al*., 2012).

Table 1. Response of tomato genotypes to the *M. incognita* infection at different temperature

Tomato genotypes	28±2 °C		30±2 °C		32±2 °C	
	No. of galls Mean ± SE	**No. of J_2s penetrated Mean ± SE**	**No. of galls Mean ± SE**	**No. of J_2s penetrated Mean ± SE**	**No. of galls Mean ± SE**	**No. of J_2s penetrated Mean ± SE**
EC9	1.20 ± 0.20	1.80 ± 0.37	1.00 ± 0.00	2.00 ± 0.32	1.00 ± 0.00	2.00 ± 0.00
EC42	0.60 ± 0.24	1.00 ± 0.32	1.60 ± 0.40	1.40 ± 0.24	1.20 ± 0.20	1.20 ± 0.20
EC43	1.40 ± 0.24	2.00 ± 0.32	1.00 ± 0.00	1.40 ± 0.24	0.60 ± 0.40	1.00 ± 0.32
EC45	1.60 ± 0.24	1.80 ± 0.37	1.80 ± 0.37	2.00 ± 0.00	0.80 ± 0.37	0.40 ± 0.24
EC46	0.60 ± 0.24	1.80 ± 0.37	1.80 ± 0.20	2.40 ± 040	0.80 ± 0.20	2.00 ± 0.32
EC21	0.80 ± 0.20	1.20 ± 0.20	1.80 ± 0.20	2.20 ± 0.37	1.80 ± 0.20	2.60 ± 0.24
EC8	1.80 ± 0.20	1.80 ± 0.37	2.00 ± 0.00	1.40 ± 0.24	1.80 ± 0.37	1.40 ± 0.24
EC28	1.60 ± 0.24	1.80 ± 0.37	1.60 ± 0.24	2.00 ± 0.45	2.20 ± 0.20	1.60 ± 0.24
EC19	1.60 ± 0.24	2.00 ± 0.32	1.20 ± 0.37	2.20 ± 0.20	1.80 ± 0.37	2.20 ± 0.20
EC30	0.80 ± 0.20	1.60 ± 0.40	2.80 ± 0.20	2.00 ± 0.32	1.60 ± 0.40	1.60 ± 0.24
EC22	2.00 ± 0.45	1.60 ± 0.24	1.40 ± 0.24	2.60 ± 0.24	2.40 ± 0.24	1.80 ± 0.37
EC32	1.60 ± 0.40	2.00 ± 0.45	1.60 ± 0.24	2.40 ± 0.24	2.20 ± 0.37	2.40 ± 0.24
EC33	1.80 ± 0.37	2.20 ± 0.20	2.60 ± 0.24	1.60 ± 0.24	1.80 ± 0.49	1.60 ± 0.24
EC27	1.20 ± 0.20	2.20 ± 0.37	2.20 ± 0.37	1.80 ± 0.37	1.00 ± 0.45	1.00 ± 0.00
EC20	0.80 ± 0.20	2.00 ± 0.32	2.00 ± 0.32	2.20 ± 0.20	0.80 ± 0.37	0.80 ± 0.37
EC24	1.40 ± 0.24	1.80 ± 0.20	1.00 ± 0.00	2.00 ± 0.32	1.80 ± 0.37	1.60 ± 0.24
EC6	2.20 ± 0.37	1.20 ± 0.20	1.60 ± 0.24	2.20 ± 0.20	1.20 ± 0.20	1.80 ± 0.20
EC31	1.80 ± 0.37	1.60 ± 0.24	1.60 ± 0.24	1.80 ± 0.37	1.20 ± 0.20	2.00 ± 0.00
EC13	3.00 ± 0.32	1.40 ± 0.24	1.40 ± 0.40	1.80 ± 0.37	2.00 ± 0.32	1.80 ± 0.20
EC5	0.00 ± 0.00	0.40 ± 0.24	0.00 ± 0.00	0.40 ± 0.24	0.00 ± 0.00	0.20 ± 0.20
EC38	1.40 ± 0.24	2.00 ± 0.00	1.40 ± 0.24	2.60 ± 0.24	1.60 ± 0.24	1.60 ± 0.24
EC17	0.40±0.24	1.00 ± 0.45	1.40 ± 0.24	2.40 ± 0.24	1.00 ± 0.00	1.40 ± 0.24
EC20	0.80 ± 0.20	1.00 ± 0.32	2.00 ± 0.32	2.20 ± 0.20	2.40 ± 0.24	1.60 ± 0.24
EC35	0.60 ± 0.24	1.40 ± 0.40	1.60 ± 0.51	2.00 ± 0.32	2.40 ± 0.24	1.60 ± 0.24
EC4	0.00 ± 0.00	0.60 ± 0.24	0.00 ± 0.00	1.20 ± 0.20	0.00 ± 0.00	0.40 ± 0.24
EC36	0.00 ± 0.00	0.80 ± 0.20	1.80 ± 0.37	2.00 ± 0.00	0.00 ± 0.00	0.80 ± 0.20
EC3	0.00 ± 0.00	0.80 ± 0.20	1.60 ± 0.40	1.40 ± 0.24	0.00 ± 0.00	0.00 ± 0.00
EC68	2.20 ± 0.37	1.80 ± 0.20	1.40 ± 0.24	2.40 ± 0.24	2.40 ± 0.24	1.40 ± 0.24
EC67	0.00 ± 0.00	1.00 ± 0.32	0.00 ± 0.00	1.40 ± 0.24	0.00 ± 0.00	0.20 ± 0.20
EC69	0.80 ± 0.20	1.60 ± 0.24	0.80 ± 0.20	1.80 ± 0.20	2.00 ± 0.32	1.80 ± 0.20
EC35	1.60 ± 0.24	1.40 ± 0.40	1.20 ± 0.20	1.80 ± 0.20	1.80 ± 0.37	2.20 ± 0.20
EC51	4.00 ± 0.32	1.60 ± 0.24	0.80 ± 0.20	1.80 ± 0.20	1.60 ± 0.40	2.40 ± 0.24
EC61	0.60 ± 0.24	1.60 ± 0.24	0.80 ± 0.20	1.60 ± 0.24	1.40 ± 0.24	2.40 ± 0.40
EC53	2.20 ± 0.37	1.40 ± 0.24	0.80 ± 0.20	1.60 ± 0.24	2.00 ± 0.45	1.60 ± 0.24
EC58	1.40 ± 0.24	2.00 ± 0.32	1.60 ± 0.24	2.40±0.24	2.00 ± 0.55	2.00 ± 0.32
EC49	0.60 ± 0.24	1.20 ± 0.20	1.00 ± 0.32	2.40 ± 0.51	2.00 ± 0.32	2.20 ± 0.37
EC70	1.20 ± 0.37	2.00 ± 0.32	1.00 ± 0.00	2.40 ± 0.24	0.80 ± 0.37	1.80 ± 0.20
EC66	0.40 ± 0.24	1.20 ± 0.37	0.80 ± 0.20	2.00 ± 0.32	1.40 ± 0.24	1.60 ± 0.40

Tomato genotypes	28±2 °C		30±2 °C		32±2 °C	
	No. of galls Mean ± SE	No. of J_2s penetrated Mean ± SE	No. of galls Mean ± SE	No. of J_2s penetrated Mean ± SE	No. of galls Mean ± SE	No. of J_2s penetrated Mean ± SE
EC57	0.00 ± 0.00	0.20 ± 0.20	0.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.40 ± 0.24
EC64	1.60 ± 0.24	1.60 ± 0.24	0.60 ± 0.24	1.80 ± 0.37	1.20 ± 0.37	1.20 ± 0.37
EC65	1.40 ± 0.24	1.60 ± 0.24	1.40 ± 0.24	2.00 ± 0.32	0.60 ± 0.24	1.40 ± 0.24
EC63	2.80 ± 0.20	1.80 ± 0.20	1.00 ± 0.32	1.80 ± 0.20	0.40 ± 0.24	0.60 ± 0.24
EC62	1.40 ± 0.24	2.00 ± 0.00	0.80 ± 0.20	2.40 ± 0.24	0.40 ± 0.24	0.80 ± 0.20
EC16	1.80 ± 0.20	1.60 ± 0.24	2.80 ± 0.37	2.00 ± 0.45	1.40 ± 0.24	1.00 ± 0.55
EC18	1.20 ± 0.20	2.20 ± 0.20	0.80 ± 0.20	2.20 ± 0.20	0.60 ± 0.24	1.60 ± 0.40
EC59	1.80 ± 0.37	1.40 ± 0.24	0.00 ± 0.00	0.80 ± 0.20	0.00 ± 0.00	0.20 ± 0.20
EC56	1.20 ± 0.20	2.00 ± 0.00	0.40 ± 0.24	0.80 ± 0.37	0.00 ± 0.00	0.60 ± 0.24
EC40	1.80 ± 0.20	1.60 ± 0.24	1.20 ± 0.20	1.40 ± 0.40	0.80 ± 0.20	1.40 ± 0.51
EC52	1.40 ± 0.24	2.40 ± 0.24	1.20 ± 0.37	1.80 ± 0.20	1.60 ± 0.60	1.20 ± 0.37
EC60	0.00 ± 0.00	0.80 ± 0.37	0.00 ± 0.00	0.80 ± 0.20	0.00 ± 0.00	0.20 ± 0.20
IC-A	3.00 ± 0.32	1.60 ± 0.24	1.80 ± 0.20	2.20 ± 0.37	1.60 ± 0.40	2.00 ± 0.00
IC-B	1.40 ± 0.24	2.00 ± 0.32	1.60 ± 0.40	1.80 ± 0.20	0.80 ± 0.20	1.20 ± 0.20
IC-C	1.60 ± 0.24	1.60 ± 0.24	2.20 ± 0.37	1.80 ± 0.37	2.40 ± 0.40	1.60 ± 0.40
IC-D	0.60 ± 0.24	1.80 ± 0.20	1.80 ± 0.20	2.20 ± 0.37	1.60 ± 0.51	1.60 ± 0.24
G30	1.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.32	1.80 ± 0.20	3.40 ± 0.24	1.20 ± 0.20
G13	3.00 ± 0.32	4.20 ± 0.20	1.40 ± 0.24	1.20 ± 0.20	2.40 ± 0.24	2.00 ± 0.00
G39	2.20 ± 0.37	3.60 ± 0.24	2.00 ± 0.32	1.80 ± 0.20	3.00 ± 0.45	1.40 ± 0.24
G3	1.40 ± 0.24	2.20 ± 0.20	1.40 ± 0.40	1.20 ± 0.20	1.80 ± 0.37	2.00 ± 0.00
G14	3.40 ± 0.75	2.40 ± 0.24	1.20 ± 0.20	1.80 ± 0.20	2.00 ± 0.45	2.60 ± 0.24
G34	2.20 ± 0.20	1.80 ± 0.20	1.20 ± 0.20	2.00 ± 0.00	2.20 ± 0.58	1.00 ± 0.00
G19	3.00 ± 0.32	1.80 ± 0.20	0.40 ± 0.24	0.60 ± 0.40	2.80 ± 0.58	1.80 ± 0.20
G42	1.80 ± 0.58	2.20 ± 0.20	1.40 ± 0.24	2.00 ± 0.00	2.00 ± 0.45	1.80 ± 0.20
G25	2.00 ± 0.63	2.40 ± 0.24	1.20 ± 0.20	1.80 ± 0.20	0.80 ± 0.37	2.20 ± 0.20
G57	1.80 ± 0.37	1.80 ± 0.20	1.00 ± 0.00	1.00 ± 0.00	2.00 ± 0.45	1.80 ± 0.20
G15	1.20 ± 0.20	2.00 ± 0.00	1.00 ± 0.00	2.00 ± 0.00	1.20 ± 0.37	1.00 ± 0.00
G61	1.80 ± 0.37	2.40 ± 0.24	1.00 ± 0.00	1.80 ± 0.20	1.80 ± 0.37	1.20 ± 0.20
G2	2.60 ± 0.24	2.40 ± 0.40	1.80 ± 0.37	1.60 ± 0.24	2.20 ± 0.20	1.80 ± 0.20
G24	2.20 ± 0.49	1.80 ± 0.20	1.00 ± 0.00	1.60 ± 0.24	3.20 ± 0.37	1.80 ± 0.20
G35	1.40 ± 0.24	1.60 ± 0.24	1.20 ± 0.20	1.00 ± 0.00	2.40 ± 0.24	1.60 ± 0.24
G9	2.60 ± 0.24	1.40 ± 0.24	1.80 ± 0.66	1.20 ± 0.37	2.40 ± 0.24	1.40 ± 0.24
G60	2.60 ± 0.24	1.40 ± 0.40	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
G21	3.60 ± 0.40	1.80 ± 0.20	1.00 ± 0.00	0.80 ± 0.20	2.20 ± 0.37	1.20 ± 0.20
G4	2.20 ± 0.37	1.60 ± 0.24	1.00 ± 0.00	1.00 ± 0.00	2.00 ± 0.45	2.20 ± 0.20
G50	2.20 ± 0.37	1.20 ± 0.20	2.00 ± 0.63	1.40 ± 0.24	3.60 ± 0.24	1.80 ± 0.20
G38	2.80 ± 0.58	2.00 ± 0.32	1.20 ± 0.20	1.20 ± 0.20	2.00 ± 0.32	2.20 ± 0.20
G20	1.80 ± 0.37	2.00 ± 0.32	1.00 ± 0.00	1.40 ± 0.24	0.80 ± 0.37	2.00 ± 0.32
G01	3.00 ± 0.45	1.80 ± 0.20	0.60 ± 0.24	1.20 ± 0.20	1.20 ± 0.58	1.20 ± 0.20

Tomato genotypes	**28±2 °C**		**30±2 °C**		**32±2 °C**	
	No. of galls Mean ± SE	**No. of J_2s penetrated Mean ± SE**	**No. of galls Mean ± SE**	**No. of J_2s penetrated Mean ± SE**	**No. of galls Mean ± SE**	**No. of J_2s penetrated Mean ± SE**
G17	2.20 ± 0.37	2.20 ± 0.37	1.00 ± 0.00	1.60 ± 0.24	1.60 ± 0.24	1.40 ± 0.24
G32	1.20 ± 0.20	1.80 ± 0.20	0.40 ± 0.24	0.60 ± 0.40	1.80 ± 0.37	1.80 ± 0.20
G26	3.60 ± 0.24	2.00 ± 0.32	0.00 ± 0.00	0.20 ± 0.20	0.00 ± 0.00	0.00 ± 0.00
G45	2.20 ± 0.58	1.80 ± 0.20	1.20 ± 0.20	1.40 ± 0.24	2.20 ± 0.37	1.20 ± 0.20
G43	0.00 ± 0.00	0.20 ± 0.20	1.00 ± 0.32	1.40 ± 0.24	1.80 ± 0.20	1.80 ± 0.20
G27	2.20 ± 0.20	1.60 ± 0.24	1.40 ± 0.24	1.60 ± 0.24	3.20 ± 0.66	1.80 ± 0.20
G22	1.20 ± 0.20	2.00 ± 0.00	1.40 ± 0.24	2.00 ± 0.45	3.40 ± 0.68	1.60 ± 0.24
G29	3.00 ± 0.55	2.00 ± 0.32	1.20 ± 0.20	1.80 ± 0.20	1.80 ± 0.37	1.20 ± 0.20
G37	1.80 ± 0.37	1.60 ± 0.24	1.20 ± 0.20	1.20 ± 0.20	2.00 ± 0.45	1.40 ± 0.24
G12	2.80 ± 0.20	1.80 ± 0.20	2.00 ± 0.00	2.20 ± 0.20	0.80 ± 0.37	0.80 ± 0.20
G51	2.00 ± 0.32	2.00 ± 0.32	0.40 ± 0.24	1.60 ± 0.24	1.80 ± 0.37	1.80 ± 0.20
G16	2.20 ± 0.49	1.80 ± 0.20	1.40 ± 0.24	2.60 ± 0.24	0.60 ± 0.40	0.00 ± 0.00
G54	1.40 ± 0.24	2.20 ± 0.37	1.00 ± 0.00	2.20 ± 0.20	1.80 ± 0.37	2.00 ± 0.00
G23	2.60 ± 0.68	2.00 ± 0.32	1.40 ± 0.24	2.60±0.24	2.80 ± 0.37	1.00 ± 0.00
G53	3.00 ± 0.32	2.00 ± 0.00	0.60 ± 0.24	0.80 ± 0.37	2.80 ± 0.37	2.20 ± 0.20
G49	1.00 ± 0.00	1.80 ± 0.37	0.60 ± 0.24	1.00 ± 0.45	1.00 ± 0.00	1.00 ± 0.00
G40	1.40 ± 0.24	1.60 ± 0.24	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
G44	2.00 ± 0.32	1.00 ± 0.00	1.40 ± 0.24	1.60 ± 0.40	2.20 ± 0.20	1.40 ± 0.24
G18	2.00 ± 0.00	1.60 ± 0.24	2.20 ± 0.86	1.40 ± 0.40	1.80 ± 0.37	1.60 ± 0.24
G33	1.80 ± 0.20	1.60 ± 0.40	0.40 ± 0.24	1.00 ± 0.00	1.60 ± 0.24	1.80 ± 0.20
G07	1.00 ± 0.00	1.20 ± 0.20	2.00 ± 0.45	1.40 ± 0.24	2.80 ± 0.20	1.80 ± 0.20
G36	3.00 ± 0.45	1.60 ± 0.24	2.40 ± 0.24	1.40 ± 0.24	1.80 ± 0.20	1.80 ± 0.20
G10	3.60 ± 0.51	1.80 ± 0.20	3.20 ± 0.20	1.80 ± 0.20	1.80 ± 0.37	1.60 ± 0.24
G11	3.40 ± 0.81	1.80 ± 0.20	1.00 ± 0.00	2.20 ± 0.20	0.60 ± 0.24	1.20 ± 0.20
G06	2.20 ± 0.49	1.00 ± 0.00	1.40 ± 0.24	2.00 ± 0.00	2.00 ± 0.32	1.80 ± 0.20
G55	3.00 ± 0.71	2.40 ± 0.24	0.80 ± 0.20	1.40 ± 0.24	0.80 ± 0.37	1.80 ± 0.20
G08	2.00 ± 0.32	2.60 ± 0.24	1.80 ± 0.37	1.80 ± 0.20	1.00 ± 0.32	1.00 ± 0.00
G59	3.00 ± 0.32	1.80 ± 0.37	1.40 ± 0.40	1.60 ± 0.24	1.40 ± 0.24	1.60 ± 0.24
G28	2.60 ± 0.24	1.60 ± 0.24	0.80 ± 0.37	2.40 ± 0.24	1.80 ± 0.20	1.80 ± 0.20
G46	1.40 ± 0.24	2.00 ± 0.00	1.00 ± 0.32	2.40 ± 0.24	2.80 ± 0.58	1.60 ± 0.24
NS 4266	4.60 ± 0.24	3.20 ± 0.20	3.00 ± 0.32	2.40 ± 0.24	4.40 ± 0.40	2.80 ± 0.20
Abhilash	2.80 ± 0.20	3.40 ± 0.40	1.60 ± 0.24	2.40 ± 0.40	2.60 ± 0.24	3.20 ± 0.37
F1.T.H (CRQ)	2.20 ± 0.37	2.40 ± 0.24	2.40 ± 0.24	3.20 ± 0.37	2.40 ± 0.24	2.00 ± 0.00
Arka Rakshak	2.80 ± 0.20	3.40 ± 0.24	1.60 ± 0.24	2.60 ± 0.24	2.40 ± 0.24	1.40 ± 0.24
PCT	2.80 ± 0.20	3.80 ± 0.20	1.20 ± 0.20	2.40 ± 0.24	3.00 ± 0.32	1.40 ± 0.24
TR-48	3.60 ± 0.24	2.00 ± 0.32	2.80 ± 0.20	2.60 ± 0.40	3.20 ± 0.37	2.80 ± 0.20

Data shown correspond to the mean of five replicates ± SE. SE=Standard error

Screening of tomato genotypes for *M. incognita* resistance in pots and naturally infested field condition

Ninety nine tomato genotypes were screened in pot experiment under polyhouse condition to investigate the response of tomato genotypes against *M. incognita*. Experimental results (Table 2) revealed that, in reference to the gall index, 16 genotypes were recorded moderately resistant, 81 genotypes as susceptible, and 2 genotypes were highly susceptible. The number of galls were maximum in NS-4266 (111.25 galls/root) and Arka Rakshak (137.25 galls/root) which were included as positive controls followed by 81 genotypes which were susceptible to *M. incognita* with gall index of 4. Out of 16 lines which were showed moderately resistant, G-46 recorded maximum gall formation (29.75 galls/root) with 22.00 egg masses/root and RF of 0.87. Minimum gall formation, egg masses/root and reproduction factor were recorded in moderately resistant genotype, EC-38 (19.50 galls/root, 11.00 egg masses/root and RF of 0.32).

About 12 tomato genotypes recorded moderately resistant to *M. incognita* in pot experiment under polyhouse condition were selected to investigate their response under naturally infested polyhouse beds. Experimental results (Table 3) revealed that, in reference to the gall index, 5 genotypes (G-21, G-28, G-35, G-40 and G-92) were recorded moderately resistant, 7 genotypes previously showed moderately resistant in pot experiment were become susceptible in naturally infested polyhouse beds due to the more nematode population density (5-6 J_2s/cc soil) in the polyhouse beds. The number of galls were maximum in the tomato cv, NS-4266 (246.50 galls/root system), which were classified as highly susceptible followed by 7 susceptible genotypes (gall index of 4). Out of 5 genotypes which showed moderately resistant, G-35 recorded maximum gall formation (27.50 galls/root system) with 16.75 egg masses/root and reproduction factor of 0.66. Minimum gall formation, egg masses/root and RF were recorded in moderately resistant genotype, G-92 (24.00 galls/root system, 12.50 egg masses/root and RF of 0.80).

The reproduction of *M. javanica* populations on resistant tomato cultivars, together with the fact that *M. javanica* is one of the most common root-knot nematode species in many tomato growing areas (Ornat*et al*., 2001) raise concerns about the durability of the resistance mediated by the *Mi* gene in tomato cultivars and rootstocks. Resistance governed by *Mi* is lost at temperatures beyond 28°C (Dropkin, 1969). Repeated cultivation of resistant tomato cultivars or rootstocks may lead to the selection of virulent nematode populations (Verdejo-Lucas *et al*., 2009). Our results are in conformity with the Dharani *et al*. (2019) where they evaluated 30 tomato accessions for resistance against *M. incognita* under lab and glasshouse conditions. Among 30 accessions two accessions (EC 631364, EC 164863) were identified as highly resistant and 8 accessions (EC 620394, EC 617051, EC 620288, EC 145615, EC 636874, EC 151568, EC 163606, EC 620498) were identified as resistant. Similarly, Guleria *et al*. (2020) screened 51 tomato germplasm against *M. incognita* in net house condition out of 51, 3 genotypes, EC 620394, EC 620427 and EC 617047 were recorded resistant having 1.1 to 2.0 root gall index. Ten exhibited moderately resistant reaction having root gall index between 2.1 to 3.0. Among the remaining genotypes, 20 were found susceptible showing root gall index between 3.1 to 4.0 and 18 lines were highly susceptible having root gall index between 4.1 to 5.0.

Our data revealed the potential of several tomato genotypes as new tool for effective management of *M. incognita*. The temperature impacted the efficacy of *M. incognita* resistance in the tomato genotypes screened in PF-127 medium. The genotypes, G21, G28, G35, G40 and G92 recorded moderately resistant response when exposed to high population density (5-6 J2s/cc soil) in severely infected polyhouse field plots can be used for

Table 2. Response of tomato genotypes to *M. incognita* infection in pots under polyhouse condition

Tomato genotypes	No. of galls/root (Mean ± SE)	Gall index	Resistance reaction	No. of egg masses/root (Mean ± SE)	RF (Mean ± SE)
EC67	35.50 ± 1.71	4	S	23.00 ± 1.78	0.39 ± 0.10
EC56	52.75 ± 1.38	4	S	31.50 ± 0.96	0.56 ± 0.14
EC64	60.75 ± 1.93	4	S	28.75 ± 0.95	0.59 ± 0.15
EC35	45.75 ± 2.06	4	S	21.50 ± 0.65	0.57 ± 0.15
EC51	36.25 ± 1.25	4	S	24.00 ± 1.22	0.55 ± 0.14
EC53	43.75 ± 1.11	4	S	22.25 ± 0.48	0.42 ± 0.11
EC69	54.00 ± 1.08	4	S	33.00 ± 1.22	0.56 ± 0.14
EC66	60.25 ± 1.11	4	S	29.25 ± 1.93	0.49 ± 0.12
EC59	61.25 ± 0.63	4	S	38.50 ± 1.19	0.63 ± 0.16
EC57	59.75 ± 1.38	4	S	32.00 ± 2.04	0.79 ± 0.20
EC55	52.00 ± 0.91	4	S	27.50 ± 1.55	1.17 ± 0.30
EC61	60.25 ± 0.48	4	S	43.50 ± 2.47	0.96 ± 0.24
EC21	70.75 ± 0.85	4	S	42.0 ± 1.35	0.85 ± 0.22
EC45	64.75 ± 0.75	4	S	47.50 ± 3.30	0.65 ± 0.17
EC9	60.25 ± 0.48	4	S	32.50 ± 1.94	0.86 ± 0.22
EC40	46.25 ± 0.48	4	S	28.00 ± 1.47	1.29 ± 0.34
EC30	48.00 ± 3.76	4	S	51.00 ± 0.41	0.96 ± 0.24
EC17	51.00 ± 0.71	4	S	41.75 ± 0.75	1.24 ± 0.32
EC42	63.50 ± 1.26	4	S	51.00 ± 0.41	0.95 ± 0.24
EC28	63.25 ± 1.44	4	S	53.50 ± 1.44	1.24 ± 0.32
EC24	72.50 ± 0.65	4	S	60.00 ± 2.35	1.02 ± 0.26
EC33	35.00 ± 1.83	4	S	22.75 ± 1.18	1.18 ± 0.30
EC32	34.75 ± 1.93	4	S	21.25 ± 2.02	0.54 ± 0.14
EC27	43.25 ± 1.31	4	S	24.75 ± 1.49	0.63 ± 0.16
EC16	37.50 ± 0.65	4	S	27.75 ± 1.97	0.39 ± 0.10
EC31	27.25 ± 2.36	3	MR	18.50 ± 1.04	0.30 ± 0.08
EC20	25.25 ± 0.85	3	MR	19.25 ± 0.85	0.50 ± 0.13
EC68	28.75 ± 0.63	3	MR	16.00 ± 1.08	0.53 ± 0.14
EC38	19.50 ± 0.96	3	MR	11.00 ± 0.82	0.32 ± 0.08
EC46	23.50 ± 1.04	3	MR	10.00 ± 1.08	0.52 ± 0.13
EC65	33.00 ± 0.71	4	S	21.50 ± 0.65	0.42 ± 0.11
EC43	23.50 ± 2.02	3	MR	12.75 ± 1.65	0.27 ± 0.07

Tomato genotypes	No. of galls/root (Mean ± SE)	Gall index	Resistance reaction	No. of egg masses/root (Mean ± SE)	RF (Mean ± SE)
EC57	57.50 ± 0.65	4	S	36.75 ± 1.65	0.54 ± 0.14
EC5	26.75 ± 1.25	3	MR	18.25 ± 1.80	0.45 ± 0.12
EC60	69.25 ± 0.85	4	S	50.00 ± 3.34	0.41 ± 0.12
EC53	47.00 ± 0.82	4	S	34.50 ± 1.71	0.34 ± 0.09
EC58	53.25 ± 1.65	4	S	33.75 ± 3.15	0.57 ± 0.15
EC18	64.00 ± 1.78	4	S	38.75 ± 2.17	0.62 ± 0.16
EC8	71.25 ± 0.48	4	S	50.75 ± 3.01	0.37 ± 0.10
EC6	63.75 ± 2.87	4	S	39.25 ± 2.69	0.54 ± 0.14
IC-A	45.00 ± 0.91	4	S	30.75 ± 0.85	0.62 ± 0.16
IC-B	60.75 ± 1.93	4	S	45.25 ± 1.49	0.52 ± 0.13
IC-C	63.25 ± 2.25	4	S	45.75 ± 1.93	0.61 ± 0.15
IC-D	58.00 ± 1.47	4	S	48.50 ± 1.55	1.15 ± 0.31
G07	42.50 ± 1.71	4	S	30.75 ± 0.85	1.06 ± 0.27
G08	33.00 ± 1.41	4	S	26.50 ± 2.10	0.60 ± 0.15
G09	42.50 ± 1.71	4	S	41.75 ± 1.80	0.81 ± 0.20
G10	42.00 ± 1.29	4	S	34.00 ± 1.29	0.68 ± 0.17
G11	32.50 ± 1.71	4	S	24.75 ± 1.55	0.94 ± 0.24
G12	32.00 ± 0.58	4	S	18.00 ± 1.47	0.68 ± 0.17
G14	43.75 ± 1.25	4	S	23.25 ± 1.31	0.53 ± 0.13
G15	46.50 ± 1.89	4	S	28.75 ± 1.11	0.70 ± 0.18
G16	51.25 ± 0.85	4	S	29.00 ± 1.87	0.64 ± 0.16
G17	33.00 ± 1.58	4	S	26.00 ± 1.08	0.53 ± 0.13
G18	34.25 ± 1.03	4	S	31.00 ± 0.71	0.33 ± 0.09
G20	71.75 ± 2.17	4	S	54.25 ± 2.29	0.47 ± 0.12
G21	28.75 ± 1.11	3	MR	31.75 ± 1.11	0.89 ± 0.23
G22	42.50 ± 2.22	4	S	26.75 ± 1.89	1.20 ± 0.30
G23	45.25 ± 2.66	4	S	32.25 ± 1.49	1.14 ± 0.29
G24	26.75± 2.46	3	MR	19.00 ± 1.08	0.42 ± 0.11
G25	32.50 ± 1.32	4	S	24.75 ± 1.80	0.56 ± 0.14
G26	50.00 ± 1.29	4	S	31.75 ± 1.11	1.19 ± 0.30
G27	40.00 ± 1.29	4	S	26.00 ± 1.47	1.11 ± 0.29
G28	22.50 ± 1.71	3	MR	12.25 ± 1.49	1.31 ± 0.39
G29	31.50 ± 0.96	4	S	25.50 ± 2.33	0.88 ± 0.22

Tomato genotypes	**No. of galls/root (Mean ± SE)**	**Gall index**	**Resistance reaction**	**No. of egg masses/root (Mean ± SE)**	**RF (Mean ± SE)**
G30	26.50 ± 1.26	3	MR	20.25 ± 0.75	0.82 ± 0.21
G33	34.00 ± 1.73	4	S	27.75 ± 1.65	0.92 ± 0.24
G05	28.25 ± 2.14	3	MR	21.00 ± 0.41	0.63 ± 0.16
G34	31.50 ± 2.22	4	S	18.50 ± 1.55	0.91 ± 0.23
G35	22.25 ± 0.75	3	MR	12.75 ± 1.18	0.60 ± 0.15
G36	60.75 ± 0.85	4	S	39.25 ± 2.53	1.18 ± 0.30
G37	32.50 ± 1.71	4	S	19.25 ± 2.17	1.03 ± 0.26
G38	37.00 ± 1.47	4	S	24.00 ± 1.29	0.94 ± 0.25
G39	63.50 ± 1.76	4	S	39.75 ± 1.38	1.35 ± 0.34
G40	21.00 ± 0.82	3	MR	13.25 ± 1.03	0.89 ± 0.23
G42	41.25 ± 2.02	4	S	33.00 ± 1.47	1.08 ± 0.27
G43	40.75 ± 0.85	4	S	31.00 ± 0.82	0.81 ± 0.20
G44	50.25 ± 1.25	4	S	38.00 ± 1.08	1.23 ± 0.31
G45	44.50 ± 2.10	4	S	34.50 ± 1.55	1.17 ± 0.30
G46	29.75 ± 1.11	3	MR	22.00 ± 1.08	0.87 ± 0.22
G51	40.50 ± 1.66	4	S	29.00 ± 1.08	0.47 ± 0.12
G53	39.75 ± 3.50	4	S	25.00 ± 1.29	0.52 ± 0.13
G54	44.25 ± 1.11	4	S	22.75 ± 1.18	0.67 ± 0.17
G55	35.50 ± 1.19	4	S	24.25 ± 2.32	1.26 ± 0.32
G57	30.25 ± 1.25	4	S	20.25 ± 0.48	1.32 ± 0.33
G59	43.25 ± 1.65	4	S	27.00 ± 1.68	0.94 ± 0.24
G60	35.25 ± 1.55	4	S	26.75 ± 1.49	1.24 ± 0.31
G61	48.50 ± 2.63	4	S	32.75 ± 0.63	0.80 ± 0.20
G92	25.50 ± 1.55	3	MR	14.50 ± 1.71	1.06 ± 0.27
G01	42.25 ± 1.49	4	S	25.75 ± 1.70	1.27 ± 0.32
G02	30.50 ± 1.26	4	S	17.75 ± 1.70	1.02 ± 0.26
G03	56.00 ± 2.08	4	S	37.00 ± 1.22	1.34 ± 0.34
G04	55.25 ± 2.02	4	S	33.75 ± 2.50	1.18 ± 0.30
NS 4266	111.25 ± 1.11	5	HS	92.75 ± 4.59	1.52 ± 0.38
Abhilash	79.75 ± 2.29	4	S	69.00 ± 3.24	0.54 ± 0.14
F1.T.H (CRQ)	59.75 ± 2.69	4	S	41.50 ± 1.32	0.93 ± 0.24
Arka Rakshak	137.25 ± 4.35	5	HS	114.00 ± 4.14	1.27 ± 0.32
PCT	64.50 ± 1.32	4	S	49.50 ± 2.25	1.05 ± 0.27
TR-48	64.50 ± 2.33	4	S	46.25 ± 2.63	1.03 ± 0.26

Data shown correspond to the mean of four replicates ± SE. SE: Standard error, RF: Reproduction factor, S: Susceptible, MR: Moderately resistant, HS: Highly susceptible

Table 3. Response of tomato genotypes to the *M. incognita* infection under naturally infested protected cultivation

Tomato genotypes	No. of galls/root (Mean ± SE)	Gall index	Resistance reaction	No. of egg masses/root (Mean ± SE)	RF (Mean ± SE)
EC5	35.75 ± 2.29^{de}	4	S	25.00 ± 2.97^{de}	1.10 ± 0.06^{de}
EC31	52.25 ± 1.89^{bc}	4	S	30.25 ± 0.48^{cd}	1.28 ± 0.07^{c}
G2	57.50 ± 2.75^{bc}	4	S	36.25 ± 2.75^{c}	1.18 ± 0.05^{cd}
G21	24.75 ± 1.75^{e}	3	MR	15.25 ± 1.65^{ef}	0.57 ± 0.03^{g}
G24	49.50 ± 2.50^{bcd}	4	S	36.00 ± 2.12^{c}	1.01 ± 0.09^{e}
G28	24.75 ± 1.31^{e}	3	MR	17.00 ± 2.27^{ef}	0.64 ± 0.03^{fg}
G30	62.50 ± 1.55^{b}	4	S	49.25 ± 1.93^{b}	1.29 ± 0.04^{c}
G35	27.50 ± 1.55^{e}	3	MR	16.75 ± 0.85^{ef}	0.66 ± 0.05^{fg}
G40	25.00 ± 1.08^{e}	3	MR	13.75 ± 1.11^{f}	0.53 ± 0.08^{g}
G46	46.75 ± 2.17^{cd}	4	S	38.75 ± 2.02^{bc}	1.14 ± 0.03^{cde}
G57	55.75 ± 2.43^{bc}	4	S	39.50 ± 2.33^{bc}	1.60 ± 0.05^{b}
G92	24.00 ± 1.87^{e}	3	MR	12.50 ± 1.32^{f}	0.80 ± 0.09^{f}
NS 4266	246.50 ± 17.17^{a}	5	HS	152.50 ± 11.95^{a}	2.10 ± 0.01^{a}
F value	134.792		94.540	54.124	
CV	18.008		20.298	11.426	
CD ($P \leq 0.05$)	14.551		10.809	0.175	

Data shown correspond to the mean of four replicates ± SE.Means with the same alphabet letters on each columns are not significantly ($P < 0.05$) different. CD: Critical difference, CV: Coefficient of variation.SE: Standard error, RF: Reproduction factor, S: Susceptible, MR: Moderately resistant, HS: Highly susceptible

the identification of gene(s) involved in *M. incognita* resistance that can be used for the development of resistant tomato cultivars.

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Two Known Species of *Hemicriconemoides* (Nematoda: Criconematidae) from Aizawl District, Mizoram

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ABSTRACT: A survey was conducted in six different localities of Aizawl district i.e Zemabuak, Thumpui, Haulawng, Lungleng, Mizoram University campus and Aizawl with elevation ranging between 1132 to 1500 msl in December, 2018 and September, 2019. Soil samples were randomly collected from cultivated and forest cover area. The collected soil were processed through Cobb's sieving and decanting methods followed by Baerman's funnel method (Thorne,1961). Among the plant parasitic and predatory nematode collected *Hemicriconemoides mangiferae* (Siddiqi, 1961) and *Hemicriconemoides chitwoodi* (Esser, 1960) associated with vegetables crops and wild plants were found in large numbers. The morphometric description and data of *Hemicroconemoides mangiferae* (Siddiqi, 1961): Body length= 217.48-236.42 (224.65±6.04), R=127-132(130±1.83), V=90.15-91.65(90.7±0.49), Stylet=35.86-51.53 (39.6±5.35), Oesophagus = 42.69-54.26 (49.83±3.86), ABD= 8.91-13.30 (11.5±1.62), Tail=17.52-19.90 (18.75±0.86), shorter in body and stylet length when compared to the existing species and tail elongate and conoid. *Hemicriconemoides chitwoodi* (Esser, 1960): Body length=450.39-491-54 (472.21±15.58), R= 125-126 (125.7±0.43), V= 88.43-89.32 (89.28±0.46), Stylet= 84.26-85.58 (85.12±0.52),Oesophagus=117.90-122-08 (119.69±2.19), ABD= 21.61-23.48 (22.59±0.76),Tail= 43.54-48.00 (45.40±1.68), vulval opening with vulval sheath and tail sharply conoid, gradually tapering to an acute tip. The detail morphological description and data with host, localities, photomicrograph and camera lucida diagram are shown. The work recorded the first report of the species from Mizoram.

Keywords: Aizawl, *Hemicriconemoides*, Mizoram, nematodes, vegetables

Mizoram lies in North-Eastern region of India, extending from 21^0 56AA N to 24^0 31A N and 92^0 16A E to 93^0 26A E. About 76% of the state is covered by forest, 8% is fallow land, 3% is barren and considered uncultivable area, while the rest constitute cultivable and sown area. Agriculture is the main occupation; about 60% of the population is engaged in agricultural and allied activities. Because of the hilly state the pattern of agriculture followed is Jhum or Slash and burn cultivation. Among the agricultural pest, plant parasitic nematode is also one of the main threat that caused harm or various diseases in both cultivable crops and wild plants. Plant parasitic nematodes are microscopic worms which are generally filiform, sexually dimorphic and affect plants and give significant economic impact on commercial crops. There are various groups of plant parasitic nematodes that belong to different orders e.g Dorylaimida, Aphelenchida, Tylenchida etc. Those plant parasitic nematodes belonging to superfamily *Criconematoidea* (Taylor,1936) are commonly known as Criconematid, belonging to the order *Tylenchida,* Criconematid nematode is a group of plant parasitic nematode infesting various plants such as vegetable crops, wild plants, perennial woody tree, etc with habits ranging from migratory through semi-sedentary to sedentary ectoparasitism (Christie,1959, Thorne,1961, Jenkin & Taylor,1967). They belong to superfamily *Criconematoidea* (Taylor,1936) and are widely distributed. They are cosmopolitan and indigenously occurring (Heyn,1970) and obligatory tylenchid nematodes, possess poorly developed labial region, labial disc often consist of four submedian lobes. Stylet usually elongated but the length of conus is not proportionate to the length of Shaft. The posterior part of oesophagus or post corpus is pear in shape and clearly set off from intestine. They may possess retrorse annules, spines, scales or an extracuticular body sheath. Lateral field present or absent. Female reproductive system is mono-prodelphic type i,e single anteriorly directed genital branch without post uterine extension. The genus *Hemicriconemoides* belong to family *Criconematidae,* was proposed by Chitwood and Birchfield (1957) to keep those species which neither fit the present status of Criconemoides (Taylor,1936) nor that of *Hemicycliophora* (De man,1921). Goodey, 1963 placed the genus *Hemicriconemoides* in synonym with *Hemicycliophora* but it's again revive the genus by Siddiqi and Goodey (1964). *Iota squamous* (Cobb,1913) was considered to be as a member of *Hemicriconemoides*, so by Siddiqi & Goodey,1963 synonymized *Hemicriconemoides mangfera* and *Hemicriconemoides strictathecatus* with *Squamosus.* According to Siddiqi & Goodey (1964) the generic characters of *Hemicriconemoides* and *Hemicycliophora* is given as "Spear base anchor shaped" in *Hemicriconemoides* and "basal knobs of spear spheroid" in *Hemicycliophora.* Based on this difference in knob shape, *Hemicriconemoides biformis* and *Hemicriconemoides floridensis* were removed from *Hemicriconemoides* and placed in *Hemicycliophora.*

Those species belonging to genus *Hemicriconemoides* are commonly known as Sheathoid nematodes because the cuticle of the female body are covered by a loosened outer cuticular sheath, which is attached to the main body, at the anterior end or head and at the vulva. But such cuticular sheath is absent in male and juvenile stages. *Hemicriconemoides* (Chitwood & Birchfield,1957) are ectoparasite that feeds on the root of various crops & caused a damage to it (Sikora et al,2018), mainly found in the warm climatic region. According to Geraert, 2010 there are 52 species in genus *Hemicriconemoides* but 54 valid species (Maria et al, 2018) as a worldwide.

The genus *Hemicriconemoides* are greatly different from other nematodes in possessing small to medium sized body, elongated, cylindrical and tapering towards both ends, slightly ventrally curved when fixed. Body cuticle with two detached layers and closedly depressed. The annules of the sheath is flat and round, not retrorse and lateral field is not marked. Total body annules is in the range of 51-164 in numbers. Lip region consist of two annules, continuous or offset, variable in shape and submedian lobes absent. Presence of elongated, strong stylet with basal knobs directed anteriorly. Reproductive system mono-prodelphic type with outstretched ovaries. Vulva posterior with or without lateral cuticular flaps. Tail short, conoid to conoid round. The male body slender, cylindrical and slightly curved to ventrally. Head region conoid to rounded. Oesophagus degenerated. Stylet absent. Presence of slender and arcuate spicules. Gubernaculum small and simple. Tail conoid to subcylindrical.

Analysis of soil samples collected from Zemabuak, Haulawng, Thumpui, Aizawl, Lunglen and Mizoram University campus reveals the presence of large population of the two species of *Hemicriconemoides,* they are *Hemicriconemoides mangifera* (Siddiqi,1961) and *Hemicriconemoides chitwoodi* Esser,1960). *Hemicriconemoides mangifera* (Siddiqi,1961) is one of the smallest plant parasitic nematodes. This generic group of nematodes is characterized by transparent sheath which is closely appeared to the inner cuticle (Chitwood & Birchfield,1957, Dasgupta, Raski and Van Gundy,1969, Heyns.J.1970). *Hemicriconemoides mangifera* described as a parasite from the root of the mango from India and is believed to be nearly cosmopolitan in warm climatic condition area of the world (Siddiqi,1961, Siddiqi, 1977). It is one of the most important parasite that are found in tropical and subtropical fruits and also widely distributed in Mangroove in South Florida (Mesorley,J.L Parrado & Goldweber 1981,Heyns 1970,Siddiqi 1977). It is also considered as one of the major pest of Litchi and Mango (Mesorley R. 1981, Mesorley, C.W. Campbell & S. Goldweber, 1980, Mesorley J.L. Parrado & S. Goldweber, 1981, Milne D.L. 1982). *Hemicriconemoides mangifera* (Siddiqi 1961) re-described and considered it to a senior synonym of *Hemicriconemoides strictathecatus* Esser 1960 by Decraemer & Geraert 1992 *Hemicriconemoides chitwoodi* (Esser 1960), the generic key for identification is the lip region truncate, partly set off, first annule angular, directed outward, second annule much smaller than first (Chitwood & Birchfield, 1957). They possess transparent cuticular sheath which attached to the anterior end and at the vulva, not well separated. Presence of labial disc, elevated and rounded at the top. Absence of vulvar sheath. Tail conoid, gradually tapered into somewhat angular tip. Thus, these species *Hemicriconemoides mangifera* (Siddiqi 1961) and *Hemicriconemoides chitwoodi* (Esser 1960) are reported for the first time from Mizoram. They are described with morphometric data, photomicrograph, locality and their host.

MATERIAL AND METHODS

Soil samples were collected in the month of December, 2018 and September 2019 from four different localities viz Zemabuak, Haulawng, Aizawl and Mizoram University campus. The nematodes were extracted from the collected soil samples by Cobb's (1918) sieving and decanting methods followed by Baerman funnel technique. The extracted nematodes were killed and fixed in warm F.A(4:1) and processed by G.A method of Sienhorst (1959) and kept in a dessicator for slow dehydration. The specimen were permanently mounted by using anhydrous glycerine and sealed. Measurement, morphological observation and photomicrograph were taken by using NIKON Eclipse E200 microscope equipped with digital camera and VUE2017 software. Camera lucida diagram were also drawn using NIKON Eclipse E200 mounted with drawing tube. Identification were made by using keys developed by Esser (1960), Siddiqui (1961) and Siddiqi & Goodey (1964)

For scanning electron microscopy (SEM), specimens were fixed in 2.5% glutarldehyde. The fixed specimen is washed in 0.1M Sodium Cacodylate buffer and subsequently dehydrated in a graded series of acetone solutions. After dehydration specimens were air dried with Trimethylsilane(TMS), Dey et al (1989), mounted on stubs with double conductive tapes, coated with 25nm gold and photographed with a JSM – 6360 (JEOL) at 20kv.

RESULT AND DISCUSSION

1. *Hemicriconemoides mangiferae* Siddiqi,1961 (Table 1,2, Figure 1,2,3)

Description:

Female: Body elongate, cylindrical, slightly curved ventrally with a length of 217.48-236.42µm (224.65±6.04)

Table 1: Morphometric data of female species of *Hemicriconemoides mangiferae*. All measurement in µm

Sl.No	Character	Female
1.	N	6
2.	Length	217.48-236.42 (224.65±6.04)
3.	A	11.90-13.72 (12.6±0.65)
4.	B	3.93-4.53 (4.18±0.22)
5.	C	11.27-12.67 (11.9±0.59)
6.	V	90.15-91.65 (90.7±0.49)
7.	Total no. of body annule	127-132 (130±1.83)
8.	Rst	27-29 (27.5±0.95)
9.	Roes	32-39 (35.8±2.83)
10.	Rv	11-16 (13.16±1.80)
11.	Annule between vulva and anus	4-5(4.6±0.47)
12.	Ran	10-14 (12.3±1.49)
13.	VL/VB	1.10-1.90 (1.58±0.41)
14.	Stylet	35.86-51.53 (39.6±5.35)
15.	Oesophagus	42.69- 54.26 (49.83±3.86)
16.	St%L	16.05- 17.10 (16.65±0.44)
17.	Oeso%L	18.05-24.07 (22.05±2.07)
18.	Knob width/height	2.44-3.65 (3.2±0.5), 1.19-1.84 (1.4±0.2)
19.	Tail	17.52-19.90 (18.75±0.86)
20.	Max.body width	16.35-19.85 (17.76±1.12)
21.	Anal body width	8.91-13.30 (11.5±1.62)

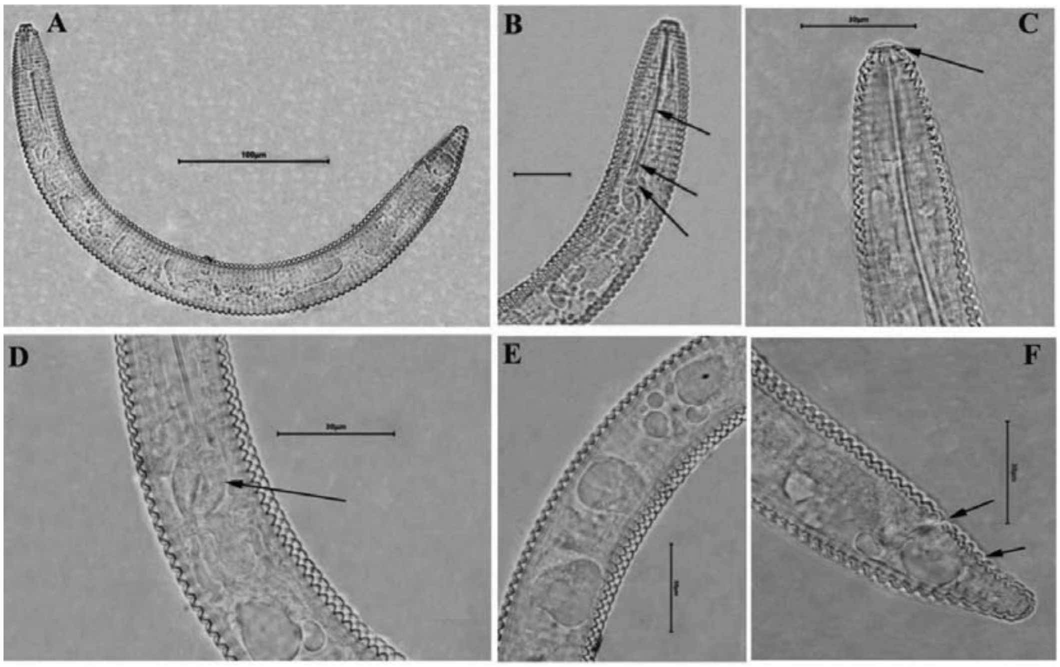

Fig. 1. Light microscopy images of *Hemicriconemoides mangiferae*; A. Female whole body B. Anterior portion (1st arrow showing conus, 2nd arrow showing shaft and 3rd one shows the basal knob of stylet), C. Head region (arrow showing lip region), D. Oesophageal region (Arrow showing median bulb of the oesophagus), E. Mid region of the body and F. Tail region (1st arrow showing vulval opening and 2nd arrow show anal opening). Scale bar: b = 30µm

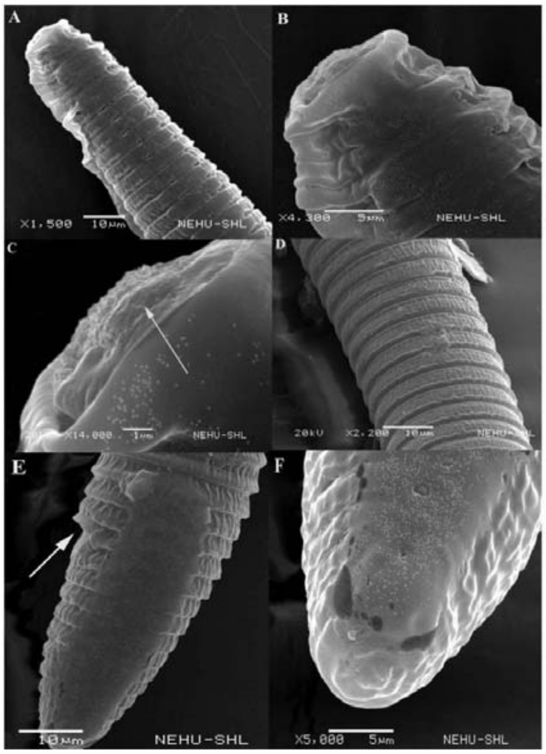

Fig. 2. Scanning electron microscopy images of *Hemicriconemoides mangiferae*. A. Anterior region; B. Head region; C. Labial region showing lobe; D. Body annulation; E. Posterior portion showing vulval region & F. Tail tip.

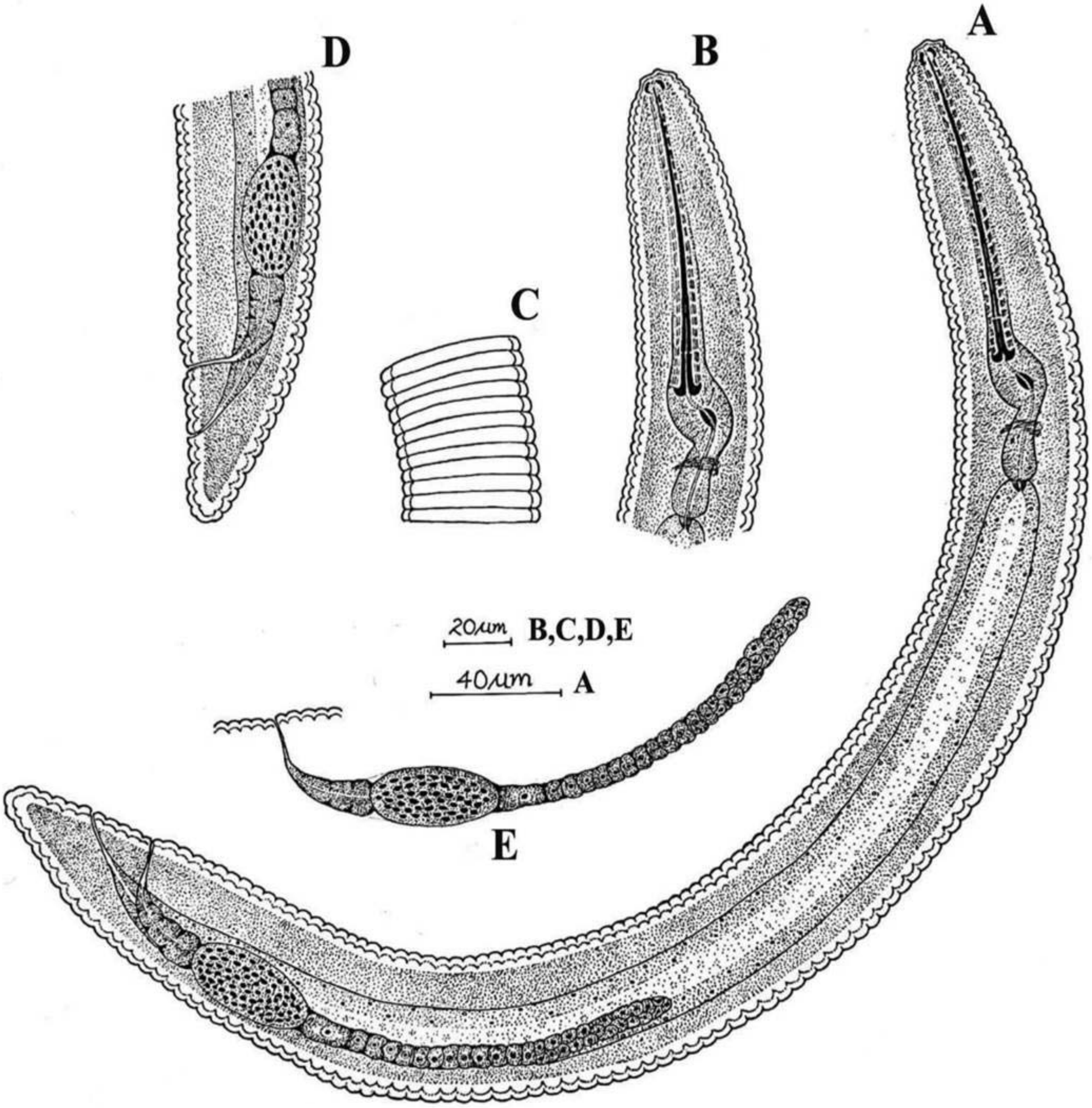

Fig. 3. Illustration of *Hemicriconemoides mangiferae*, A. Female entire body, B. Anterior end, C. Body annulation, D. Posterior end, E. Female reproductive system

Table 2: Comparative account of morphometric data of present species with *Hemicriconemoides mangiferae* , Siddiqi, 1961

Sl.No	Character	*Hemicriconemoides mangiferae* (present)	*Hemicriconemoides mangiferae*, Siddiqi, 1961
1.	Length	0.21-0..23 (0.22±0.00)	0.41-0.59 (0.50±0.0)
2.	A	11.90-13.72 (12.6±0.65)	12.2-21 (17.6±2.6)
3.	B	3.93-4.53 (4.18±0.22)	3.5-5.2 (4.4±0.4)
4.	C	11.27-12.67 (11.9±0.59)	11-24.1 (21.3±6.8)
5.	V	90.15-91.65 (90.7±0.49)	90.9-94 (92.1±1.1)
6.	R	127-132 (130±1.83)	119-147 (131.9±7.4)
7.	Rst	27-29 (27.5±0.95)	17-28 (19.5±2.5)
8.	Roes	32-39 (35.8±2.83)	28-36 (32.6±2.6)
9.	Rv	11-16 (13.16±1.80)	10-15 (12.7±1.8)
10.	Rvan	4-5 (4.6±0.47)	2-7 (4.4±1.4)
11.	Ran	10-14 (12.3±1.49)	5-12 (8±2.2)
12.	VL/VB	1.10-1.90 (1.58±0.41)	1.5-1.6 (1.5±0.0)
13.	Stylet	35.86-51.53 (39.6±5.35)	57.6-75 (67.7±9.3)
14.	Oesophagus	42.69-54.26 (49.83±3.86)	96-124.2 (112.9±9.3)
15.	St%L	16.05-17.10 (16.65±0.44)	11.8-15.5 (13.6±0.9)
16.	Tail	17.52-19.90 (18.75±0.86)	11.2-40.3 (6±7.8)
17.	Max.body width	16.35-19.85 (17.76±1.12)	25.7-33.6 (28.6±2.4)
18.	ABD	8.91-13.30 (11.5±1.62)	11.2-16 (14.4±1.6)

upon fixation. Round and coarse type of body annules are present ranging from 127-132 in numbers. Lip region consist of 2 annules about equal width (4.65±0.27) and height (1.84±0.6) in an outward direction. Labial disc slightly upward with strong sclerotized cephalic framework. Presence of elongated stylet 35.86-51.53µm (39.67±5.35) with anchor shaped basal knobs with outer margin directed forward and long flexible spear facing anteriorly. Oesophagus 42.69- 54.26µm (49.83±3.86) with large median bulb anteriorly extending into pre-corpus a short isthmus merging with a small rounded basal bulb. Female genital system is monoprodelphic. Ovary outstretched with oocytes arranged in a row. Vulva, a transverse slit with smooth lip. Anus located 2-5annules behind the vulva on 12th -14thannules from the tip of the tail. Tail elongate conoid or rounded with a length of 17.52-19.90µm (18.75±0.86).

Male: Not found

Habitat and localities: Soil collected from around the roots of *Antidesma* sp. From Zemabauk, 23°43´49.7´´N 92°45´17.7´´E, *Mangifera indica* from Aizawl, 23°39´56.6´´N 92°39´40.8´´E, *Ananas comosus* from Lunglen 23°39´31.9´´N 92°40´43.1´´E, *Phyllanthus emblica* from Thumpui and Rubber tree from Haulawng and unknown tree and grasses from Mizoram University campus, 23°44´17.2´´N 92°40´02.8´´E, Aizawl district, Mizoram, India.

Specimen: Female on the slide MU/HM/1-5 deposited in the nematode collection center of Parasitology section, Department of Zoology, Manipur University, Canchipur 795003, Manipur, India.

Discussion: *Hemicriconemoides* is a group of plant parasitic nematodes belonging to Order- Tylechida. It is a group proposed by Chitwood & Birchfield (1957). Under the genus *Hemicriconemoides*, several numbers of different species has been discovered, showing its diversity. Survey on soil soil sample of five different localities of Aizawl district viz, Zemabuak, Haulawng, Aizwal, Thumpui and Mizoram University campus reveal the presence of various plant parasitic nematodes. Among them *Hemicriconemoides mangiferae* Siddiqi (1961) is one and reported as new record from Mizoram. *Hemicriconemoides mangiferae* is a commonly and widely distributed species. On comparision with the morphometric data and description of Siddiqi (1961), there is slight variation in the length of the whole body, stylet, oesophagus, body width at the mid region, anal body width, the value of a,b,c,v, number of the annule from tail terminus to vulva and tail length but they lie in the range of data given for *Hemicriconemoides mangiferae* Siddiqi (1961). However the present specimen is shorter in body and stylet length, which may be likely due to the variation of the topography of different regions. Since the measurement comes within the range of intraspecific variation and body structure are similar with those given by Siddiqi (1961) the present specimen has been retained as *Hemicriconemoides mangiferae*. Male species are not found during survey. It is reported for the first time from the state of Mizoram.

2. *Hemicriconemoides chitwoodi* Esser, 1960 (Table 3, 4, Figure 4,5)

Description:

Female: Body ventrally arcuate with a length of 450.39-491-54µm(472.21±15.58) when fixed. Cuticular sheath covered the body annules, flat and smooth with a total 126 in numbers. Labial disc rounded and there is absence of submedian lobes. Lip consist of two annules, 1st annule is wider than the 2nd annule, which is in outward direction, lip width is 9.18(±0.310) and height is 2.33(±0.13). Stylet long 84.26-85.58µm (85.12±0.52) and flexible, knobs anchor shaped. Oesophagus with large median bulb anteriorly extending into pre-corpus,117.90-122.08µm(119.69±2.19) in length. Reproductive system mono-prodelphic type, ovary outstreched. Vulval opening without vuval sheath, anterior vulval lip not overlapping. Anus located 13th – 14th annules from the tips of tail. Tail sharply conoid, gradually tapering to an acute tip 43.54-48.00µm (45.40±1.68) in length.

Habitat and localities: Soil collected from around the roots of *Antidesma* sp. From Zemabauk, 23º43´49.7´´N 92º45´17.7´´E, *Mangifera indica* from Aizawl, 23º39´56.6´´N 92º39´40.8´´E, *Ananas comosus* from

Table 3: Parameter of female species of *Hemicriconemoides chitwoodi*. All measurement in µm.

Sl.No	Character	Female
1.	N	4
2.	Length	450.39-491-54 (472.21±15.58)
3.	A	13.24-15.92 (14.68±1.08)
4.	B	3.58-3.84 (3.71±0.08)
5.	C	10.02-10.70 (10.40±0.29)
6.	V	88.43-89.32 (89.28±0.46)
7.	R	125-126 (125.7±0.43)
8.	Rst	27 (27±0)
9.	Roes	37-38 (37.7±0.53)
10.	Rv	14-15 (14.3±0.47)
11.	Annule between vulva and anus	1 (1±0.0)
12.	Ran	13-14 (13.2±0.43)
13.	VL/VB	1,91-2.35 (2.16±0.15)
14.	Stylet	84.26-85.58 (85.12±0.52)
15.	Oesophagus	117.90-122-08 (119.69±2.19)
16.	St%L	17.31-19 (18,04±0.61)
17.	Oeso%L	23.98-26.57 (25.37±0.91)
18.	Knob width/height	6.19-6.45 (6.30±0.08), 2.50-3.32 (3.06±0.32)
19.	Tail	43.54-48.00 (45.40±1.68)
20.	Max.body width	30.85-34.01 (32.24±1.35)
21.	ABD	21.61-23.48 (22.59±0.76)

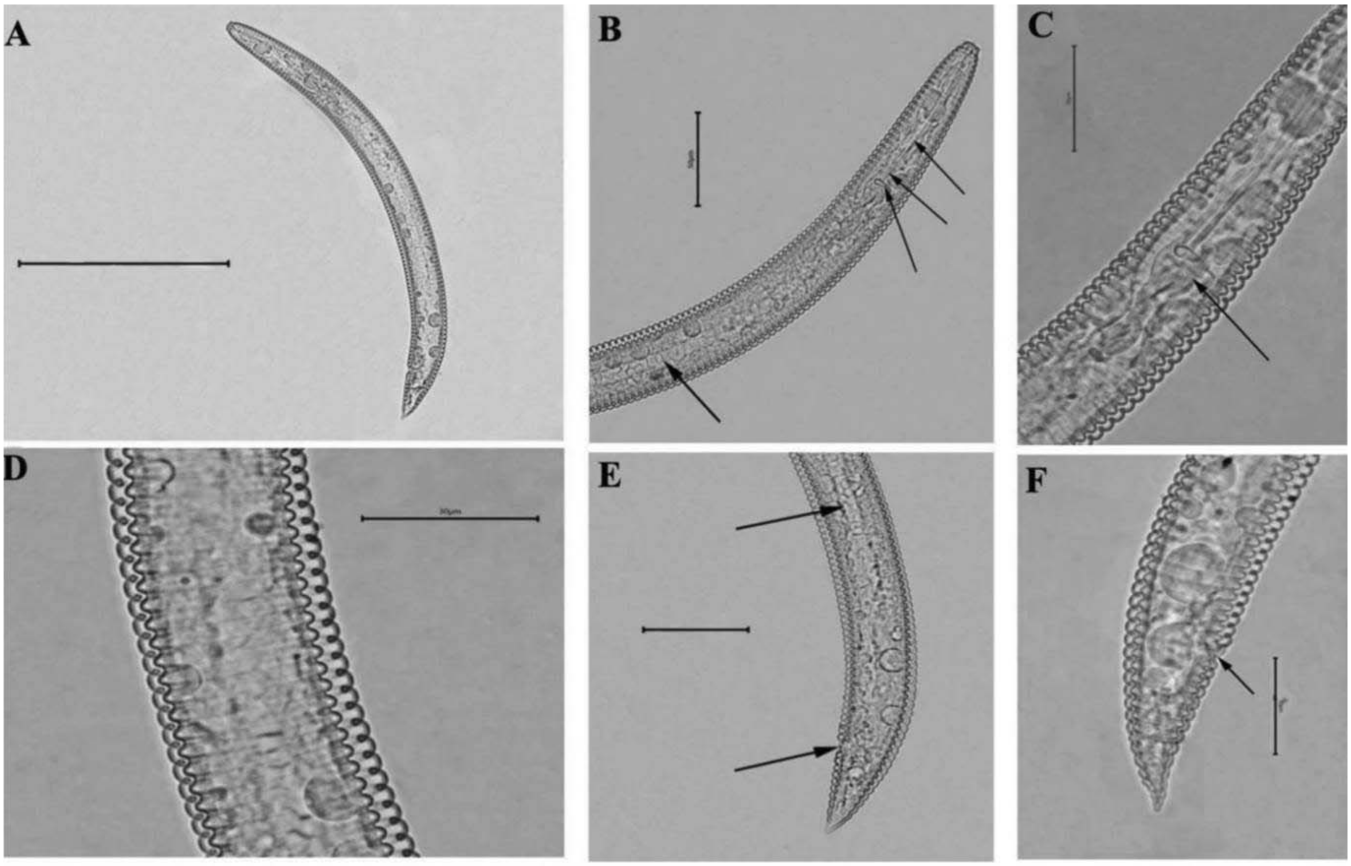

Fig. 4. Light microscopy images of *Hemicriconemoides chitwoodi*; A. Female whole body, B. Anterior region (1st arrow showing conus, 2nd arrow shaft, 3rd arrow base of stylet knob and 4th arrow ovaries), C. Oesopghageal region (arrow mark indicate median bulb of Oesopghagus), D. Mid region of the body, E. Posterior region (1st arrow showing mono-prodelphic ovary and 2nd arrow showing vulval opening) and F. Tail region (Arrow indicate vulval region). Scale: a = 100µm and e = 50µm.

Lunglen 23º39´31.9´´N 92º40´43.1´´E, and unknown tree and grass from Mizoram University campus, 23º44´17.2´´N 92º40´02.8´´E, Aizawl district, Mizoram, India.

Specimen: Female on the slide MU/HC/1-4 deposited in the nematode collection center of parasitology section, Department of Zoology, Manipur University Canchipur 795003, Manipur, India.

Discussion: *Hemicriconemoides chitwoodi*, Esser (1960) is also a plant parasitic nematode belonging to superfamily Criconematoidea of Order Tylenchida. Nematological investigation on the soil sample of four different localities of Aizawl district viz, Zemabuak, Mizoram University campus, Aizwal and Lunglen reveals the presence of *Hemicriconemoides chitwoodi*, Esser (1960). It is reported as a new record from the state of Mizoram. Based on comparative studies of morphological description and data of previous specimen *Hemicriconemoides chitwoodi*, Esser (1960) and Marco, Cordero, Robbin & Szalanski (2012) and the present specimen, all the morphometric description are similar to that of the present species and the morphometric value lies in the range of the data given by Esser (1960) except the value of C, VL/VB, number of annule from labial disc to the oesophago-intestinal junction and number of annule between vulval and anus. But these were within the range of intraspecific variation. So, the present

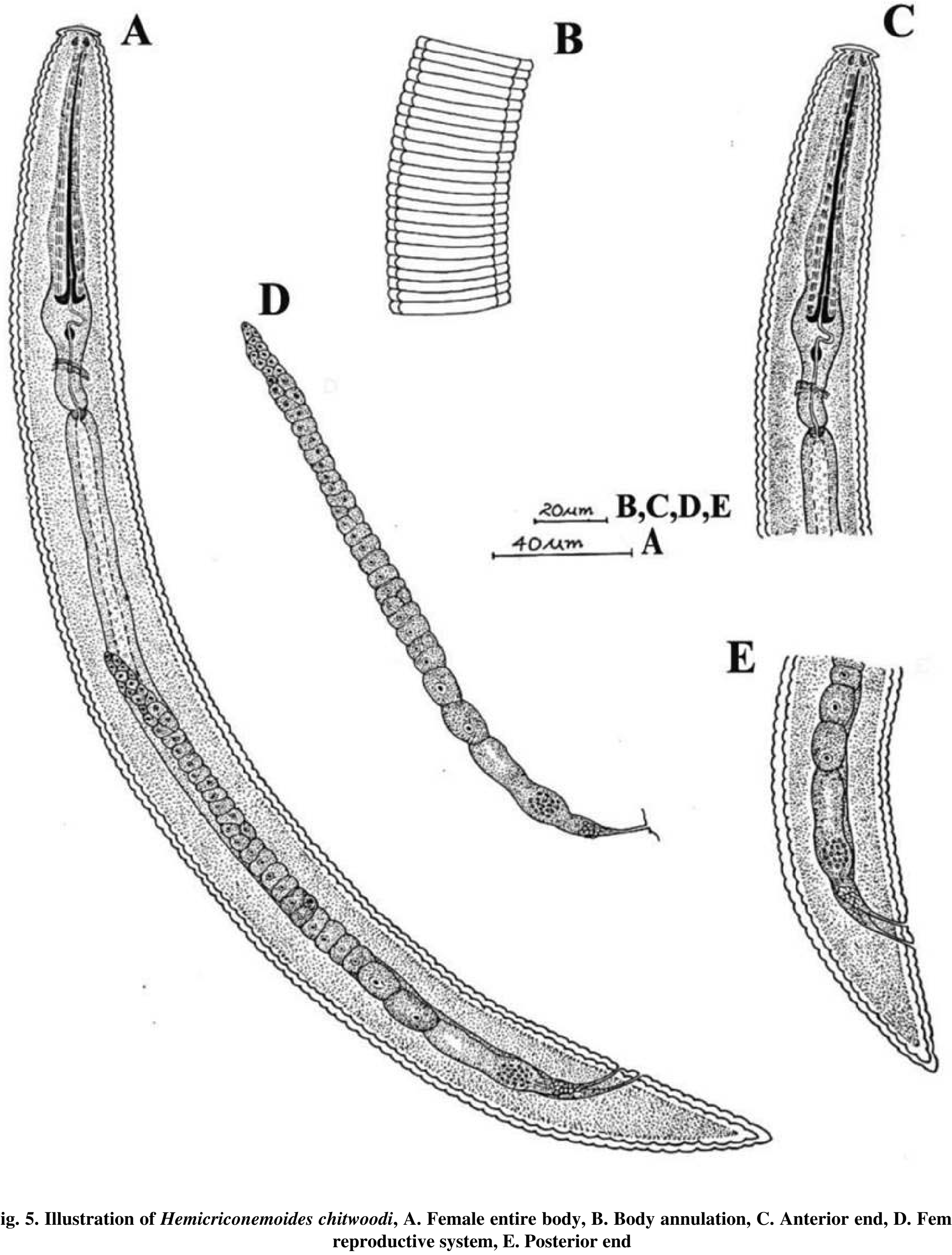

Fig. 5. Illustration of *Hemicriconemoides chitwoodi*, A. Female entire body, B. Body annulation, C. Anterior end, D. Female reproductive system, E. Posterior end

Table 4: Comparision of morphometric data of present species with *Hemicriconemoides chitwoodi*, Cordero, Robbins and Szalanski, 2012

Character	*Hemicriconemoides chitwoodi* (present)	*Hemicriconemoides chitwoodi*, M.A.Cordero, R.T. Robbins and A. L. Szalanski, 2012
Length	450.39-491-54(472.21±15.58)	442.4-606.1(503.9±40.1)
A	13.24-15.92(14.68±1.08)	14.3-18.2(16.0±1.1)
B	3.58-3.84(3.71±0.08)	3.8-4.8(4.1±0.3)
C	10.02-10.70(10.40±0.29)	14.7-24.3(17.7±2.7)
V	88.43-89.32(89.28±0.46)	89.7-92.5(91.1±0.7)
R	125-126(125.7±0.43)	113-127(119±3.8)
Roes	27(27±0)	27-37(31±2.5)
Rv	37-38(37.7±0.53)	12-16(14±1.1)
Rvan	14-15(14.3±0.47)	2-5 (3±0.7)
Ran	1(1±0.0)	8-12(10±0.1)
VL/VB	13-14(13.2±0.43)	1.5-2.0(1.7±0.1)
Stylet	1,91-2.35(2.16±0.15)	82.6-94.8(882±3.4)
Oesophagus	84.26-85.58(85.12±0.52)	113.7-132.0(122.0±40.1)
St%L	17.31-19(18,04±0.61)	14.3-19.4(17.6±1.4)
Tail	43.54-48.00(45.40±1.68)	20.3-34.9(28.9±3.5)
Max.body width	30.85-34.01(32.24±1.35)	29.2-34.9(31.4±1.4)
Anal body width	21.61-23.48(22.59±0.76)	19.5-24.4(21.6±1.3)

specimen had been regarded as *Hemicriconemoides chitwoodi*. During survey male species were not found. This study reported is the first record of the species from Mizoram.

CONCLUSION

From the above studies it can be conclude that the present species *Hemicriconemoides mangiferae* and *Hemicriconemoides chitwoodi*, have not been been reported earlier from the state of Mizoram. So, the present report is the first record of the said species from Mizoram.

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Studies on the Occurrence, Distribution and Community Analysis of Plant Parasitic Nematodes Associated with Maize, Tomato, Mulberry, Banana and Sugarcane in Erode District of Tamil Nadu

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ABSTRACT: The pathogenic ability of plant parasitic nematodes in a specified locality is determined through community analysis. This investigation involves the analysis of plant parasitic nematode populations and their associated parameters in the rhizosphere of Maize, Tomato, Mulberry, Banana and Sugarcane grown in Erode district of Tamil Nadu. There were several nematode genera identified in the plant rhizosphere, such as *Pratylenchus* spp., *Tylenchorhynchus* spp., *Meloidogyne* spp., *Helicotylenchus* spp., *Helicotylenchus* spp., *Hoplolaimus* spp., *Criconemoides* spp., *Xiphinema* spp. and *Longidorus* spp. Community analysis of nematodes associated with the aforementioned crops varied in terms of their frequency and density. In Maize, *Longidorus* spp. was reported to be the predominant genus with the absolute frequency value of 100% and the prominence value of 2.87. *Meloidogyne* spp. was the most economical nematode in tomatoes, with an absolute frequency of 72.72% and prominence value of 6.29. *Xiphinema* spp. was the more frequently occurring nematode in Mulberry, with the absolute frequency of 100% and prominence value of 21.06. In Sugarcane, *Hoplolaimus* spp., which had an absolute frequency of 90.90%, was the most prominent nematodes in sugarcane. In Banana, *Pratylenchus* spp. had the highest prominence value and density (absolute and relative).

Keywords: Community analysis, Population density, Plant parasitic nematodes, Prominence value

Plant parasitic nematodes are important hidden enemies to agriculture and horticulture crops. Despite their damage to crop plants, due to their hidden mode of life, the importance of nematodes has often been overlooked. Nematode infestation symptoms of are non-specific and often being confused with nutritional deficiencies. In India's tropical and subtropical regions, nematode damage potential is much greater, and it has been calculated that plant parasitic nematodes in 24 different crops have caused a national loss of 21068.73 million (Jain *et al.,* 2007).

Nematode density in the soil is a typical indicator of nematode infestation in crops. These nematode damages were increased only when their populations exceed the level of economic damage. Hence, studies on the occurrence of nematodes are very important parameters to estimate the role of nematodes in agriculture and horticulture crops. In the current study, different community analysis parameters of plant parasitic nematodes related to the major crops grown in Erode District, Tamil Nadu, India has been evaluated.

MATERIALS AND METHODS

Soil sample collection and extraction of nematodes

A total of 55 soil samples were randomly collected from the rhizosphere region of maize, tomato, mulberry, banana and sugarcane crops in Erode district. After thoroughly mixing the soil, a representative sample of about 250cc and a root sample (5g) were collected. In order to prevent the drying of samples, they were packed in polythene bags and maintained in the refrigerator set to 5°C.

Cobb's decanting and sieving method (Cobb, 1918) and modified Baermann's technique (Southey, 1986) were used to process soil samples and extract the nematodes. Extracted nematodes were concentrated and killed through heat fixation by distributing an equivalent quantity of TAF fixative. Root samples were carefully cleaned in tap water and then chopped into 1 cm pieces. Mechanical maceration method was used to extract migratory endoparasitic nematodes from the roots. Roots were stained with acidfuchsin lactophenol dye to observe sedentary and semi endoparasitic nematodes. Nematode population was estimated by using stereozoom microscope.

Statistical analysis

The following formulas were used to calculate the community analysis parameters (Norton, 1978).

$$\text{Absolute frequency} = \frac{\text{No. of samples containing a species}}{\text{No. of samples collected}} \text{ x } 100$$

$$\text{Relative frequency} = \frac{\text{Frequency of a species}}{\text{Sum of frequencies of all spp.}} \text{ x } 100$$

$$\text{Relative density} = \frac{\text{No. of individuals of a species in a sample}}{\text{Total of all individuals in a sample}} \text{ x } 100$$

$$\text{Absolute density} = \frac{\text{No. of individuals of a species in a sample}}{\text{Volume or mass or units of the sample}} \text{ x } 100$$

$$\text{Prominence value} = \frac{\text{Absolute density Absolute frequency}}{100}$$

RESULTS AND DISCUSSION

The frequency, density, and diversity of plant parasitic nematodes associated with maize, tomato, mulberry, banana, and sugarcane crops varied due to variations in the ecological, climatic and edaphic factors (Rawhat Un Nisa *et al*., 2021).

The major plant parasitic nematodes identified during the survey consist of two major orders *viz*., Tylenchida and Dorylaimida. Predominant plant parasitic nematodes associated with the crops grown in Erode district of Tamil Nadu were presented in Table 1. In Tylenchida, major nematode species reported were *Tylenchorhynchus* spp., *Pratylenchus* spp., *Meloidogyne* spp., *Helicotylenchus* spp., *Hoplolaimus* spp., *Criconemoides* spp. and *Helicotylenchus* spp. The major nematode species *viz*., *Xiphinema* spp. and *Longidorus* spp. were commonly recorded from Dorylaimida.

Four plant parasitic nematodes, namely *Meloidogyne* spp., *Helicotylenchus* spp., *Longidorus* spp., and *Hoplolaimus* spp., identified through a community analysis of plant parasitic nematodes associated with tomato. According to Sagu *et al.* (2011), the nematodes associated with tomato, eggplant, cowpea and bottle gourd crops were *Meloidogyne* spp., *Rotylenchuus reniformis*, *Tylenchorhynchus indicus*, *Pratylenchus* spp. and *Helicotylenchus* spp. Among them, *Meliodogyne* spp. was reported as most prevalent one.

In the present study, *Meloidogyne* spp. was the more frequently observed nematode genera in tomato with an absolute frequency of 72.72%. This was followed by *Helicotylenchus* spp. (63.63%) and *Longidorus* spp. (45.45%). *Hoplolaimus* spp. recorded the least absolute frequency of 18.18% in tomato (Table 2). Relative frequency was recorded to be maximum for *Meloidogyne* spp. (14.55 %), followed by *Helicotylenchus* spp. (12.73 %), *Longidorus* spp. (9.09 %) and *Hoplolaimus* spp. (3.64 %). Absolute density and relative density were recorded to be maximum for *Meloidogyne* spp., followed by *Helicotylenchus* spp., *Longidorus* spp. and *Hoplolaimus* spp. Prominence value (PV) of 6.29, 1.27, 0.61 and 0.2 were recorded for *Meloidogyne* spp., *Helicotylenchus* spp., *Longidorus* spp. and *Hoplolaimus* spp. respectively. The findings of Firake *et al.* (2019), who examined the

Table 1. Predominant plant parasitic nematodes associated with the major crops grown in Erode district, Tamil Nadu

S.No.	Host Crops	Nematode genus recorded
1.	Maize	*Longidorus* spp., *Tylenchorhynchus* spp., *Pratylenchus* and *Xiphinema* spp.
2.	Tomato	*Meloidogyne* spp., *Helicotylenchus* spp., *Longidorus* spp. and *Hoplolaimus* spp.
3.	Mulberry	*Meloidogyne* spp. , *Xiphinema* spp., *Longidorus* spp. and *Helicotylenchus* spp.
4.	Sugarcane	*Hoplolaimus* spp., *Criconemoides* spp., *Helicotylenchus* spp., *Pratylenchus* spp. and *Tylenchorhynchus* spp.
5.	Banana	*Pratylenchus* spp., *Helicotylenchus* spp. , *Hoplolaimus* spp. and *Criconemoides* spp.

Table 2. Community analysis of soil inhabiting plant parasitic nematodes associated with Tomato in Erode district, Tamil Nadu

Nematodes	Population (250 cc soil)	Frequency %		Density %		Prominence value
		Relative	Absolute	Relative	Absolute	
Meloidogyne spp.	184.45	14.55	72.72	71.37	73.78	6.29
Helicotylenchus spp.	39.82	12.73	63.63	15.41	15.93	1.27
Longidorus spp.	22.73	9.09	45.45	8.79	9.09	0.61
Hoplolaimus spp.	11.45	3.64	18.18	4.43	4.58	0.2

host ranges of the major nematode species in Meghalaya supported the present study. They found that *Helicotylenchus* spp., *Rotylenchulus* spp., and *Meloidogyne* spp. were the most prominent genera, each of which had a wide host range.

Community analysis in maize (Table 3) reported four genera of nematodes associated with the crop. With an absolute frequency of 100%, *Longidorus* spp. was reported to be the most prevalent nematode, followed by *Xiphinema* spp. (90.90%) and *Tylenchorhynchus* spp. (63.63%). *Pratylenchus* spp. recorded least absolute frequency of 36.36%. The highest absolute density was found in *Longidorus* spp. (28.66%), followed by *Xiphinema* spp. (25.86%) and *Tylenchorhynchus* spp. (16.11%). *Pratylenchus* spp. had the least absolute density of 7.89%. Similarly, *Longidorus* spp. has the highest relative density (36.50%), followed by *Xiphinema* spp. (32.93%), and *Tylenchorhynchus* spp. (20.52%). Least relative density was observed in *Pratylenchus* spp. (10.05%). Based on the frequency and density values, prominence value (PV) was calculated, which was reported to be higher in *Longidorus* spp. (2.87), followed by *Xiphinema* spp. (2.47) and

Table 3. Community analysis of soil inhabiting plant parasitic nematodes associated with Maize in Erode district, Tamil Nadu

Nematodes	Population (250 cc soil)	Frequency %		Density %		Prominence value
		Relative	Absolute	Relative	Absolute	
Longidorus spp	71.64	18.18	100	36.5	28.66	2.87
Xiphinema spp.	64.64	16.36	90.9	32.93	25.86	2.47
Tylenchorhynchus spp.	40.27	12.73	63.63	20.52	16.11	1.29
Pratylenchus spp.	19.73	7.27	36.36	10.05	7.89	0.48

Tylenchorhynchus spp. (1.29). *Pratylenchus* spp. has the lowest prominence value (0.48). The current results supported the findings of CABI (2020), which reported the association of numerous taxa of plant parasitic nematodes in maize *viz*., *Criconemella spp., Ditylenchus spp., Globodera spp., Hoplolaimus spp., Helicotylenchus spp., Hemicriconemoides spp., Heterodera spp., Longidorus spp., Meloidogyne spp., Paratrichodorus spp., Pratylenchus spp., Radophulus spp., Rotylenchulus spp., Scutellonema spp., Trichodorus spp., Tylenchorhynchus spp.* and *Xiphinema spp.*

Analysis of nematode population in mulberries revealed four distinct genera of plant parasitic nematodes associated with the crop (Table 4). Ramakrishnan and Senthilkumar (2003) reported about 42 nematode species from 24 genera were associated with mulberry in different mulberry growing regions of the world. The crop recorded highest population of *Meloidogyne* spp. (306.36 J2/250 cc soil) followed by *Xiphinema* spp. (52.64 J2/250 cc soil), *Longidorus* spp. (37.27 J2/250 cc soil), and *Helicotylenchus* spp. (10.27 J2/250 cc soil). However, *Xiphinema* spp. had the highest absolute frequency (100%), followed by *Meloidogyne* spp. (72.72%), *Longidorus* spp. (45.45%), and *Helicotylenchus* spp. (18.18%).The relative frequency was also found to be maximum for *Xiphinema* spp. (18.18%) followed by *Meloidogyne* spp. (14.55%), *Longidorus* spp. (12.73%) and *Helicotylenchus* spp. (3.64%). On the other hand, absolute density and relative density were maximum for *Meloidogyne* spp. followed by *Xiphinema* spp., *Longidorus* spp. and *Helicotylenchus* spp. PV was calculated to be 12.25, 1.68, 1.0 and 0.18 for *Meloidogyne* spp., *Xiphinema* spp., *Longidorus* spp., and *Helicotylenchus* spp. respectively. Severity of damage relies on the soil conditions and meterological factors of the different places. *M. incognita* is more dangerous to mulberry not only because of the direct damage it cause to the crop but also, it also makes the plants more susceptible to certain soil-borne plant diseases (Bhagrathy *et al*., 2000; Ishitha Naik *et al*., 2003).

Present study showed that, *Hoplolaimus* spp. was the prominent plant parasitic nematode associated with sugarcane with high absolute (90.90) and relative (18.18) frequency, absolute (41.50) and relative (47.52) density and prominence value (3.96) (Table 5). This was followed by *Helicotylenchus* spp., *Criconemoides* spp., *Tylenchorhynchus* spp. and *Pratylenchus* spp. Abdul Rashid (2001) reported the occurrence of *Tylenchorhynchus* spp., *Helicotylenchus* spp., *Pratylenchus* spp., and *Hoplolaimus* spp. on sugarcane with low to high population densities. *Pratylenchus* and *Meloidogyne* spp. were recorded to be extremely morbific to sugarcane crop (Luc et al., 2005). *Meloidogyne incognita* was the most frequently observed plant parasitic nematode in the sugarcane fields of Uttar Pradesh, India, according to Akhtar and Wani (1992), followed by *Helicotylenchus indicus*, *Hoplolaimus indicus*, *Tylenchorhynchus mashhoodi*, *Longidorus* spp., and *Xiphinema* spp.

Table 4. Community analysis of soil inhabiting plant parasitic nematodes associated with Mulberry crop in Erode district, Tamil Nadu

Nematodes	Population (250 cc soil)	Frequency %		Density %		Prominence value
		Relative	Absolute	Relative	Absolute	
Meloidogyne spp.	306.36	14.55	72.72	75.36	122.54	12.25
Xiphinema spp.	52.64	18.18	100	12.95	21.06	1.68
Longidorus spp.	37.27	12.73	45.45	9.17	14.91	1.00
Helicotylenchus spp.	10.27	3.64	18.18	2.53	4.11	0.18

Table 5. Community analysis of soil inhabiting plant parasitic nematodes associated with Sugarcane in Erode district, Tamil Nadu

Nematodes	Population (250 cc soil)	Frequency %		Density %		Prominence value
		Relative	Absolute	Relative	Absolute	
Hoplolaimus spp.	103.73	18.18	90.9	47.52	41.5	3.96
Criconemoides spp.	59.09	16.36	81.82	27.07	23.64	2.14
Helicotylenchus spp.	36.73	16.36	81.82	16.83	14.69	1.33
Tylenchorhynchus spp.	13.09	14.55	72.73	6.00	5.24	0.45
Pratylenchus spp.	5.64	5.45	27.28	2.58	2.26	0.12

Table 6. Community analysis of soil inhabiting plant parasitic nematodes associated with Banana in Erode district, Tamil Nadu

Nematodes	Population (250 cc soil)	Frequency %		Density %		Prominence value
		Relative	Absolute	Relative	Absolute	
Pratylenchus spp.	418.36	18.18	100	63.28	167.35	16.74
Criconemoides spp.	70.55	18.18	100	10.67	28.22	2.82
Helicotylenchus spp.	156.82	18.18	100	23.72	62.73	6.27
Hoplolaimus spp.	15.36	10.91	72.73	2.32	6.14	0.52

Presence of four plant parasitic nematodes, namely *Pratylenchus* spp., *Helicotylenchus* spp., *Hoplolaimus* spp., and *Criconemoides* spp., were confirmed through the community analysis studies in banana (Table 6). Similarly, Nimisha and Nisha (2019) observed about nine nematode species *viz*., *Meloidogyne incognita*, *Rotylenchulus reniformis, Helicotylenchus dihystera*, *Heterodera oryzicola, Radopholus similis, Pratylenchus coffeae, Hoplolaimus indicus, Criconema* sp. and *Xiphinema* sp. in five different varieties of banana (Nendran, Poovan, Robusta, Red Banana, and Rasakadali).

The current survey revealed that *Pratylenchus* spp. that was more common in the banana-growing areas of Erode district, Tamil Nadu. This result was consistent with that of Srinivasan *et al.* (2011) who found that the *Pratylechus coffeae* was the prominent species present in the majority of banana root samples obtained from 8 Taluks in Thanjavur district, Tamil Nadu with the highest prominence value.

According to the results of the current study, the association of plant parasitic nematodes, particularly the most significant nematodes like *Meloidogyne* spp., *Pratylenchus* spp., and *Helicotylenchus* spp., would cause a significant yield loss in Erode district of Tamil Nadu, India. To address these issues, an Integrated Nematode Management programme should be implemented as soon as possible to increase agricultural and horticulture crop production in those areas.

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Status and Population Diversity of Plant-Parasitic Nematodes in Medicinal Plants of Uttarakhand

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ABSTRACT: Plant-parasitic nematodes (PPN) are the most important group of soil-borne pathogens prevailing around the root of medicinal plants causing a reduction in plant growth and yield. Because Uttarakhand is known as India's herbal state, this work aimed to assess the diversity of PPN communities with medicinal plants in the soils of Uttarakhand. Herein, nematode communities were characterized in soil samples collected from four districts with an altitude range of 180-3000 meters above mean sea level. Eleven PPN genera belonging to 10 families were identified among which two genera *Meloidogyne* spp. and *Heliocotylenchus* spp. were most dominant. At elevations below 500 meters above mean sea level, the most common nematodes were *Pratylenchus* spp. and *Meloidogyne* spp., and among plants Safed Musli (*Chlorophytum borivilianum*) and Sarpagandha (*Rauvolfia serpentia*) recorded the highest population density of nematodes. At 900-1900 meters above mean sea level range all associated nematodes exhibited 100% frequency except for *Tylechorhynchus* spp., *Trichodorus* spp., and *Rotylenchulus* spp. and Tulsi (*Ocimum tenuiflorum*) plant showed the highest population of these associated nematodes. At range above 2000 meters above mean sea level, Chopcheeni (*Samilax china*) had the largest population of PPNs, with *Meloidogyne* spp. being the most frequent nematode. Rich diversity of plant parasitic nematodes have been discovered in association with medicinal plants, which might pose a severe danger to their production.

Keywords: Altitude, frequency, medicinal plants, plant-parasitic nematodes (PPNs), survey

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The Uttarakhand state of India is a natural habitat of a variety of herbs, medicinal and aromatic plant species, therefore declared as a herbal state. It is located at the foothills of the Himalayas and it has been divided into two regions- the western region- Garhwal Mandal and the eastern region Kumaon Mandal. The state has nearly 700 species of medicinal plants of which 175 species are being commercially exploited and traded. About 42000 registered farmers in more than 15000 ha of land are cultivating 38 prioritized medicinal and aromatic plants (MAPs), and the state fetching turnover of 120 Crore from pure and natural essential oil suitable for aromatherapy and spa industry (http://www.hrdiuk.org; shm.uk.gov.in; forest.uk.gov.in). High returns and intercropping of aromatic and medicinal plants with food grains provide a positive attitude for farmers and also help diversify the income basket for small and marginal farmers. Aromatic plants like Lemongrass (*Cymbopogon flexuosus*), Citronella (*Cymbopogon nardus)*, Palmarosa (*Cymbopogon martini*), Chamomilla (*Matricaria recutita*), Tulsi (*Ocimum tenuiflorum*), Geranium (*Pelargonium hortorum*), Nagarmotha (*Cyperus rotundus),* Japanese mint (*Mentha Canadensis*), Khas (*Chrysopogon zizanioides*), and Genda (*Tagetes erecta*) are used extensively in the cosmetics industry. There is further scope for medicinal plants and medicinal trees like Tejpatta (*Cinnamomum tamala*), Amla (*Phyllanthus emblica*), Harad (*Terminalia chebula*), and Bhagera (*Passer domesticus*) are being planted. There is huge commercial value in the oils and essences extracted from aromatic and medicinal plants (Mittal *et al.* 2008).

Unfortunately, the medicinal plants are also susceptible to soil bio-aggressors including plant-parasitic nematodes (PPNs), which are considered the most deleterious parasites (Abtahi and Bakooie 2017). The intensity of the loss caused by them depends upon their population densities and the effect of abiotic factors. Several studies of PPNs associated with medicinal plants have been executed, and are known to be associated with almost all medicinal plants studied till date (Khanzada *et al*. 2012; Abtahi and Bakooie 2017; Eapen and Pandey 2018). Remarkably, yield losses caused by plant-parasitic nematodes on medicinal plants were up to 30% (Eapen and Pandey 2018). Although a survey of the PPNs community with medicinal plants and identification has been done in some parts of India like Karnataka, West Bengal, Manipur, Lucknow and Bareilly but Uttarakhand is still untouched. Keeping in view the current status of medicinal plants in the state, the present study aims to investigate the population diversity of PPNs based on altitudes, their identification and frequency in association with medicinal plants from different districts of the Kumaon regions. An extensive survey was conducted at an altitude ranging from 180 to 2190 meters above mean sea level on rhizospheric soil of 64 medicinal plants to analyse the diversity and distribution and a total of 11 genera of Plant Parasitic Nematodes (PPNs) viz; *Meloidogyne* spp., *Pratylenchus* spp., *Trichodorus* spp., *Helicotylenchu*s spp., *Tylenchorhynchus* spp., *Ditylenchus* spp., *Rotylenchulus* spp., *Criconemoides* spp., *Aphelenchus* spp., *Xiphinema* spp., and *Tylenchus* spp. were found. The dominant taxa were represented by *Helicotylenchu*s spp. and *Meloidogyne* spp. with 45-100% frequency of occurrence.

MATERIAL AND METHODS

Survey: Four districts Udham Singh Nagar (US), Nainital (N), Almora (A), and Pithoragarh (P) of Uttarakhand state with an altitude range of 180-3000 meters above mean sea level were surveyed (Fig. 1).

From US (180-220 meters above mean sea level), five sub-locations namely MRDC, Pantnagar, Narayanpur, Kashipur and CIMAP, N (857-2190 meters

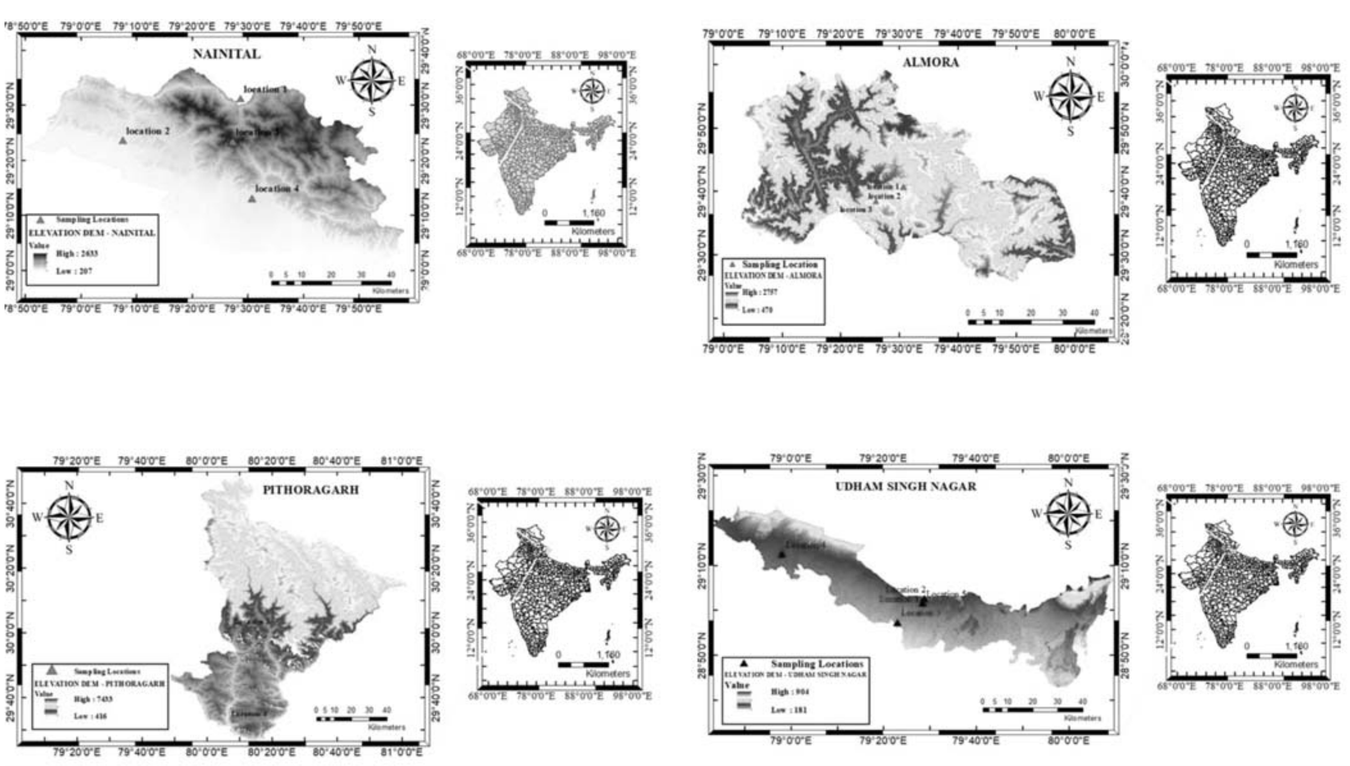

Fig. 1. ArcGIS map representing different locations of survey

above mean sea level), four sub-locations viz; Khairna, Ramnagar, and Nainital and Haldwani, A (1855-1989 meters above mean sea level), three sub-locations Ranikhet, Majkhali, and Chillayanuala, P (2465 meters above mean sea level) Forest nursery Munsiyari, Govt. Potato Seed Multiplication Farm Balati (Munsiyari), Tiksen Farm and Pithoragarh were surveyed. The districts were further divided on the basis of altitude ranging from 180-3000 meters above mean sea level. A total of 64 medicinal plants were collected from four districts, 46 plants at an altitude above 2000 above mean sea level, 8 plants from 900-1900 meters above mean sea level, and 26 plants below 500 meters above mean sea level were surveyed. Plants varied depending on the location and altitude while some were common (Table 1). From March to September, soil samples were taken from the plant root zone up to the depth of 15-20 cm from each location. Properly labelled soil samples were kept in polythene bags which were then stored at 5°C in the refrigerator in the Nematology laboratory, Plant Pathology Department, Pantnagar.

Table 1: List of surveyed medicinal plants in Uttarakhand

S. No.	Medicinal Plant	Family
1	*Acorus calamus* (Vach)	Acoraceae
2	*Allium humile* (Small alpine onion)	Alliaceae
3	*Aloe barbadensis* (Aloevera)	Asphodelaceae
4	*Amomum bulatum* (Bari Ilaychi)	Zingiberaceae
5	*Andrographis paniculata* (Kalmegh)	Acanthaceae
6	*Artemisia absinthium* (Mugwort)	Asteraceae
7	*Asparagus racimosus* (Sataver)	Asparagaceae
8	*Bacopa monnieri* (Bhrami)	Plantaginaceae
9	*Barleria Prionitis* (Bajradanti)	Acanthaceae
10	*Bergenia ligulata* (Pashanbhed)	Saxifragaceae
11	*Caesalpinia pulcherrima* (Chura /Amesh)	Fabaceae
12	*Cardiocrinum giganteum* (Himalayan lily)	Liliaceae
13	*Carthamus tinctorius* (Saffola)	Asteraceae
14	*Carum carvi* (Wild caraway)	Apiaceae
15	*Centella asiatica* (Gotu kala)	Mackinlayaceae
16	*Chlorophytum borivilianum* (Safed musli)	Asparagaceae
17	*Cichorium intybus* (Kasni)	Asteraceae
18	*Citrus limon* (Neebu)	*Rutaceae*
19	*Corylus colurna* (Turkish hazel)	Betulaceae
20	*Cuminum cyminum* Kala Jeera (Himanchali)	Apiaceae.
21	*Cuminum cyminum* Kala Jeera (Lokal)	Apiaceae.
22	*Cymbopogon flexuosus* (Lemon grass)	Poaceae
23	*Cymbopogon martini* (Palmarosa)	Poaceae
24	*Cymbopogon witerianus* (Citronella)	Cardiopteridaceae
25	*Dactylorhiza hatagirea* (Salampanja)	Orchidaceae

26	*Digitalis purpurea* (Common foxglove)	Plantaginaceae
27	*Elwendia persica* (Kalajeera)	Apiaceae.
28	*Hedychium spicatum* (Spiked ginger tail)	Zingiberaceae
29	*Heracleum candicans* (Hogweed)	Apiaceae
30	Hippi	Amaryllidaceae
31	*Inula racemose* (Puskar Mool)	Asteraceae
32	*Lawsonia alba* (Henna tree, Mignonette tree)	Lythraceae
33	*Lepidium sativum* (Chandarsur)	Cruciferae
34	*Lewisia rediviva* (Bitter root)	Portulacecae
35	*Mahonia napaulensis* (Kesari, Khasi)	Berberidaceae
36	*Matricaria recutita* (Cmomile)	Asteraceae
37	*Mentha piperita* (Mint)	Lamiaceae
38	*Miscanthus nepalensis* (Silvergrass)	Poaceae
39	*Ocimum tenuiflorum* (Tulsi)	Lamiaceae
40	*Paris polyphylla* (Satuwa)	Melanthiaceae
41	*Pelargonium graviolunce* (Geranium)	Geraniaceae
42	*Picrophiza kurrooa* (Kutki)	Plantaginaceae
43	*Piper nigrum* (Pepper)	Piperaceae
44	*Pogostemon cablin* (Pachouli)	Lamiaceae
45	*Polygonatum verticillatum* (Mahameda)	Asparagaceae
46	*Polygonatum verticillatum* (Salammishri)	Liliaceae
47	*Potentilla fulgens* (Silverweed)	Rosaceae
48	*Primula vulgaris* (Primroses)	Primulaceae
49	*Rheum webbianum* (Indian rhubarb)	Polygonaceae
50	*Rhodollia trifolia* (Roseroot, Golden root)	Crassulaceae
51	Valeriana jatamansii (Sameva)	Caprifoliaceae
52	*Samilax china* (Chopcheeni)	Liliaceae
53	*Saussurea costus* (Indian costus, kuth)	Asteraceae
54	*Saussurea costus* (Koot)	Asteraceae
55	*Schizostachyum dulloa* (Dolu)	Poaceae
56	*Solanum nigrum* (Makoi or black nightshade)	Solanaceae
57	*Stevia rebaudiana* (Meethi Tulsi, Candyleaf).	Asteraceae
58	*Syzygium cumini* (Jambu)	Myrtaceae
59	*Taxus bacata* (Yew)	Taxaceae
60	*Tinospora cordifolia* (Guduchi or Giloy)	Menispermaceae
61	*Valeriana hardwickii* (Indian valerian)	Caprifoliaceae
62	*Viola spp.* (Wild pansy)	Violaceae
63	*Withania somnifera* (Ashwagandha, Indian gingsen)	Solanaceae
64	*Zenthoxylum armatum* (Winged prickly ash)	Rutaceae

Processing of the soil samples

The nematodes were isolated from soil using modified Cobb's sieving and decanting method (Cobb, 1918) followed by Schindler modification, a substitute to the Modified Baermann funnel technique. The nematodes were identified on basis of morphology.

Community analysis

$$\text{Absolute frequency} = \frac{\text{No. of samples containing a genus}}{\text{No. of total sample collected}} \times 100$$

$$\text{Relative frequency} = \frac{\text{Frequency of a genus}}{\text{Sum of frequencies of all genus}} \times 100$$

$$\text{Absolute density} = \frac{\text{No. of individual of a genus in a sample}}{\text{Volume of sample}} \times 100$$

RESULTS

Results of the study revealed the presence of 11 genera of PPNs as identified on a morphological basis namely; *Aphelenchus* spp., *Criconemoides* spp., *Ditylenchus* spp., *Helicotylenchu*s spp., *Meloidogyne* spp., *Pratylenchus* spp., *Trichodorus* spp., *Tylenchorhynchus* spp., *Tylenchus* spp., *Rotylenchulus* spp., and *Xiphinema* spp. and classified accordingly belonging to 10 families (Table 2, 3) in 64 medicinal plants. PPNs associated with different medicinal plants showed variability between the plants and altitudes also.

At an altitude below 500 meters above mean sea level (180-383 meters above mean sea level), eight genera of PPNs namely *Pratylenchus* spp., *Helicotylenchus* spp., *Meloidogyne* spp., *Rotylenchulus* spp., *Tylenchorhynchus* spp., *Tylenchus* spp., *Circonemoides* spp., *Aphelenchus* spp. and *Trichodorus*

Table 2: Identification of plant-parasitic nematodes associated with the rhizospheric soil of medicinal plants

S. No	Nematode Identified	Important diagnostics characteristics	Body length (µm)	Stylet length (µm)
1	*Helicotylenchus* spp.	Stylet (robust, knobs flattened and anteriorly cupped), Body shape coiled when fixed and tail conoid-rounded	680-750	25-28
2	*Pratylenchus* spp.	Head is continuous with the body, heavy cephalic sclerotization, tail conoid or cylindrical extended up to tail tip, posterior vulva 70-80% of the body.	550-630	14-15
3	*Circonemoides* spp.	Large stylet with anchor- shaped knobs, Large ring-like annuli	420-450	30-32
4	*Trichodorus* spp.	Body(straight to slightly curved), Stylet Onchiostylet, Bursa absent	590-630	30-35
5	*Aphelenchus* spp.	Body(cylindrical\Slender), median bulb/metacarpus (large, filled the whole diameter of body), tail (blunt, round terminus)	627-725	16-18
6	*Tylechorhynchus* spp.	Stylet (short, cone- nneedle-like, tail (cylindroid with round tip)	750-840	15-20
7	*Tylenchus* spp.	Body bent ventrally, head continuous with bthe ody, short stylet, tail (curved tapering to end and filiform)	550-630	10-13
8	*Meloidogyne* spp.	Cephalic sclerotization (poorly developed), stylet (weak, cylinder, rounded knobs), tail (elongated, pointed tip without hyaline portion).	200-300	11-15
9	*Rotylenchulus* spp.	Body shape coiled when fixed, (stylet knobs rounded/anteriorly cupped), tail conoid	500-650	15-20
10	*Xiphinema* spp.	Body (long), Long stylet(flanged odontophore), guiding ring located in the ear base of odontostyle, males have paired spicules but no gubernaculum and bursa	600-750	25-50
11	*Ditylenchus* spp.	Shape (vermiform), stylet (short), Tail (Conoid and elongated), vulva (75 % of body length)	830-850	12-15

Table 3: Generalized classification of the plant-parasitic nematodes identified to be associated with medicinal plants in Uttarakhand state

Phylum	Class	Order	Family	Genera
Nematoda	Chromadorea	Tylenchida		
			Hoplolaimidae	*Helicotylenchus, Ditylenchus*
			Meloidogynidae	*Meloidogyne*
			Pratylenchidae	*Pratylenchus*
			Tylenchidae	*Tylenchus*
			Belonolaimidae	*Tylenchorynchus*
			Aphelenchidae	*Aphelenchus*
			Rotylenchidae	*Rotylenchulus*
			Criconematidae	*Criconemoides*
	Tylenchidae	Dorylaimida		
			Longidoridae	*Xiphinema*
			Trichodoridae	*Trichodorus*

spp., were harbouring in the rhizospheric soil of 26 medicinal plants. *Pratylenchus* spp. and *Meloidogyne* spp*.,* exhibited 96% frequency of occurrence while the least was recorded in *Circonemoides* spp. (65%). The highest population density of the PPNs was recorded in Safed Musli (*Chlorophytum borivilianum)* and Sarpagandha (*Rauvolfia serpentia*) whereas, least in Centella (*Centella asiatica*) and Pepper (*Piper nigrum*). Among all the tested plants, only six plants viz., Aloe-vera (*Aloe barbadensis*), *Saffola* (*Carthamus tinctorius*)*,* Safed Musli (*Chlorophytum borivilianum*)*,* Tulsi (*Ocimum tenuiflorum*), Lemon grass (*Cymbopogon flexuosus*), Mentha (*Mentha piperita*) and Ashwagandha (*Withania somnifera*) showed association with all the recorded PPNs (Fig. 2).

At an altitude of 900-1900 meters above mean sea level, nine PPN genera *Aphelenchus* spp.*, Circonemoides* spp. *Helicotylenchus* spp., *Meloidogyne* spp. and *Pratylenchus* spp., *Rotylenchulus* spp., *Tylenchorhynchus* spp., *Tylenchus* spp., *Circonemoides* spp*.,* and *Trichodorus* spp., were observed from eight medicinal plants. All nematodes showed (100%) frequency except *Tylechorhynchus* spp*., Trichodorus* spp. and *Rotylenchulus* spp. (87.5%). were found. Tulsi (*Ocimum tenuiflorum*) was the plant with a maximum population of all the PPNs followed by Aloe-vera (*Aloe barbadensis*), while minimum in Lemongrass (*Cymbopogon flexuosus*). All the eight plants showed association with all the PPNs except for Giloy (*Tinospora cordifolia*), Lemongrass (*Cymbopogon flexuosus*)

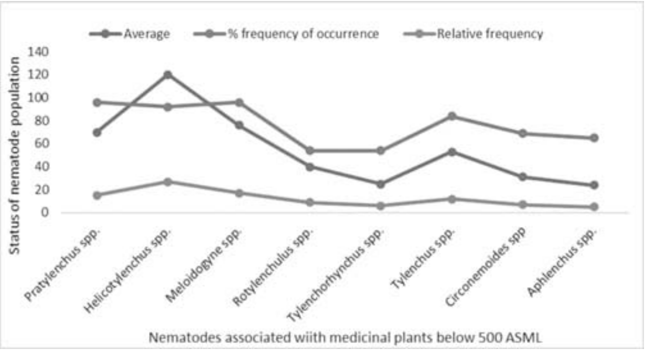

Fig. 2. Graphical representation of nematode population in per 200cc soil

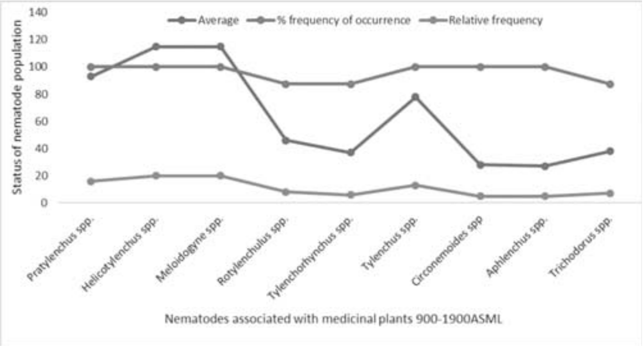

Fig. 3. Graphical representation of nematode population in per 200cc soil

Table 4: Plant parasitic nematodes associated with medicinal plants at 500 meters above mean sea level

S. No	Host Plants	*Pratylenchus* spp.	*Helicotylenchus* spp.	*Meloidogyne* spp.	*Rotylenchulus* spp.	*Tylenchorynchush* spp.	*Tylenchus* spp.	*Circonemoides* spp.	*Aphelenchus* spp.	Total
1.	Tulsi	40	179	150	94	8	64	37	10	582
2.	Stevia	55	155	153	125	58	39	122	0	707
3.	Alovera	33	110	110	83	23	66	13	33	471
4	Mentha	61	224	241	53	11	108	42	30	770
5	Bahrami	95	178	133	10	0	133	25	21	595
6	Lemongrass	71	179	71	7	4	29	29	58	448
7	Giloy	39	186	6	0	0	30	3	5	269
8	Ashwagandha	50	97	120	46	13	70	43	33	472
9	Patchouli	33	166	0	0	0	83	0	33	315
10	Sarpagandha	108	232	149	0	0	91	183	25	788
11	Chamomile	191	108	116	0	125	33	0	33	606
12	Chandarsur	66	0	0	116	0	16	50	83	331
13	Lawsonia	66	216	83	0	33	100	16	50	564
14	Safola	33	83	33	150	16	33	50	0	398
15	Artemisia	233	0	100	0	0	33	0	0	366
16	SafedMusli	100	200	16	233	150	83	33	83	898
17	Geranium	83	67	100	0	0	117	0	17	384
18	Centella	33	50	13	0	0	0	17	33	146
19	Sataver	17	83	33	0	0	83	13	50	279
20	Vach	0	100	67	50	0	67	0	17	301
21	Solanum	50	117	50	0	0	17	33	0	267
22	Kalmegh	33	83	33	0	33	0	33	0	215
23	Palmarosa	17	100	17	33	50	0	0	0	217
24	Citronella	150	50	117	13	0	50	0	0	380
25	Pepper	0	116	0	0	33	0	0	0	149
26	Kasni	83	50	67	17	100	33	67	17	434

Table 5: Plant-parasitic nematodes associated with medicinal plants below 900-1900 meters above mean sea level

S. No	Host Plants	*Pratylenchus* spp.	*Helicotylenchus* spp.	*Meloidogyne* spp.	*Rotylenchulus* spp.	*Tylenchorhynchus* spp.	*Tylenchus* spp.	*Circonemoides* spp.	*Aphelenchus* spp.	*Trichodorus* spp.	Total
1.	Aloevera	130	154	69	27	11	195	40	47	133	806
2.	Tulsi	133	138	233	154	40	89	40	50	17	894
3.	Stevia	157	90	191	43	44	85	40	3	45	698
4.	Giloy	69	193	71	0	14	30	46	13	13	449
5.	Mentha	120	55	158	59	38	77	9	24	33	573
6.	Lemongrass	29	37	54	8	0	50	13	29	17	237
7.	Bahrami	54	22	35	27	26	40	2	41	50	297
8.	Ashwagandha	50	233	93	47	118	56	33	8	0	638

Table 6: Plant-parasitic nematodes of associated with medicinal plants above 2000 meters above mean sea level

S. No.	Host Plants	*Helicotylenchus* spp.	*Tylenchorhynchus* spp.	*Tylenchus* spp.	*Aphelenchus* spp.	*Rotylenchulus* spp.	*Meloidogyne* spp.	*Pratylenchus* spp.	*Trichodorus* spp.	*Ditylenchus* spp.	*Criconemoides* spp.	*Xiphinema* spp.	Total
1	Mentha	133	167	17	0	0	100	83	0	0	0	0	500
2	Tulsi	350	17	100	50	50	217	50	0	0	33	0	867
3	Bhrami	50	67	0	0	0	350	17	0	0	0	0	484
4	Giloy	250	0	83	0	17	67	50	0	0	17	0	484
5	Lemon grass	83	50	0	83	0	17	83	0	0	33	0	349
6	Stevia	0	367	233	67	0	83	217	0	0	0	0	967
7	Aloe vera	0	50	33	83	0	133	67	0	0	17	0	283
8	Patharchata	150	0	17	0	0	50	100	0	0	33	0	350
9	Puskar Mool	0	1200	87	17	0	0	0	0	0	0	0	1460
10	Hippi	0	13	193	67	0	0	0	07	0	0	0	280
11	Neebu	13	47	33	173	0	0	207	0	0	0	0	473
12	Dolu	0	47	93	53	0	0	13	0	40	0	0	246
13	Bari Ilaychi	0	0	327	73	0	0	20	07	0	0	0	427
14	Chura /Amesh	0	53	40	20	0	0	453	0	0	0	0	566
15	Koot	27	0	413	287	0	0	47	0	87	0	0	861
16	Tulsi	40	13	253	160	0	0	33	0	0	0	0	499
17	Sameva	27	0	107	140	0	0	33	0	0	0	0	307
18	Kala Jeera (Himanchali)	13	0	160	13	07	0	0	0	20	0	0	213
19	Kala Jeera (Local)	20	0	333	73	0	213	07	53	0	0	0	699
20	Wild caraway	907	0	533	13	0	667	0	67	13	0	0	2200
21	Yew	53	07	60	0	0	40	0	0	0	07	0	167
22	Golden root	73	0	227	20	0	12	0	0	0	0	0	332
23	Costus	07	0	133	07	0	940	0	0	0	0	0	1087
24	Gilgiti rhubarb	120	27	440	07	0	20	40	07	0	0	0	661
25	Jambu	333	0	252	52	0	127	0	0	27	0	0	791
26	Whorled Solomon's Seal	07	0	160	87	0	0	0	0	20	0	0	274
27	Hogweed	0	0	100	487	0	73	0	87	07	0	0	754
28	Bitter-Root	07	07	67	07	0	20	0	20	0	0	0	128
29	Fairy Grass	73	0	20	0	0	0	0	07	0	0	0	100
30	Foxglove	47	0	40	0	0	07	0	0	0	0	0	94
31	Primrose	0	453	213	120	0	147	0	0	0	0	0	933
32	Mahonia	12	0	12	12	07	20	0	0	0	0	0	63
33	Bajradanti	54	200	187	27	167	553	0	07	0	0	07	1202
34	Sandharlika	07	0	20	32	0	0	0	0	0	0	0	59
35	Timur	27	0	153	07	0	0	0	12	0	0	0	199
36	Corylus	07	0	167	0	0	0	0	07	0	0	0	181
37	Viola	47	200	187	27	167	553	0	07	0	0	07	1195
38	Giant lily	0	0	1107	20	0	12	0	07	0	0	07	1153
39	Sugandhbala	12	20	07	27	54	133	0	0	0	0	0	253
40	Small Alpine Onion	20	0	47	0	0	0	0	07	07	0	0	81
41	Satuwa	07	12	87	07	0	0	0	0	0	0	0	113
42	Salammishri	07	0	22	33	0	0	107	0	13	0	0	586
43	Pashanbhed	0	0	0	0	0	0	0	0	0	0	0	233
44	Satavar	40	0	87	113	0	0	53	0	07	0	0	300
45	Chopcheeni	3973	27	73	67	0	0	40	0	0	0	0	4180
46	Salampanja	200	0	07	180	0	0	0	0	07	0	0	394

and Ashwagandha (*Withania somnifera*) which lacks the presence of *Rotylenchulus* spp., *Tylenchorhynchus* spp., and *Trichodorus* spp., respectively (Fig. 3).

A total of 46 medicinal plants were surveyed at an altitude above 2000 meters above mean sea level, where 11 genera of PPNs namely *Aphelenchus* spp., *Circonemoides* spp. *Helicotylenchus* spp., *Meloidogyne* spp. and *Pratylenchus* spp., *Rotylenchulus* spp., *Tylenchorhynchus* spp., *Tylenchus* spp., *Trichodorus* spp., *Ditylenchus* spp., and *Xiphinema* spp. were recorded. *Meloidogyne* spp. was most prevalent with maximum frequency (96%) followed by *Pratylenchus* spp. (80%), while minimum by *Xiphinema* spp. (6%). Chopcheeni (*Samilax china*) recorded the highest population of PPNs with the maximum density of *Helicotylenchus* spp. followed by Wild Caraway (*Carum carvi*) with a higher population density of *Meloidogyne* spp. while there were only three genera of PPNs parasitizing Sandharlika (*Hedychium spicatum*) with the lowest population. Two new genera of PPNs namely *Ditylenchus* spp. were found in association with only four plant and *Xiphinema* spp., with two plants (Fig. 4).

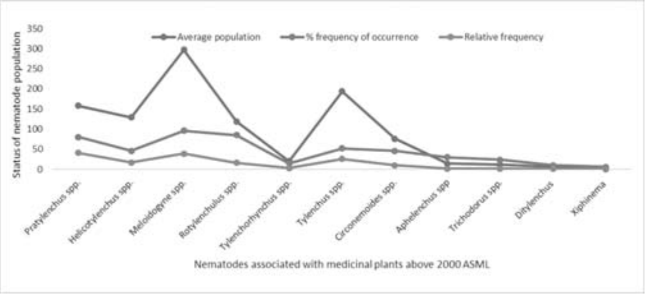

Fig. 4. Graphical representation of nematode population in per 200cc soil

DISCUSSION

The growth and productivity of plants are greatly affected by PPNs (Mateille *et al.* 2016). Nematodes of the order Tylenchida are considered as a major pest of the crop (Bernard *et al.* 2017). Jones *et al.* (2013) listed *Aphelenchoides bessseyi*, *Bursaphelenchus xylophilus*, *Ditylenchus dispaci*, *Globodera* spp., *Heterodera* spp., *Meloidogyne* spp., *Naccobus aberrans, Pratylenchus* spp., *Radopholus similis*, *Rotylenchulus reniformis* and *Xiphinema index* as the most damaging top 10 PPNs because of their scientific and economic significance. Shukla *et al.* (1986) observed 8 nematode species viz. *Hoplolaimus indicus*; *Helicotylenchus indicus*; *Hemicriconemoides communis*; *Tylenchorhynchus brassicae*; *Tylenchus filiformis*; *Rotylenchulus reniformis*; *Xiphinema* spp. and *Longidorus* spp. infesting medicinal plants in Allahabad district of Uttar Pradesh. Rathour *et al.* (2003) in their report, mentioned the occurrence of *Hoplolaimus indicus, Helicotylenchus dihystera, Meloidogyne incognita, M. javanica* and *Rotylenchulus reniformis, Hemicriconemoides cocophilus, B. spectabilis, Xiphinema insigne* and *Longidorus elongatus* from some perennial ornamental and medicinal plants. Gupta and Mondal (2018) revealed the presence of plant parasitic nematode species, *viz. Rotylenchulus reniformis, Helicotylenchus dihystera, Helicotylenchus indicus, Tylenchorhynchus brassicae, Tylenchorhynchus mashoodi, Hoplolaimus indicus, Hemicriconemoides communis, Hemicriconemoides mangiferae, Helicolylenchus crenacauda, Xiphinema americanum*, and *Hoplolaimus indicus* associated with the medicinal plants of West Bengal. Rathour *et al.*, (2006) reported nine genera of PPNs *viz. Meloidogyne* spp., *Helicotylenchus* spp., *Pratylenchus* spp., *Tylenchorhynchus* spp., *Trichodorus* spp., *Quinisulcius* spp., *Rotylenchulus* spp., *Hemicriconemoides* spp. and *Hoplolaimus* spp. from Champawat district (1600m) of Uttarakhand. Most of the above mentioned PPNs were also recorded from our study also as *Meloidogyne* spp., *Pratylenchus* spp., *Trichodorus* spp., *Helicotylenchu*s spp., *Tylenchorhynchus* spp., *Ditylenchus* spp., *Rotylenchulus* spp., *Criconemoides* spp., *Aphelenchus* spp., *Xiphinema* spp., and *Tylenchus* spp. associated with the medicinal plants surveyed from different altitudes of Uttarakhand.

Pattharchaat (*Bryophyllum pinnatum*), Tulsi (*Ocimum tenuiflorum*), Stevia (*Stevia rebaudiana*), Safed musali (*Cholrophytum borivilianum*), and Mentha (*Mentha piperita*) plants recorded root knot nematode population in the soil from our study which are in harmony with Sultan *et al.* (2009) from Punjab who reported root knot nematode infection in roots of Pattharchaat (*Bryophyllum pinnatum*), Kali tulsi (*Ocimum tenuiflorum*), Ashwagandha (*Withiana somnifera*), Patchouli (*Pogostemon cablin*), and Mentha (*Mentha piperita*). Similarly Shivalingappa *et al*. (2017) from Karnataka, revelead the occurrence of *Meloidogyne incognita*, *Helicotylenchus dihystera* and *Pratylenchus penetrans* with plant Patchouli (*Pogostemon cablin)* which coincides with our records while *Aphelenchus* spp. was observed during our study. This represents the presence of *Aphelenchus* spp. in the surveyed area of Uttarakhand which has to be compared with other states related to medicinal plants and it needs further attention.

Pandey, 2003 from CIMAP experiment station Lucknow mentioned *Meloidogyne incognita, Helicotylenchus, Pratylenchus*, *Tylenchorhynchus* and *Tylenchus* from Safed musli (*Chlorophytum borivillianum*), as same PPNs were recorded from our studies also however 3 new species *Rotylenchulus, Criconemoides* and *Aphelenchus* were also obsereved during the present study from the same crop.

Furthermore, the results revealed that *Meloidogyne* spp. and *Heliocotylenchus* spp. are one of the most damaging pathogens of nematode group which needs to be monitored carefully. When it comes to frequency of nematodes on medicinal plants, genus *Helicotylenchus* was found on all medicinal plants excluding 11 plants. Samathanam and Chawla (1982) reported that *Helicotylenchus* spp. and *Meloidogyne* spp. are associated with field crops, medicinal plants and trees in the hilly areas of a southern district of Tamil Nadu. This means these two genera are frequent in the rhizospheric soil of medicinal plants.

CONCLUSION

The outcome of this study provides knowledge of PPNs status and the high diversity of medicinal plants in Uttarakhand at various altitudes. *Helicotylenchus* spp. was most predominant followed by *Meloidogyne* spp. among all the nematode population whereas the least was shown by *Tylenchorhynchus* spp. At higher altitudes of Munsiyari *Tylenchus* spp., was predominant along with the presence of *Xiphinema* spp. and *Ditylenchus* spp. population in that area.

The present study provides a brief overview regarding the rich diversity of plant parasitic nematodes in association with medicinal plants, which might pose a severe danger to their production and has to be of great concern. Awareness among framers and knowledge to extension workers is crucial so that it can be timely diagnosed and managed properly otherwise these plants will act as a future hosts for these nematodes. The effect of climate change has been noticed due to which new host /habitat have been recorded on which the concerned species were not reported earlier; hence, more studies are still required.

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SHORT COMMUNICATION

A Simple Technique for Culturing *Meloidogyne graminicola* in the Laboratory

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Meloidogyne graminicola is considered as one of the major constraints of successful cultivation of rice. This endoparasitic nematode produces hook shaped galls at the terminal end of rice roots, resulting in reduction in growth, reduced tillering, chlorosis of young plants, and production of unfilled grains at maturity (Babatola, 1984; Hunt and Handoo, 2009). This pest is responsible for a loss in yield of rice throughout the world, which may range from 15-80 percent (Prot *et al.,* 1994 and Plowright and Bridge, 1990). This pest was considered as major threat for direct seeded rice, but extensive monoculture of rice with need based irrigation makes it a major threat to transplanted rice in Northern India (Haque *et al.,* 2018). The importance of this pest has been increasing throughout the world owing to present day water conservation rice cultivation.

Culturing of nematodes is one of the prerequisites for conducting the laboratory studies like screening of germplasm under inoculated condition, biology of the organism, host-parasitic relationship etc. Being obligate parasite, nematodes are to be cultured in living host plant, which is generally done in pots or microplots. Kumar *et al.* (2017) have developed one protocol for soil less system for culturing *M. graminicola*. The present technique enables to culture this nematode in laboratory on Petri Plates.

A clean 90mm or 160mm diameter PetriPlates is taken. At the bottom of the plates around 50 number of rice seeds of susceptible variety is placed (Fig.1A). The seeds are covered with a mixture of sand and field soil at 3:1 ratio (Figs. 1B and 1C). Seeds should be placed at the bottom of the PetriPlates (Fig. 1D). Soil is moistened by sprinkling water. Inundation or over watering should be avoided. The seedlings are inoculated with galled rice roots (galled roots may be collected from field or pure culture; chopped into smaller pieces and then placed on the soil around seedlings) when the seedlings are about 1 inch long (Fig. 1E) . The plates are kept as such at room temperature. Sprinkling of water to be done, as and when necessary. After five to six days of inoculation (at 28-30^0C), tiny terminal galls can be seen at the bottom of the PetriPlates. After 30 days of inoculation, the root mat will bear matured gall and can be seen easily at the bottom of Petri Plate through the glass (Fig. 1F). Seedlings can be pulled out very easily without detachment of the terminal galls from the root system, which is most common problem in conventionally used technique of culturing *M. graminicola,* and can be used for conducting further studies. The culturing of *M. graminicola* following this technique is very handy, easily transferable and can easily be maintained on the desk of laboratory with minimum space.

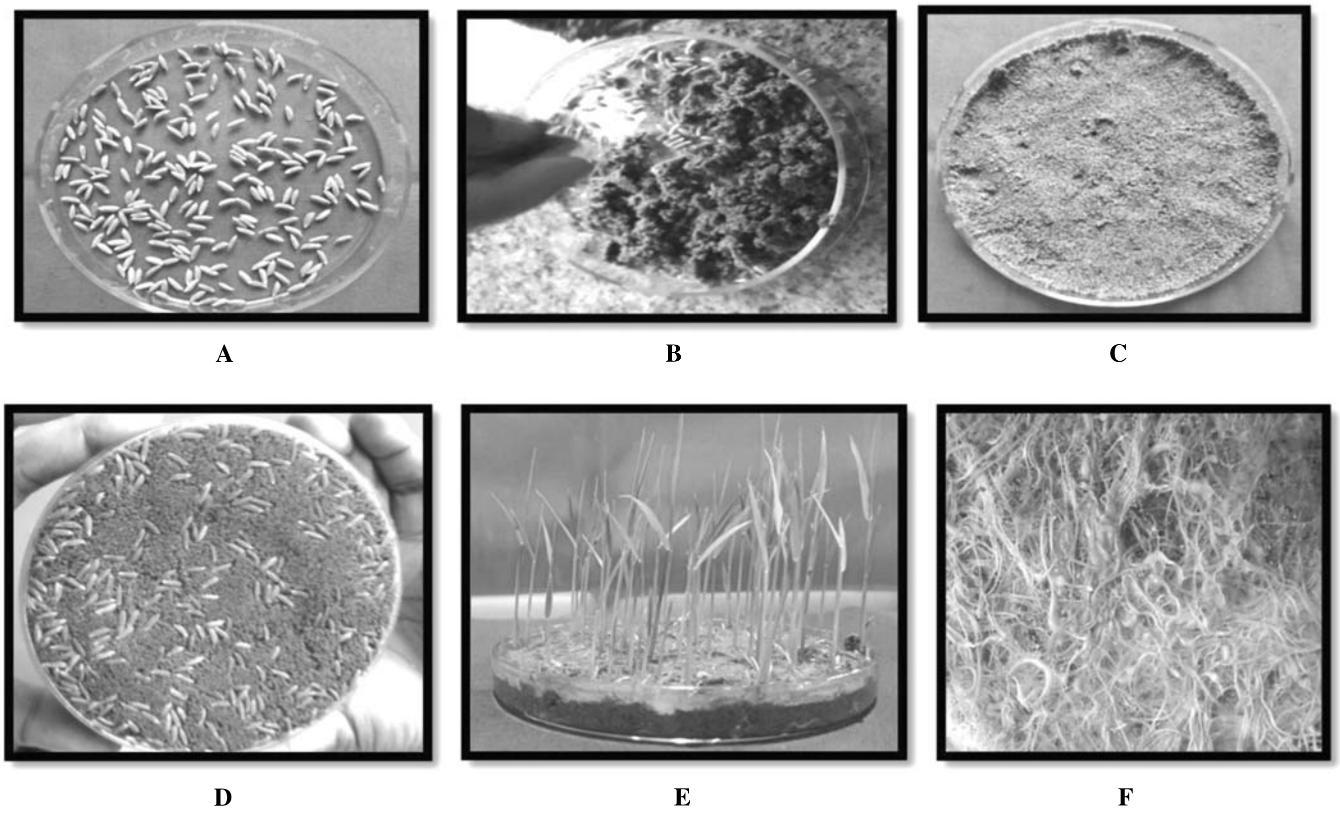

Fig. 1. Photographs of various stages in culture of *M. graminicola* on rice in Petri dishes

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Migration of Infective Juveniles of Entomopathogenic Nematode, *Heterorhabditis indica* in Soil after Application through Fully Automatic High Pressure Drip Irrigation System

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Entomopathogenic nematode (EPN), *Heterorhabditis indica* confers high virulence against soil insect pests and have been used widely against many economically important insect pests of crops (Grewal *et al.,* 2005; Mohan *et al.,* 2016). As EPNs are tolerant to shear stress, they can survive under high pressure (Fife *et al.*, 2003). Studies showed that EPN species can resist up to 14 bar (Wright *et al.,* 2005). In the current agricultural scenario, there is a shift towards precision farming and resource conservation, use of drip irrigation technology, wherein precise and slow application of water in the form of discrete or continuous drops through mechanical devices called emitters in the root zone of the plants, provides more efficient utilization of water. The objective of this study was to evaluate the migration behaviour of infective juveniles of *H. indica* after their passing through Fully Automatic High Pressure Drip Irrigation System in the field to facilitate *H. indica* to establish itself as a potent bio-control agent under Indian conditions.

The experiment was conducted at CPCT, ICAR-IARI, New Delhi during 2020. Infective juveniles (IJs) of *H. indica* were multiplied in the laboratory on *Galleria mellonella* larvae *in vivo*, following the standard procedures (Kaya and Stock, 1997). Freshly emerged infective juveniles from the wax moth larval cadavers were collected in sterilized distilled water using White's trap (White, 1927) and used for subsequent studies within 2-4 weeks.

The field dimensions were 100 m × 30 m. The drip irrigation system was installed by Netafim Irrigation Co. Fallow plots used for small scale injection of IJs consisted of six drip laterals each 10 m long. The pressure compensating emitters were 1.0 m apart with a flow rate of 1.0 L/ h. Three release pressures (200, 300 and 400 kPa) and two types of pipes having diameters of 16 mm and 20 mm were evaluated. Treatments were assigned in a completely randomized design and were replicated three times. A 100 ml dilution of one million *H. indica* IJs was prepared. The IJs were injected by removing the filter at the point where the drip line left the main line. The injection port was then sealed to avoid nematode leakage. The irrigation pump was stopped during the nematode injection process and the lines were pressurized after application for 15 minutes. Each drip lateral was a replicate.

To evaluate migration of IJs of *H. indica* applied via drip irrigation system in the field, nematodes release points on the soil by emitters of each of the six laterals (total 60 emitters) were marked. Thirty emitters were marked for horizontal migration studies and rest thirty for vertical migration studies. The migration of IJs was observed at 2, 3 and 4 inch distance at four time intervals viz. 24, 48, 72 and 96 h post release. Three replicates of each treatment were maintained. After 24, 48, 72 and 96 hours, about 20 g of soil was taken in plastic tubes at a distance of 2, 3 and 4 inch from the release point of 30 emitters randomly on all the four sides for horizontal migration studies. Soil samples were also collected at a depth of 2, 3 and 4 inch from the release point of 30 emitters for vertical migration studies. Samples were collected destructively from seven release points at a time for each type of migration study. Each sample set up was accompanied with control, in which soil from the same field was taken where no EPNs were released. Additionally, a check of zero pressure (approximately 1500 IJs in an aqueous suspension of 10 ml were released in the same field on pre-determined spots marked 1m apart along the drip lines) was also taken. This arrangement was replicated thrice. Infectivity profile of IJs of *H. indica* was developed following a filter paper technique reported by Miller (1989). Factorial CRD using OP Stat software was used for the statistical analysis of the data on vertical and horizontal migration studies.

The data obtained on the mortality of *Galleria mellonella* larvae as an indicator of migration of IJs of *Heterorhabditis indica* applied in the field via fully automated high pressure drip irrigation system after 96 h is presented in table 1.

IJs were intercepted up to 4 inch horizontally as well as vertically after 96 hrs, but they were more concentrated at a horizontal distance of 3 inch compared to 2 and 4 inches from the point of release and is revealed by pooled mean (A) for horizontal migration. Significantly maximum mortality at a horizontal distance of 3 inches from the point of release was recorded compared to 2 and 4 inch distances. Pooled mean (A) for vertical migration revealed that highest number of IJs migrated up to 4 inch vertically causing maximum mortality (48.57%) as compared to 2 and 3 inch. The interactions of all the three factors with each other and among themselves were non-significant.

Nematodes need a thin water film in soil pore spaces for movement. If thickness of the water film is approximately half the thickness of the nematodes' body, it is optimum for nematode movement (Wallace, 1958). Inactivity of EPNs in the soil is a behavioral strategy for their persistence in the soil. They become active in response to various mechanical and chemical stimuli. Migration of EPNs in the soil increases their chances for encountering a susceptible host as well as permits them escape from unfavourable habitats (Ishibashi and Kondo, 1990). Choo and Kaya (1991) demonstrated that shape and size of the root system of the plant influence the distribution of target insects as well as migration and host finding of nematodes.Paunikar and Kulkarni (2020) evaluated the effect of soil texture, moisture and depths on survival and infectivity of *Steinernema dharanaii* against *G. mellonella*. The IJs could move easily at soil moisture level of 10 to 20% up the distance of 3.5 cm, whereas at moisture level 40.0%, there was no IJ movement. Since the physical edaphic properties (temperature, moisture content etc.) affect the nematode migration behaviour to a large extent.

It can be concluded that after 96 h, horizontally maximum number of IJs were concentrated at 3 inch distance compared to 2 and 4 inch distance; while vertically, maximum concentration IJs was recorded at 4 inch distance compared to 2 and 3 inch distance as evident by maximum mortality of *G. mellonella* larvae at these points. Also, movement of IJs was positively correlated with time. The rate of downward migration was faster compared to lateral movement, suggesting that IJs showed positive geotropism. Regulating pressure of drip irrigation system or use of pipes having diameters of 16 or 20 mm had no effect on the migration of *H. indica* IJs post application in the field.

Table 1: Horizontal and vertical migration of *Heterorhabditis indica* in the soil after 96 h of application via fully automated high pressure drip irrigation system as indicated by mortality of *Galleria mellonella* larvae after

(Mean of 3 replications)

Distance (inch)(A)	Pipe diameter (mm)(B)	Horizontal migration: Pressure (^{a}kPa) (C) 0	200	300	400	Control	Mean (A×B)	Pooled mean (A)	Vertical migration: Pressure (kPa) (C) 0	200	300	400	Control	Mean (A×B)	Pooled mean (A)
	16	9.53 (14.81)	14.29 (18.18)	9.52 (10.78)	9.53 (14.81)	0.00 (0.00)	8.57 (11.72)		9.52 (10.78)	14.29 (18.18)	19.05 (21.04)	19.05 (25.59)	0.00 (0.00)	12.38 (15.12)	
2	20	9.53 (14.81)	14.29 (18.18)	9.52 (10.78)	9.53 (14.81)	0.00 (0.00)	8.57 (11.72)	8.57 (11.72)	9.52 (10.78)	14.29 (18.18)	14.29 (18.18)	19.05 (25.59)	0.00 (0.00)	11.43 (14.55)	11.91 (14.83)
	Mean (A×C)	9.53 (14.81)	14.29 (18.18)	9.52 (10.78)	9.53 (14.81)	0.00 (0.00)			9.52 (10.78)	14.29 (18.18)	16.67 (19.61)	19.05 (25.59)	0.00 (0.00)		
	16	28.57 (31.82)	14.29 (18.18)	28.57 (31.82)	28.57 (31.82)	0.00 (0.00)	20.00 (22.73)		23.81 (28.96)	38.10 (38.05)	38.10 (38.05)	38.10 (38.05)	0.00 (0.00)	27.62 (28.62)	
3	20	28.57 (32.33)	14.29 (18.18)	23.81 (28.96)	23.81 (28.96)	0.00 (0.00)	18.10 (21.69)	19.05 (22.21)	23.81 (28.96)	33.34 (34.68)	33.33 (35.19)	38.10 (38.05)	0.00 (0.00)	25.72 (27.38)	26.67 (28.00)
	Mean (A×C)	28.57 (32.08)	14.29 (18.18)	26.19 (30.39)	26.19 (30.39)	0.00 (0.00)			23.81 (28.96)	35.72 (36.37)	35.72 (36.62)	38.10 (38.05)	0.00 (0.00)		
	16	14.29 (18.18)	4.76 (7.41)	14.29 (18.18)	14.29 (18.18)	0.00 (0.00)	9.52 (12.39)		57.14 (49.13)	57.14 (49.25)	66.66 (55.36)	76.19 (61.09)	0.00 (0.00)	51.43 (42.97)	
4	20	9.53 (14.81)	4.76 (7.41)	14.29 (18.18)	9.53 (14.81)	0.00 (0.00)	7.62 (11.04)	8.57 (11.72)	52.38 (46.39)	47.62 (43.65)	61.90 (51.99)	66.67 (54.86)	0.00 (0.00)	45.71 (39.38)	48.57 (41.18)
	Mean (A×C)	11.91 (16.50)	4.76 (7.41)	14.29 (18.18)	11.91 (16.50)	0.00 (0.00)			54.76 (49.26)	52.38 (46.45)	64.28 (53.68)	71.43 (57.98)	0.00 (0.00)		

Mean (B×C)

	0	200	300	400	Control	Pooled mean B
16	17.46 (21.60)	11.11 (14.59)	17.46 (20.26)	17.46 (21.60)	0.00 (0.00)	12.70 (15.61)
20	15.88 (20.65)	11.11 (14.59)	15.87 (19.31)	14.29 (19.53)	0.00 (0.00)	11.43 (14.81)
Pooled mean C	16.67 (21.13)	11.11 (14.59)	16.67 (19.78)	15.88 (20.57)	0.00 (0.00)	

Factors	SE(m)	C.D. at 5%
Distance (A)	1.71	4.84
Pipe diameter (B)	1.40	NS
A × B	2.42	NS
Pressure (C)	3.21	NS
A × C	4.82	NS
B × C	3.12	NS
A × B × C	5.40	NS

Mean (B×C)

	0	200	300	400	Control	Pooled mean B
16	30.16 (29.62)	36.51 (35.16)	41.27 (38.15)	44.45 (41.58)	0.00 (0.00)	30.48 (28.90)
20	28.57 (28.71)	31.75 (32.17)	36.51 (35.12)	41.27 (39.50)	0.00 (0.00)	27.62 (27.10)
Pooled mean C	29.36 (29.17)	34.13 (33.67)	38.89 (36.64)	42.86 (40.54)	0.00 (0.00)	

Factors	SE(m)	C.D. at 5%
Distance (A)	2.24	6.35
Pipe diameter (B)	1.83	NS
A × B	3.17	NS
Pressure (C)	3.90	NS
A × C	5.02	NS
B × C	4.10	NS
A × B × C	7.10	NS

^{a}kilo Pascal. Values in parentheses are *arc sine* transformed. SE (m): Standard Error of Mean; CD: Critical difference; NS: Non-significant

It is, therefore, concluded from this study that the EPNs that survived the hydrodynamic conditions of drip irrigation system were not damaged and were able to maintain their migration equivalent to those EPNs that had not been applied through drip irrigation besides the use and relevance of application of their formulations through drip irrigation. The results of present study are only indicative of the migration potential of IJs of *H. indica* applied via drip irrigation system. Nematode migration in field conditions depends on a plethora of other factors such as the presence of the host insect, physical and chemical properties of the soil, soil temperature, and characteristic of root systems etc.

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Effect of Ecological Factors on *Meloidogyne incognita* Population Dynamics Associated with Okra Based Cropping Sequences

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Okra (*Abelmoschus esculentus* L. Moench) is an important vegetable crop commercially grown in most of tropics and subtropics with an extension to the Mediterranean climate (Duzyaman and Vural, 2003). It is also called a "perfect villager's vegetable" because of its robust nature, dietary fibers and distinct seed protein balanced in essential amino acids like lysine and tryptophan, necessarily required in human diet (Kumar *et al*., 2010). It is grown extensively under various agro-climatic zones of India, adversely affected by the root-knot nematode, *Meloidogyne incognita* (Khan and Pariari, 2013), causing approximately 50% of the overall damage. In India, loss in okra due to *M. incognita* has been reported in monetary terms to the tune of US$ 8.7 million and estimated over all annual yield losses more than 14% (Jain *et al.,* 2007). Soil temperature, soil moisture and rainfall play a vital role in the population build up of plant parasitic nematodes (Das *et al.,* 2013). Information on population structure of nematodes is important for the development of an effective management schedule and advisory service (Chawla & Mittal, 1995). In the above-mentioned context, an experiment regarding seasonal variation of *M. incognita* population in relation to different edaphic factors in okra-based cropping sequence was carried out to provide a valuable data base for the development of effective management schedule and advisory services.

The study was carried out in a field naturally infested with root knot nematode, *M. incognita* at Department of Nematology, OUAT, Bhubaneswar, Odisha, India from February, 2014 to January, 2016, located at 20°26' North Latitude and 85° 80' East Longitude at an elevation of 25.9 meters above the mean sea level. Soil was sandy loam in texture, slightly acidic (pH 5.70), low in organic carbon (0.47%), low in available nitrogen (238.5 kg/ha), high in available phosphorus (38.5 kg/ha) and medium in available potassium (261.4 kg/ha).

The sampling area for cropping sequence (Okra - Green gram - French bean) was represented by five different fix locations (replications) of one meter square area in a okra growing field of 0.1 ha in size (40.0 m x 25.0 m) by placing four stakes in each corner. The cropping sequence during the both the year was- okra (February to May), green gram (August to October) and french bean (November to January). The varieties "Utkal Gaurav", "Dhauli" and "Arka komal" were used for growing of Okra, Green gram and French bean respectively during both the year. Standard crop production practices and crop protection measures were followed as per crop production guide.

The composite soil samples (200 cc) were collected at a depth of 15-20 cm during first week of every month, from February, 2014 to January 2016.These composite samples were processed for extraction of nematodes by Cobb's sieving and decanting technique (Cobb, 1918) followed by 'Modified Baerman's funnel technique' (Christie & Perry, 1951). The nematodes were extracted for 48 h at room temperature and fixed in 4% formalin and counted subsequently in a counting dish using a stereoscopic zoom microscope.

Soil temperature was recorded at a depth of 20 cm below the ground during the time of sampling with the help of a soil thermometer. At the time of sampling, soil was collected for determination of soil moisture following Gravimetric method (Khanna & Yadav, 1979). The data on mean monthly atmospheric maximum and minimum temperature, relative humidity, rainfall data was collected from the Department of Meteorology, Orissa University of Agriculture and Technology, Bhubaneswar. The data on population of root knot nematode was correlated with different abiotic parameter recorded during the period of experimentation. The correlation coefficient (r) was calculated as per Karl Pearson correlation coefficient (Snedecor and Cochran, 1967).

The data on the population growth pattern of *M. incognita* in okra based cropping sequence during both the year (2014-15 and 2015-16) revealed that, the nematode population was higher and lower during the warmer and winter months respectively. During both the year, the average soil temperature was lowest in December and highest in May. After October, soil temperature declined and reached the minimum level during December. Thereafter, temperature increased till May. The lowest and highest average atmospheric temperature were recorded in December and May respectively during both the year. It maintained a high level during April to June (>30^{0}C) and then declined till December. *M. incognita* exhibited a pattern of population fluctuation with a prominent peak during March and a low peak during October every year (Fig.1 & 2). Likewise, there was a minimum during December. The highest peak (3657.0 J_2/200 cc soil) nematode population was recorded in March in okra crop followed by other peak (2853.0 J_2/200 cc soil) in October in green gram (Fig.1) during the year 2014-15. The same trend in nematode population was observed during 2015-16. The highest peak (4211 J_2/200 cc soil) nematode population was recorded in March in okra crop followed by other peak (2544 J_2/200 cc soil) in October in green gram (Fig.2). The nematode population exhibited a significant positive correlation with atmospheric temperature, soil moisture and soil temperature during both the years (Table 1 &2). The present study clearly indicated that yearly there were two peaks of *M. incognita* population in okra based cropping sequence. These peaks were observed during October and March in the both the years which were linked to host crop, atmospheric temperature, soil temperature and soil moisture. When there was maximum rainfall observed during the month July and August, the nematode population was comparatively lower than other months. The population increased after rainfall but declined during post monsoon period and maintained a low level during winter. During early spring population again increased but summer caused a decline in the number. Present investigation revealed that populations

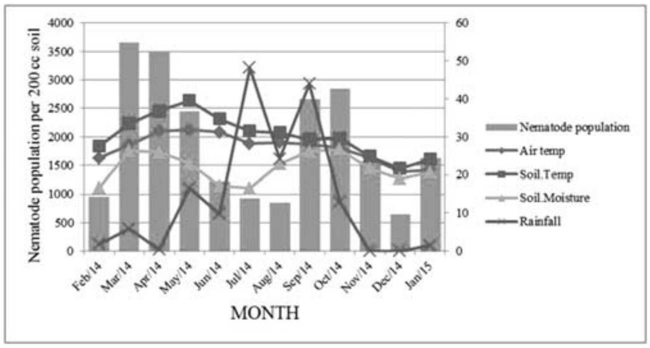

Fig. 1. Population growth of root knot nematode in okra based cropping sequence during the year 2014-15

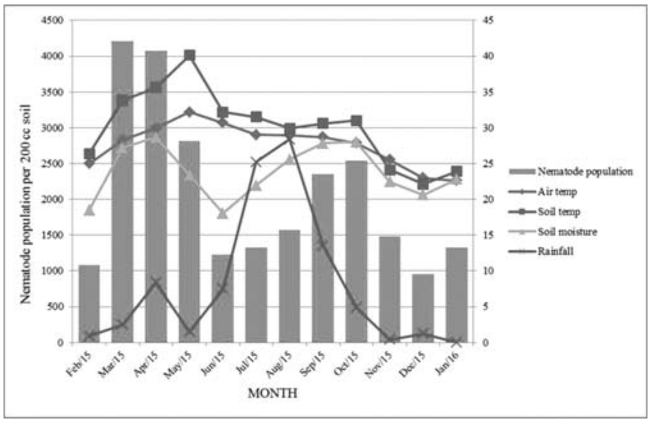

Fig. 2. Population growth of root knot nematode in okra based cropping system during the year 2015-16

Table 1. Correlation coefficient (r) of root-knot nematode population with abiotic parameters in okra based cropping sequence during the year 2014-16

Root knot nematode (*M. incognita*) population	Abiotic parameters			
	Atmospheric temperature	Soil temperature	Soil moisture	Rainfall
2014-15	0.398	0.45	0.87	-0.097
2015-16	0.468	0.664	0.767	-0.13

of root knot nematode was low in December and January due to low temperature and low moisture content of soil.

However, the root-knot nematode population did not show any significant correlation with rainfall in 2014-15, but showed negative correlation in 2015-16. Temperature, humidity, light, aeration of the soil, age and nutritional status of the host may influence the biological activities in the life cycle and development of *Meloidogyne* spp. while both the host plant and its environment will influence the population dynamics of these parasites. This finding on population fluctuations is in conformity with the findings of Deori and Das (2013) who reported the maximum nematodes population of *M. incognita* during the warmer months of the year in rhizosphere of banana. The low temperature and moisture also might have had adverse effects on the nematode population (Pandey, 1999). As the temperature increased in March, the nematode populations tended to increase. Boag (1980) reported that temperature influences feeding rate of nematodes, reproduction and population level also increased. The higher soil temperature might have cause desiccation and dryness of soil due to scanty soil moisture waiving necessary thin film of water around them for the movement and nematodes are subjected to increased stress and consume a considerable amount of energy stored resulting reduction of their population density (Gaur, 1994). The studies conducted by Kamra and Sharma (2000) and Khan & Sharma (1990) also indicated that *M. incognita* having a positive correlation with temperature *i.e.,* population densities increased with the increase in temperature and maximum population was recorded at 29ºC. During rain, the soil moisture when increases, reduce the temperature of soil. Thus, it resulted in decrease in the nematode population build up as soil pores were over saturated with moisture causing sweeping out of the nematode in the flooded situation during August. Thereafter, population goes on increasing after August (post monsoon period) onward reaching peak in October. This might be due to optimum moisture conditions of soil and moderate temperature. Thus the present study suggested that, soil temperature and moisture favored nematode population growth but the extremes of these had an adverse effect on the population. The result was also supported by findings of other workers with regard to seasonal fluctuations of root-knot nematodes. In support of the present finding Eapen (1993) reported that *M. incognita* population differed significantly from period to period due to seasonal production of new root biomass. The same author also concluded that crop phenology appeared to be the major factor influencing the seasonal fluctuation of nematode in cardamom. Poornima and Vadivelu (1999) also reported that the nematode population was found to increase with the age of the host crop because of availability of more feeder roots to feed by nematodes and then started to decrease the population in the crop itself as observed in the present study. Thus, the edaphic factors appear to interact for the development, multiplication and seasonal behavior of nematodes associated with okra based cropping sequence. However, effective control measures against the nematodes should also be adapted during these periods and the production of crop will be increased significantly.

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Short communication

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Short communications

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