International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(2): 2836-2838

Received: 15-01-2020
Accepted: 19-02-2020

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Investigation on potential of different bio-agents as soil application against root-knot nematode, *Meloidogyne incognita* infecting tomato

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DOI: https://doi.org/10.22271/chemi.2020.v8.i2aq.9179

Abstract
Anexperiment trial was conducted during 2018-19 for the management of root-knot nematode, *Meloidogyne incognita* through bio-agents as soil application on tomato in micro plots. Micro-plot was filled with root-knot nematode infested soil (520 IJs/100 cc soil) brought from the pure culture field. Bio-agents *viz.*, *Purpureocillium lilacinum*, *Trichoderma harzianum* and *Pseudomonas fluorescens* were added to soil @ 2.5 and 5.0 g per m^2. Standard check (*Trichoderma viride* @ 2.5 kg/ha) and control were also maintained for comparison. Results of bio-agent and dose interaction have indicated that *P. lilacinum* @ 5.0 g per m^2 was found best followed by *P. fluorescens* @ 5.0 g per m^2 and *T. harzianum* @ 5.0 g per m^2. However, *T. viride* 2.5 kg/ha was found superior in respect to plant yield and reducing nematode reproduction on tomato in comparison to all other treatments.

Keywords: Bio-agents, soil application, *Meloidogyne incognita*, tomato

Introduction
Tomato [*Lycopersicum esculentum* (Mill.)] is a most important vegetable crop in the universe. It is cultivated in both temperate and tropical regions of the world and consumed in a various ways like fresh in salad and sandwiches, cooked or processed in ketchup, sauce, juice or dried powder. Tomato plays an important role in human diet which is providing essential nutrients like amino acids, vitamins and minerals etc. It also contains lycopene, which is very important antioxidant and can prevent cancer (Agarwal and Rao, 2000) [2]. During 2018-19 India has covered 789 thousand hectare in vegetables and production of 197.59 lakh MT and productivity of 25 ton per ha which contribute about 11 % of their world production (Anonymous, 2017-18a) [3]. The Rajasthan shares 21 thousand ha area of tomato with a production of 85 thousand MT (Anonymous, 2017-18b) [3].

The crop having such economic value is affected by fungi, bacteria, viruses, insects and nematodes. Among various biotic factors plant parasitic nematodes play a major rolefor yield decline in tomato. The crop suffers huge qualitative and quantitative losses due to plant parasitic nematodes like *Meloidogyne* spp., *Rotylenchulus* spp., *Pratylenchus* spp., and *Tylenchorhynchus* spp. Among these, root-knot nematode, *Meloidogyne spp*., being obligate sedentary endoparasites with an inherent capacity to interact with other fungal and bacterial pathogens to cause complex diseases, these are the most difficult pests to manage (Fourie and McDonald, 2000) [2]. Internationally a study was conducted on sample estimates from 75 countries which showed that *M. incognita*, *M. javanica*, *M. areneria*, *M. hapla* and other spp., were widespread with 53%, 50%, 8%, 8% and 2%, respectively (Johnson and Fassuliotis, 1984). In India, about 350 plants are identified as the host of *Meloidogyne* spp. (Sen and Dasgupta, 1982). It is mostly attacked by *M. incognita* a pre-dominant as well as widely prevalent species inflicting serious loss in tomato (Sasser, 1990).

Materials and methods
The experiment was laid in micro-plot filled with infested soil (520 IJs/200 cc soil) carried from the pure culture field. Talc-based formulationof *P. lilacinum*, *T. harzianum* and *P. fluorescens* were added to soil @ 2.5 and 5g per m^2. Each treatment was replicated three times.

Standard check (*Trichoderma viride* @ 2.5 kg/ha) and control were also maintained for comparison. Plants were harvested after 3 month of transplanting.

Observation on the root was wash 'carefully under tap water and stained with 0.1 per cent acid fuschinlac to phenol and after wash set aside in clear lacto phenol for 24 hrs. There after the roots were examined scrupulously under a stereoscopic binocular microscope for number of gall per plant, number of females per 5g root, number of egg masses per 5g root and number of eggs and larvae per egg mass. After removing the plant from the micro-plot, soil was carefully mixed and 200cc soil from each micro-plot were taken and process by Cobb´s sieving and decanting technique (Cobb, 1918) [7] followed by Baermann´s funnel technique (Christie and Perry, 1951) [6] for estimation of nematode population in soil. The data were subjected to statistical analysis.

Results and Discussion

Talc-based formulation of bio-agents *P. lilacinum, T. harzianum* and *P. fluorescens* were used as soil application @ 2.5 g and 5g per m^2 for the managing of root knot nematode, *M. incognita* on tomato. Different bio-agents were found to influence the plant yield and nematode reproduction to a varied degree. However, among these, *P. lilacinum,* was found most effective, compared to *P. fluorescens* and *T. harzianum*in improving plant yield and suppressing the nematode reproduction(the as well as number of gall per plant, number of females per 5g root, number of egg masses per 5g root and number of eggs and larvae per egg mass andfinal nematode population/200cc soil).

Among doses, *P. lilacinum* @ 5g per m^2 was found significantly better over other treatments. Results of bio-agent and dose interaction have indicated that *P. lilacinum* @ 5g per m^2 was best over @ 2.5g and 5 g per m^2 *P. fluorescens* and *T. harzianum* in improving plant yield and reducing nematode reproduction. However, *T. viride* 2.5 kg/ha was superior in respect to plant yield and reducing nematode reproduction on tomato in comparisons to all other treatment.

Results showed in the table-1 is that application of *P. lilacinum, T. harzianum* and *P. fluorescens* as soil application @ 2.5 and 5 g per m^2 effectively reduced number of galls per plant, number of females per 5 g root, number of egg masses per 5 g root, number of eggs & larvae per egg masses and final nematodes population 200cc soil on tomato infected with *M. incognita*. It has been observed in this table that higher dose of bio-agents give superior protection from nematode infection over lower dose. Among following treatments, minimum number of galls perplant (30.88), number of females per 5 g root (34.43), number of egg masses per 5 g root (46.56), number of eggs & larvae per egg mass (16.72) and final nematode population per 200cc soil (19.90) were reduced by*P. lilacinum* followed by *P. fluorescens* at 22.06, 31.10, 41.39, 12.54 and 18.88 per cent and *T. harzianum* at 19.12, 25.57, 36.21, 10.45 and 16.33 per cent in nematode population.

These findings are in concurrence with the conclusion of Zaki and Uzma (2009) [17], who reported that use of *P. lilacinus* against root-knot nematode, *M. incognita* caused higher enlarge in plant growth than *P. fluorescens*, *T. harzianum* and *P. lilacinus* also caused higher reduction in galls and nematode multiplication over*P. fluorescens*, *T. harzianum*. De Leij and Kerry (1991) [8] report the potential of *P. lilacinus* as a biological control agent against *M. arenaria* on tomato plants. Significant reduction in the population *i.e.* more than 80 per cent after the first nematode generation was achieved.

Khalil *et al*. (2012) [12] reported that *P. lilacinus* most valuable treatment on both galls and egg masses triumph 88.23 and 76.94% decrease, While, less effective treatment *P. fluorescens*getting 57.53% galls decrease. Joshi *et al*. (2012) [11] were weathered fungalbio-agents *P. lilacinus* @ 2 g/kg soil was set up best treatment in greater than ever plant growthand in reduced nematode reproduction over other fungal bio-agents treatments**.** Hanawi (2014) [10] also reported that *T. harzianum* was the best bio-control agent in increase shoot length and root length 16.2% and 26.1%, respectively. The *P. lilacinus* was the best bio-agent in reduction of root galls (57.53%).

These findings also similar to Baheti *et al*. (2015) [4], Sen *et al*. (2016) and Bhati *et al*. (2019) [5]. Sen *et al*. (2016) [16] found thatbio-agents *viz. Trichoderma viride, Trichoderma harzianum* and *Pseudomonas fluorescens* were used at 2 g and 3 g per kg soil. Soil application with *T. harzianum* at 3 g per kg soil was found best treatment followed by *T. Viride* and *P. fluorescens* 3 g/kg soil to enhanced plant growth of brinjal and management of *M. incognita*. Bhati *et al*. (2019) [5] found that *Paecilomyces lilacinus* at 12 g/kg seed was found most effective followed by *Pochonia chlamydosporia* at 12 g/kg seed and *Glomus fasiculatum* at 12 g/kg seed to enhancing plant growth of bitter gourd and to reduce the infection of *M. incognita*.

Table 1: Efficacy of bio-agents against root-knot nematode (*M. incognita*) at two different doses as soil application on tomato.

S. No.	Detail of Treatment	No. of galls per plant	No. of females per 5 g root	No. of egg masses per 5 g root	No. of eggs and larvae per egg mass	Final nematodes population per 200 cc soil	Yield (kg/plot)
T_1	*Purpureocillium lilacinum* @ 2.5 g/m^2	57.00 (16.18)	22.67 (24.43)	13.67 (29.28)	87.67 (8.36)	830.00 (15.31)	2.50
T_2	*Purpureocillium lilacinum* @ 5 g/m^2	47.00 (30.88)	19.67 (34.43)	10.33 (46.56)	79.67 (16.72)	785.00 (19.90)	3.35
T_3	*Trichoderma harzianum* @ 2.5 g/m^2	62.00 (8.82)	28.00 (6.67)	16.67 (13.76)	90.00 (5.93)	850.00 (13.27)	2.40
T_4	*Trichoderma harzianum* @ 5 g/m^2	55.00 (19.12)	22.33 (25.27)	12.33 (36.21)	85.67 (10.45)	820.00 (16.33)	3.15
T_5	*Pseudomonas fluorescens* @ 2.5 g/m^2	60.00 (11.76)	27.00 (10.00)	15.67 (18.93)	88.67 (7.32)	840.00 (14.29)	2.45
T_6	*Pseudomonas fluorescens* @ 5 g/ m^2	53.00 (22.06)	20.67 (31.10)	11.33 (41.39)	83.67 (12.54)	795.00 (18.88)	3.25
T_7	*Trichoderma viride* @ 2.5 kg/ha	40.00 (41.18)	17.00 (43.33)	9.00 (53.44)	76.33 (20.22)	760.00 (22.45)	3.80
T_8	Control	71.00	34.00	19.33	98.67	980.00	1.70

SEm±		1.93	0.74	0.44	2.57	24.55	1.32
CD 5%		5.84	2.25	1.33	7.79	74.46	0.40

Data are the average of three replications, Initial Nematode Population 520 larvae/ 200 cc Soil, Values in parentheses are (%) increase or decrease over control, Plot size = $2m^2$

Acknowledgement
The authors are highly thankful to The Dean, Rajasthan College of Agriculture and The Director Research, MPUAT, Udaipur (Raj.) to provide necessary facilities and technical guidance during the course of investigation.

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