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Management of root knot nematode (*Meloidogyne arenaria*) in thippali

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ABSTRACT

Thippali, *Piper longum*, an important medicinal plant belonging to the family Piperaceae. An increase in root knot nematode attack was observed in thippaligrowing plots. Pot culture experiments were conducted to study the management of root knot nematode infesting thippali using bioagents, organic amendments, botanical extract and chemicals. The effect of various treatments on shoot, root characters and nematode population were evaluated. The control of root knot nematode achieved by application of *Bacillus subtilis* was superior to all other treatments. The root knot nematode population in *P. longum* could be effectively managed using the bio agents.

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INTRODUCTION

India has one of the oldest, richest and diverse cultural traditions associated with the use of medicinal plants. In recent years the cultivation of medicinal and aromatic plants has been increases and the country became a treasure house of medicinal plants. Improvement in agronomic practices and the use of high yielding superior varieties have necessitated the application of plant protection measures, which ensured better yield through the management of pest and diseases especially plant parasitic nematodes which are serious threat to the cultivation of medicinal plants. Thippali, *Piper longum* is an important medicinal plant belonging to the family Piperaceae. It is the most important species of genus Piper after black pepper and perhaps the first pepper to reach the Mediterranean. It is a slender aromatic climber with perennial woody roots and is distributed along the watercourses and over shoal lands in Assam, Kerala and Karnataka. The unripe female spikes and to a smaller extend, root and thick basal stems contribute the commercial produce. The medicinal use of dry spikes and roots of *Piper longum*in Ayurvedic system of medicine can be described with the term '*trikatu*' - black pepper, thippali and ginger (Kirtikar and Basu, 1935 and Hussain *et al*., 1992). Root knot nematodes cause areal symptoms like yellowing,

chlorosis, wilting and reduction in growth and underground symptoms like gall formation. Galls are seen through the entire length of roots and may contain many nematodes and as a result the growth of roots is arrested. A severe root knot nematode attack was observed in thippali at Vellanikkara, and the species was identified as *Meloidogyne arenaria*. The loss caused by the nematode was enormous which necessitates efficient control measures. Presently the management practices of nematodes are mainly based on systemic chemicals like nematicides (Ravi *et al*., 2000; Kerala Agricultural University, 2002 and Tiwari *et al*., 2002), which are very costly and have several side effects like residue problems, resurgence of nematode pollution, environmental pollution and health hazards. Thippali being a medicinal plant, the use of chemical pesticides is not advisable. Because of the concealed feeding habits of nematodes, bio agents [bacteria (Jyothi *et al*., 2003 and Kalairasan *et al*., 2006), fungus (Ravi *et al*., 2000; Senthilkumar and Rajendran, 2004)] and organic amendments [mulching (Prasad *et al*., 1997 and Kumar and Reddy, 2001), Botanical extracts (Sreeja and Charles, 1998), use of *Neem* cake and *Neem* based formulations (Ravindra *et al.*, 2003)] can substantially suppress its population. In this context, this study to compare the efficiency of bio agents and organic amendments for the control root knot nematode (*Meloidogyne arenaria*) infestation in Thippali.

MATERIAL AND METHODS

The study was carried out in Department of Agricultural Entomology, College of Horticulture, Vellanikkara.

Preparation of denematized potting mixture :

Sieved field soil, sand and well decomposed farm yard manure was mixed in the ratio 1:1:1 and this potting mixture was filled in earthern pots of size 25 cm. Formalin was poured into each pot in the ratio 1:20 and were covered with polythene sheets and tied firmly. After two weeks, the polythene sheets were removed and the mixture in each pot was raked well. Soil samples were taken from each pot to test the presence of nematodes. These pots with denematized potting mixture were used for further pot culture studies.

Maintenance of pure culture of root knot nematode infesting thippali :

The rooted cutting of thippali, variety Viswam were planted in pots filled with denematized mixture for the maintenance of nematode culture. After identifying the species of root knot nematode on the basis of perineal pattern, pure culture of the nematode were maintained from single egg mass collected from infested thippali roots, which were collected from College of Horticulture, Vellanikkara. The second stage juveniles of the nematodes that hatched from the egg mass were inoculated to the potted plants. Repotting and inoculation were repeated periodically for maintaining the pure culture of root knot nematode for different experiments.

Identification of nematode :

The species of root knot nematodes were identified by the perennial pattern of white females collected from root galls. The species of root knot nematode was identified as *Meloidogyne arenaria.*

Pot culture studies :

Pot culture studies were conducted to determine the efficiency of organic amendments, *Tagetserecta*, different bioagents and carbofuran in the management of root knot nematode in thippali.

Design :

T_1 – *Neem* cake @ 11 g/pot
T_2 – Mulching with *Tagetes* waste @ 250 g/pot
T_3 – Drenching with 5 per cent root extracts of *Tageteserecta*
T_4 – Soil application of *Pseudomonas fluorescens* @ 10 g/pot
T_5 – Soil application of *Trichoderma viride* @ 10 g/pot
T_6 – Soil application of *Bacillus subtilis* @ 30 ml/ pot
T_7 – Soil application of *Neem* granules (amruthguard @ 1 g/pot)
T_8 – Soil application of AMF @ 50 g/pot
T_9 – Soil application of carbofuran @ 0.1 g/pot
T_{10} – Control.

Application of bioagents :

P. fluorescence, T. viride, B. subtilis and AMF were applied one week before the inoculation of nematodes.

Talk based *P. fluorescence* and *T. viride* suspension was prepared and applied in soil. Required quantity of AMF was added to the pots and covered with a thin layer of denematized potting mixture. *B. subtilis* culture suspension having a concentration of 10^8cfu/ml was drenched in soil @ 30 ml/pot.

Application of other treatments :

Required quantities of *Neem* cake, Amruth guard and carbofuran were applied to the soil one week after inoculation of nematodes. Aerial parts of *T. erecta* was cut into small pieces and used for mulching the pots. Root extracts of *T. erecta* were also used for drenching the soil of the respective treatments.

Extraction of second stage juveniles for inoculation:

Modified Baermann funnel technique (Schindler, 1961) was used for extracting second stage juveniles for inoculation. Heavily infested plants from the culture pots were uprooted carefully, washed and egg masses from galled roots were hand-picked using forceps. The second stage juveniles were obtained after keeping the egg masses over two layers of tissue paper supported on a wire mesh, which in turn was placed over a petri dish with sufficient water just enough to touch the egg masses. Several such sets were kept for getting the required number of second stage juveniles needed for inoculation purpose. Hatched second stage juveniles were transferred to a beaker after every 24 hr.

Inoculation of nematodes :

Nematode population in suspension was assessed. Each pot was inoculated with 10 ml of suspension containing 2000 second stage juveniles of root knot nematode, after the cutting had established. At the time of inoculation the suspension was thoroughly mixed by blowing air with a pipette, to get uniform distribution of nematodes. This suspension was then poured to the root zone of plants, by making holes of about 5 cm depth in soil using glass rod.

Shoot and root characters and nematode population were observed during the course of experiments.

Estimation of nematode population from soil :

A composite sample of soil was weighed from the root zone and processed for extracting the nematodes. Nematodes were extracted from soil samples taken from different treatments, following the Cobb's decanting and sieving technique (Cobb, 1918). The nematode suspension thus obtained was made upto a constant volume (100 ml) by adding water. An aliquot of 1 ml was pipetted out into a counting dish and the number of nematodes present was counted under the stereoscopic microscope. The total population of nematodes extracted from 200 g soil sample was estimated by multiplying the average population by the appropriate factor.

Estimation of number of egg masses from 10 g of root:

The root system from each pot was carefully lifted by gentle tapping of the pots on all sides and bottom and removing the loose soil, the roots were cleaned of adhering soil particles by gentle washing in water. From this sample 10 g of root was weighed and the number of egg masses was counted.

Estimation of root knots from pots :

After counting the egg mass, the root samples was pressed gently between folds of blotting paper to remove the excess water and the number of root knots in 10 g of root sample was counted.

Root-knot index :

Based on number of galls, the root knot index was rated on a 1 to 5 scale.

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

Estimation of nematode population from root :

After counting the number of galls, the root samples were used for extracting nematodes. Modified Baermann funnel technique was used for extracting nematodes from roots (Schindler, 1961). The nematode suspension thus obtained was made to a known volume by adding water and then the population of the nematodes was assessed.

Statistical analysis :

Data collected during the study was analysed through statistical method for CRD and ANOVA using statistical package, SPSS.

RESULTS AND DISCUSSION

Pot culture experiments were conducted to study the effectiveness of management practices.

Shoot characters:

Application of *Bacillus subtilis* (T_6) resulted in an enhancement of shoot characters like number of leaves, vine length and fresh weight of shoot which were 69.83, 70.92 cm and 135 g, respectively as shown in Fig. 1. As far as the dry weight of shoot (27.88 g) and number of branches (5.67) are concerned *Trichoderma viride* (T_5) was more effective. AMF, *Pseudomonas fluorescence* and carbofuran were also found effective in reducing the impact of nematode attack on shoot parameters. *Neem* cake application was found to have least effect on fresh weight of shoot and was the inferior treatment. Plants in control pots were dwarf with less number of leaves and branches, vine length, fesh weight and dry weight. All plants except those in control pots produced fruiting branches but spike formation was observed only on plants treated with *Bacillus subtilis, Trichoderma viride,* AMF, *Pseudomonas fluorescence,* carbofuran and those mulched with *Tagetes waste*. Even though plants treated with *B.subtilis* were the first to produce spikes, an increase in spike number was noticed in *P. fluorescence* treated plants. Plants mulched with wastes of *T. erecta* produced very long spikes.

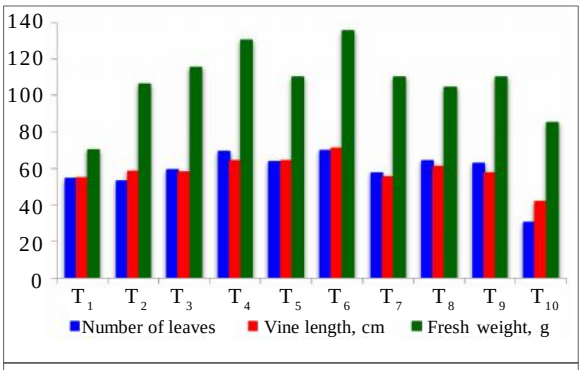

Fig. 1 : Effect of different treatments on shoot characters of thippali

Root characters:

Plants treated with *B.subtilis* (T_6) were statistically superior with all other treatments with maximum root length, fresh weight and dry weight of root asshown in Fig. 2. Pseudomonas fluorescence (T_4) was observed as the next superior treatment with respect to root length, fresh weight and dry weight of root. Application of *Neem* cake (T_1) did not shown any improvement in root characters. Control (T_{10}) plants produced only very short roots with more number of galls on it. Poor root growth attributed to the damage by second stage juveniles.

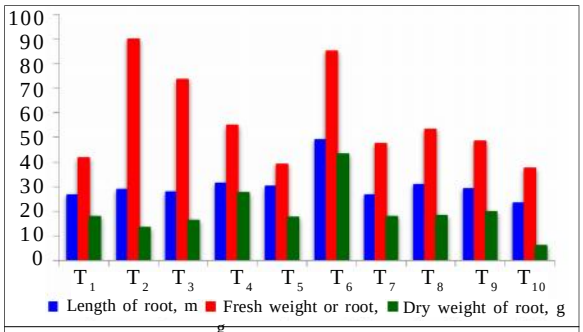

Fig. 2 : Effect of different treatments on root characters of thippali

Nematode population:

Plants treated with *B.subtilis* (T_6) recorded the maximum reduction in the number of nematodes in soil, root and gall formation as shown in Fig. 3. The percentage reduction of nematode population over control was 92.50, 93.62 and 97.84, respectively. The least value of root knot index (1.00) also observed for T_6. T_6 was closely followed by *P. fluorescence* (T_4) and carbofuran (T_9). All the treatments suppressed nematode population but the efficacy regime was not alike. The prophylactic application of the biocontrol agents produced a soil condition capable enough to suppress the population build-up of nematodes in soil and root and kept the infection at the lower level.

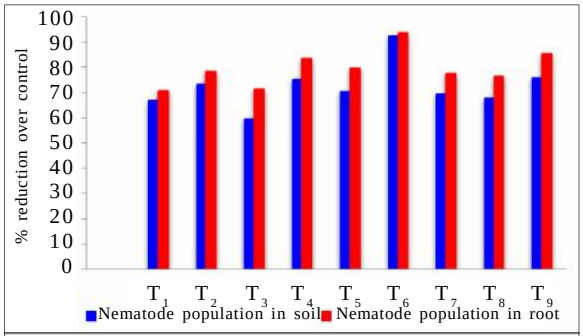

Fig. 3 : Effect of different treatments on nematode population

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