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Eco-friendly Management of Citrus Nematode (*Tylenchulus semipenetrans* Cobb.) Infecting Lemon (*Citrus limon* L.)

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ABSTRACT: Citrus nematode, *Tylenchulus semipenetrans* (Cobb, 1913), is the most concerned nematode pest of lemons, inflicting significant losses in India due to adequate soil and environmental conditions. *T. semipenetrans* causes a slow decline disease in citrus. Considering the economic importance, an eco-friendly management investigation has been conducted on *Tylenchulus semipenetrans*, which infects lemon. Vermicompost fortified with bio-agents *viz., Glomus fasciculatum*, *Purpureocillium lilacinum* and *Metarhizium anisopliae* at 5 kg bio-agents in100 kg vermicompost were used at two different doses *i.e.*5 g/kg soil and 10 g/kg soil alongwith an untreated check. All the treatments were replicated three times in completely randomized design. Initial nematode population was kept 500 juveniles/ 200 cc soil. Results exhibited that all the treatments found significantly superior over untreated control in promoting the plant vigour as well as decreasing the citrus nematode population. Maximum reduction in nematode parameters that is number of females/ 5 g roots (53.00), number of egg mass/5 g roots (33.33), number of eggs & larvae/egg mass (30.00) and final population/200 cc soil (316.67) with per cent decrease observed to be 66.67%, 74.68%, 41.17% and 69.50%, respectively with the application of *P. lilacinum* enriched vermicompostat 10.00 g/kg soil followed by *G. fasciculatum* at the rate10.00 g/kg soil and *M. anisopliae* at 10.00 g/kg soil. Experimental results advocated that these eco-friendly management options may be employed in place of pesticides against citrus nematode, *T. semipenetrans* on lemon to promote organic and economical cultivation of lemon.

Keywords: Citrus nematode, Eco-friendly management, Bio-agent, Lemon.

INTRODUCTION

Lemon (*Citrus limon* L.) is an important fruit crop with evergreen leaves and yellow edible fruits belongs to family Rutaceae. Citrus is the third largest fruit crop and is extensively grown commercially in Maharashtra, Rajasthan, Andhra Pradesh, Karnataka, West Bengal, Sikkim, Punjab and Assam in arid & semi-arid regions to humid tropical regions of India. The present area under citrus is 1.09 m ha with a production of 14.50 million tonnes (Agristat, 2020) and average productivity of 11.80 tons/ha in India. The most important commercial citrus species in India are the mandarin orange (*C. reticulata*), sweet orange (*C. sinensis* L.) and acid lime (*C. aurantifolia*)/lemon (*C. limon*) production sharing 28.36, 44.29 and 27.34 percent, respectively. In Rajasthan, Lemon occupies an area of 2.69 thousand ha with 14.47 thousand MT production and productivity of 5.66 tonnes/ha (Horticulture Statistics at a Glance 2018). Nutritional facts of citrus per 100 grams is Calories 60, Fat N/A, Sodium N/A, Fiber 3 g, Sugar 12 g, Protein 1g, Vitamin A 14 mcg, Vitamin C 70 mcg. In *C. limon* seed oil, the main ingredients are fatty acids such as arachidonic acid, behenic acid and linoleic acid and also tocopherols and carotenoids (Malacrida *et al.,* 2012; Mucci *et al.*, 2013). The *C. limon* stands out as having well-known nutritional properties, its valuable biological activities are underestimated in phytotherapy and cosmetology (Goetz, 2014).

A frightening situation in citrus cultivation has developed due to dwindling of the area under citrus as it is of late threatened with the serious disease commonly known as "Citrus decline". It is well known that citrus decline is a complex disease caused by the additive effect of fungus, bacteria, virus, nematode, insect pests and soil disorders etc. In India, seventy species within 37 genera of plant parasitic nematodes have been reported in association with citrus (Reddy and Singh 1979; Khanzada *et al.*, 2008; Kumar & Arthurs 2021). Citrus nematode, *Tylenchulus semipenetrans* Cobb, 1913 is one of the most destructive nematode associated with citrus and is known to occur in all the citrus growing areas of the world (Thorne, 1962; Duncan *et al.*, 1989; Sorribas *et al.*, 2000). The citrus nematode was first discovered in California by Cobb (1913) and

placed it in a new genus and named this species *Tylenchulus semipenetrans* on the basis of position in the root system. From India, the first report on the presence of *T. semipenetrans* was made by Siddiqi (1961). It is now known to be extensively distributed in the country including Punjab, Delhi, Rajasthan, Uttar Pradesh, Maharashtra, Assam, Orissa and Kerala (Swarup *et al*., 1964; Mani, 1994; Singh 1999). In almost all cases this species is found to be predominant wherever die-back symptoms are present. It was suggested that citrus nematode is one of the main factors responsible for slow decline of citrus and responsible to causes direct and indirect economic losses in India. *Tylenchulus semipenetrans* is known to infect all type of citrus plants (mandarin orange, sweet orange and acid lime/lemon) and responsible for a substantial amount of damage in citrus orchards. The citrus nematode is responsible for causing a substantial yield reduction of 27% (Kumar *et al*., 2020). Although some of measures are in practice to manage the plant parasitic nematodes infecting citrus but still not much work has been carried out on citrus nematode (*T. semipenetrans*) on lemon. Although the application of nematicides effectively controlled *T. semipenetrans*, it gave protection only for a few months and the nematode population also reached its original level within six months after application, possibly due to the imbalance of fauna and flora caused by the chemicals in soil ecosystems. Besides, nematicides are not only expensive but also pose serious problems of health hazards and environmental pollution. Bio-agents found to be effective for nematode control and they also don't have harmful residue like chemicals. Jatala (1985) reported use of biological control agents in reducing numbers of nematode with particular reference to the use of *Paecilomyces lilacinus* in controlling *M. incognita* on potatoes. It was found that the fungus significantly reduced the nematode population. Similarly Baheti *et al.* (2015); Bhati *et al.* (2021) reported the efficacy of *Paecilomyces lilacinus*, *Pochonia chlamydosporia, Pseudomonas fluorescence, Trichoderma harzianum* on different agri-horticultural crops. Maximum density of citrus nematode was observed at a depth of 1 ft. Nematode population decreases in horizontal and vertical distribution due to the lesser feeder roots in roots system. So bio-control management practice not required all over the orchard. Bio-agents uses in very small amount and after appropriate environment they increase their own population. Nematode population increases during high humidity and warm temperature. The population of nematode would be decline significantly in winters as the temperatures and R.H. decreases. So, management practices should be apply in months of July/August in Indian climatic conditions to prevent the nematode population when they are present in higher number (Jaiman and Baheti 2023). Hence an attempt was made to find out eco-friendly management of citrus nematode infecting lemon through vermicompost enriched with bio-agents.

MATERIALS AND METHODS

Lemon fruit stands out as having well-known nutritional properties, its valuable biological activities are underestimated in modern phytotherapy and cosmetology. It is extensively used in the food, pharma and cosmetics industries etc (Abad-García *et al.*, 2012; Russo *et al.*, 2015). Lemon juice has traditionally been used as a remedy for scurvy disease of human system. Other uses for lemon juice, known from traditional medicine include treatment of high blood pressure, common cold and irregular menstruation. Besides being rich in vitamin C, it also assists in warding off infections. Citrus crops have been affected by several pest and pathogens including phyto-nematodes in India and abroad.

Among plant parasitic nematodes, citrus nematode, *T. semipenetrans* (Cobb, 1913) is considered to be the most important nematode pest of lemon and causes severe losses in India due to favorable soil and environmental conditions. *T. semipenetrans* causes slow decline disease in citrus. Looking to the economic importance of citrus nematode, an eco-friendly management trial was conducted on *Tylenchulus semipenetrans* infecting lemon. Vermicompost fortified with bio-agents *viz., Glomus fasciculatum*, *Purpureocillium lilacinum* and *Metarhizium anisopliae* (5 kg bio-agents in100 kg vermicompost) were used at two different doses *i.e.*5 g/kg soil and 10 g/kg soil along withan untreated check. All the treatments were replicated three times in completely randomized design. Earthen pots were washed, cleaned and disinfected before use by rinsing them through four per cent formalin solution. Then pots were filled with 10 kg infested soil having an initial inoculum of 500 larvae/200 cc soil with test nematode. After completion of experiment, observations *viz.* number of females/5 g roots, number of egg masses/5 g roots number of eggs & larvae/egg masses and final nematode population/200 cc soil were recorded. Data were analysed for evaluation of different treatments as described by Panse and Sukhatmse (1989) in order to test the significant of experimental result. The critical difference was found out for comparison of treatments where 'F' test was found significant at 5% level of significance. Experimental findings have been presented in Table 1 and illustrated through Plate 1, Fig. 1-2.

RESULT AND DISSCUSSION

In present scenario, bio-agents and organic amendments play an important role in nematode management in agri-horticultural crops. Therefore in present investigation, vermicompost fortified with bio-agents (5kg per 100 kg of vermicompost), *viz. Glomus fasciculatum*@ 5 g per kg of soil (T_1), *G. fasciculatum*@ 10 g per kg of soil (T_2), *Purpureocillium lilacinum*@5 g per kg of soil (T_3), *P. lilacinum*@10 g per kg of soil (T_4), *Metarhizium anisopliae*@ 5 g per kg of soil (T_5), *M. anisopliae*@ 10 g per kg of soil (T_6) were applied for the management of citrus nematode, *T. semipenetrans*. An untreated

check was also maintained to compare and interpretate the experimental results (T_7).

Results revealed that vermicompost fortified with bio-agents *viz*. *G. fasciculatum, P. lilacinum* and *M. anisopliae* used at the rate 5 and 10 g per kg of soil credibly reduced the infection of *T. semipenetrans* (females per 5 g roots, egg masses per 5 g roots, eggs & larvae per egg mass and nematode population per 200 cc soil). Among different treatments, minimum number of females per 5 g of roots was recorded when applied vermicompost fortified with *P. lilacinum*@ 10 g per kg soil (53.00) followed by *G. fasciculatum* @ 10 g per kg soil (67.00) and *M. anisopliae* @ 10 g per kg soil (78.00) as compared to untreated check (159.00). *M. anisopliae* (93.33) @ 5 g per kg soil was recorded to be least effective. Among different bio-agents, highest reduction in females per 5g of roots was observed with *P. lilacinum* @10 g per kg of soil (66.67%) followed by *G. fasciculatum* @ 10 g per kg of soil (57.86%) and *M. anisopliae*@ 10 g per kg of soil (50.94%) as compared to untreated check (T_7). Minimum reduction (41.30%) was noticed in *Metarhizium anisopliae* @ 5 g per kg of soil over check.

Results revealed that minimum number of egg masses per 5 g roots were recorded with *P. lilacinum* @10 g per kg of soil(33.33) followed by *G. fasciculatum* @ 10 g per kg of soil (46.33) and *M. anisopliae*@ 10 g per kg of soil (57.67). *Metarhizium anisopliae* @ 5 g per kg of soil (73.33) was recorded least effective as compared to untreated check (131.67). Among vermicompost fortified with different bio-agents, highest reduction in egg masses per 5g roots was observed with *P. lilacinum* @10 g per kg of soil (74.68%) followed by *G. fasciculatum* @ 10 g per kg of soil(64.81%) and *M. anisopliae*@ 10 g per kg of soil (56.20%). *Metarhizium anisopliae* @ 5 g per kg of soil (44.30%) was found to be least effective over check.

Correspondingly, minimum number of eggs & larvae per egg mass was recorded with *P. lilacinum* at 10 g per kg of soil (30.00) followed by *G. fasciculatum* @ 10 g per kg of soil (36.00) and *M. anisopliae*@ 10 g per kg of soil (38.00). *M. anisopliae* (44.67) @ 5 g per kg soil was recorded to be least effective as compared to untreated check (51.00). Among different treatments, highest reduction in number of eggs and larvae per egg mass was observed with *P. lilacinum* @10 g per kg of soil (41.17%) followed by *G. fasciculatum* @ 10 g per kg of soil (29.41%) and *M. anisopliae*@ 10 g per kg of soil (25.49%) over untreated check. It was determined minimum (12.41%) in *Metarhizium anisopliae* @ 5 g per kg of soil.

Likewise, minimum nematode population per 200 cc soil was recorded with *P. lilacinum* @10 g per kg of soil (316.67) followed by *G. fasciculatum* @ 10 g per kg of soil (425.00) and *M. anisopliae*@ 10 g per kg of soil (525.00) over untreated check (1038.33). *Metarhizium anisopliae* @ 5 g per kg of soil (676.67) was recorded to be least effective. Among different bio-agents, highest reduction in number of nematodes per 200 cc soil was observed with *P. lilacinum* @10 g per kg of soil (69.50%) followed by *G. fasciculatum* @ 10 g per kg of soil (59.07%) and *M. anisopliae*@ 10 g per kg of soil (49.43%). Bio-agent, *Metarhizium anisopliae* @ 5 g per kg soil (34.83%) was observed to be least effective over untreated check. Experimental results revealed that all the tested bio-agents and organic amendment significantly reduced the soil and root population of *T. semipenetrans* as compared to untreated control. The nematode population declined up to 6 months and thereafter started increasing up to the harvest. This indicated that the bio-agents applications have to be further applied at 6 months interval for optimum results in nematode management.

Several workers have reported the efficacy of bio-agent for the management of plant parasitic nematode on different crops. O'bannon *et al.* (1979) revealed that the seedlings of *Citrus limon* infected with *T. semipenetrans* were transplanted into soil infested with *Glomus mosseae*, the mycorrhizal fungus infection increased seedling growth compared to non-mycorrhizal seedlings in greenhouse. Mankau (1980) carried out an experiment to evaluate the fungal antagonist of nematodes consist of nematode trapping fungi and endo-parasitic fungi. They observed that fungi can be effectively be used as bio-control agents for nematodes and may be an alternative to chemicals. Basati and Kaul (2015) observed *Paecilomyces lilacinus* as an effective bio-agent for the management of *T. semipenetrans.* The talc-formulation proved to be significantly most effective for the management of *T. semipenetrans* with 56.52% and 41.01% decrease in nematode population in vitro and in vivo. Hammam *et al.* (2016) accomplished an experiment on citrus plants with the *T. semipenetrans* to evaluate effect of various treatments on its management formulation, (*Heterorhabditis bacteriophora*, *P. lilacinus*, *Bacillus subtilis*, *Serratia* sp., *Pseudomonas* sp., *Azotobacter* sp., *B. circulans* and *B. thuringiensis*), Nema Plus Zero and Carbofuran. All the bio-agents suppressed *T. semipenetrans* populations and increased fruit yield. Ibrahim *et al*. (2019) exhibited an experiment to manage *Tylenchulus semipenetrans* by using different bio-agents and found Bio-Nematon (*P. lilacinus*) achieved the highest percentage of mortality for *T. semipenetrans* (50.0%). Bhagawati *et al*. (2021) conducted an experiment in an established lemon orchard to know the effect of bio agents and organic amendments on *T. semipenetrans*. Nagachandrabose *et al.* (2022) carried out an experiment on the efficacy of the bio-inoculants and found *P. lilacinum* with 54.9–74.4% and 53.4–74.3% reductions in population in soil and root, respectively, performed best. The egg mass parasitization by *P. lilacinum* was also 7.1–7.5 times higher than by *T. viride*. The suppression of *T. semipenetrans* by *P. lilacinum* led to an increase in the number of fruits (19.0–23.3%), fruit weight (20.2–22.3%), and fruit yield (35.4–40.3%) in acid lime trees. The cost–benefit ratio by *P. lilacinum* was also higher (1: 2.6–1: 3.0), whereas it was lower in carbofuran treatment (1: 2.3—1: 2.4).

These findings clearly indicated that the use of organic amendment fortified with bio-agents in nematode-prone regions suppressed nematode populations and enhanced plant growth of various crops. This seems to be feasible

because organic amendments enhance the physical properties of soil, boost the number of naturally existing enemies of the plant parasitic nematode and enhance the activity of beneficial microbes in soil. The nematode activity may be negatively impacted by nematotoxin generated by *P. lilacinum*, such as paecilotoxin as well as it produce appressoria on the egg shell and many enzymes (proteases & chitinase). *Glomus fasciculatum* is currently receiving more attention because of their potential to elevate host nutrition, particularly phosphate up take. Endo-mycorrhizal fungi may be valuable as "bio-fertilizers" in addition to their ability to combat nematodes. These findings demonstrated that soil application of bio-agents with organic amendments increased plant growth in nematode infested plants, which could be due to nematode population reduction via micro-parasitism, competition for food and space for nematode survival, or the release of some toxic metabolites which degrade the egg shell of nematode & ultimately reduced the nematode population.

The activity of soil microbes and the soil micro fauna which is mediated by mechanism including parasitism, predation, competition and antibiosis is believed to constitute biological control of nematodes an important part of integrated nematode management system.

Plate 1: Effect of bio-agents enriched vermicompost for the management of citrus nematode, *T. semipenetrans* on Lemon (*C. limon* L.).

Table 1: Eco-friendly management of Citrus nematode, *Tylenchulus semipenetrans* infecting lemon (*Citrus limon* L.).

Tr. No.	Detail of Treatments	No. of females/5g roots	No. of egg masses/5g roots	No. of eggs & larvae / egg mass	Final nematode population /200 cc soil	Per cent decrease in nematode population as compare to untreated check			
						Females/5 g roots	Egg masses/ 5g roots	Eggs & larvae / egg mass	Final nematode population /200 cc soil
T_1	Vermicompost fortified with *Glomus fasciculatum* 5g/kg soil	90.67	70.67	44.00	648.33	42.98	46.33	13.72	37.56
T_2	Vermicompost fortified with *Glomus fasciculatum* 10g/ kg soil	67.00	46.33	36.00	425.00	57.86	64.81	29.41	59.07
T_3	Vermicompost fortified with *Purpureocillium lilacinum* 5g/kg soil	87.67	68.33	43.33	631.67	44.86	48.10	15.04	39.16
T_4	Vermicompost fortified with *Purpureocillium lilacinum* 10g/kg soil	53.00	33.33	30.00	316.67	66.67	74.68	41.17	69.50
T_5	Vermicompost fortified with *Metarhizium anisopliae* 5g/kg soil	93.33	73.33	44.67	676.67	41.30	44.30	12.41	34.83
T_6	Vermicompost fortified with *Metarhizium anisopliae* 10g/kg soil	78.00	57.67	38.00	525.00	50.94	56.20	25.49	49.43
T_7	**Untreated check**	159.00	131.67	51.00	1038.33	-	-	-	-
SEm±		**1.047**	**0.882**	**0.520**	**26.985**	**-**	-	-	-
CD at 5%		**3.088**	**2.602**	**1.534**	**82.643**	**-**	-	-	-

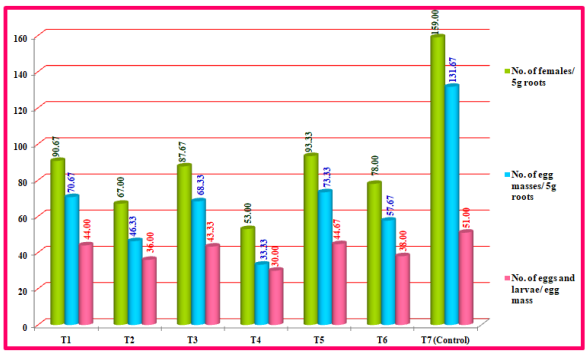

Fig. 1. Eco-friendly management of Citrus nematode (*Tylenchulus semipenetrans*) infecting lemon.

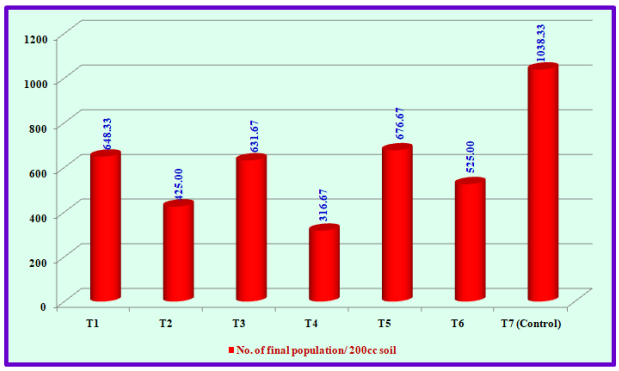

Fig. 2. Final soil population of citrus nematode, *T. semipenetrans* in eco-friendly management trial on lemon (*C.limon* L.).

CONCLUSIONS

Results of present investigation exhibited that all the treatments found significantly superior over untreated control in promoting the plant vigour as well as to decrease the infection of citrus nematode on Lemon. Maximum reduction in nematode parameters recorded with the application of *P. lilacinum* enriched vermicompost@10.00 g/kg soil followed by *G. fasciculatum* @10.00 g/kg soil and *M. anisopliae* @10.00 g/kg soil. Experimental results clearly showed that these eco-friendly management options may be employed against citrus nematode, *T. semipenetrans* on lemon to enhance plant growth as well as to promote eco-friendly and economical cultivation of lemon.

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