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Raju Dhayal
Division of Nematology, Rajasthan Agriculture Research Institute, Durgapura, Jaipur, SKNAU, Jobner, Rajasthan, India

BS Chandrawat
Department of Nematology, SKN College of Agriculture, SKNAU, Jobner, Rajasthan, India

Kavita Choudhary
Division of Nematology, Rajasthan Agriculture Research Institute, Durgapura, Jaipur, SKNAU, Jobner, Rajasthan, India

SP Bishnoi
Division of Nematology, Rajasthan Agriculture Research Institute, Durgapura, Jaipur, SKNAU, Jobner, Rajasthan, India

Hemraj Gurjar
Division of Nematology, Rajasthan Agriculture Research Institute, Durgapura, Jaipur, SKNAU, Jobner, Rajasthan, India

Hanuman Singh
Department of Plant-Pathology, College of Agriculture, Agriculture University, Kota, Rajasthan, India

Corresponding Author:
Raju Dhayal
Division of Nematology, Rajasthan Agriculture Research Institute, Durgapura, Jaipur, SKNAU, Jobner, Rajasthan, India

Bio-management of root-knot nematode, *Meloidogyne javanica* in tomato

Raju Dhayal, BS Chandrawat, Kavita Choudhary, SP Bishnoi, Hemraj Gurjar and Hanuman Singh

Abstract
Tomato (*Solanum esculentum* Mill) is related to the family Solanaceae, it is an major vegetable crop and grown globally. It is suitable host for root-knot nematode (*Meloidogyne javanica)*. Examination the efficacy of bio-agents (*viz., Trichoderma asperellum, T. harzianum, Verticillium lecanii, Metarhizium anisopliae* and *Bacillus subtilis*) against *M. javanica* in @ 3 gm/ kg soil in field condition. Experimental results revealed that the bio-agents are increased the plant growth parameter and reduced the nematode reproduction as compared to control. Among the tested bio-agents *T. harzianum* @ 3 gm/ kg soil was found most effective treatment followed by *Bacillus subtilis* and *Metarhizium anisopliae*. All bio-agents enhance plant growth parameter with in reducing nematode population in field condition.

Keywords: Tomato, root-knot nematode, *Meloidogyne* spp., bio-agents

Introduction
Tomato (*Solanum esculentum* Mill.) is the most significant and nutritive vegetable crop across many country. It is growing in both temperate and tropical regions. Its cultivated countries are China, India and Italy. After china India is second largest producer tomato crop. In India tomato are grown in area of 789.2 thousand hectare with production of 205.72 thousand million tones and productivity of 25.0 tons per hectare (NHB).

. Among all the stages of tomato crop, right from nursery to harvesting is attacked all around of disease caused by fungus, bacteria, virus and nematode. Nematode is regarded as important pest of tomato, in which RKN (*Meloidogyne* spp.) are one of the most harmful pests and causes severe economic losses losses (Kalele, *et al*., 2010) [8]. *Meloidogyne* sp caused morphological and physiological changes within roots, resulted reduced yield and quality (Khan, *et al.*, 2005) [10]. The plant infected with root-knot nematode appear symptoms of yellowing of leaves, wilting, rotting and premature shedding of the leaves with sever stunting that outcomes in great losses to the damaged crops (Saifullah, *et al.*, 1990) [17]. Root-knot infection causes up to 46.0% crop losses in Haryana (Bhatti and Jain 1977) [5], 24-26% loss in tomato (Sasser, 1979) [18], 35-39.7% loss on tomato in Karnataka (Reddy, 1985) [16]. However, heavy infestations causeover 30% yield losses in highly susceptible vegetables (Sikora, and Fernández, 2005) [21]. In tomato, 27.21% yield loss and economic loss was studied up to Rs. 2204 million (Jain 2007) [7]. Many centers AICRP$_S$ on Nematodes in Agriculture estimated crop yield losses in different cultivars of tomato which reach between 5 – 37 percent (Anonymous 2019) [2]. Management of RKN, uses of nematicides are effective, rapid, more reliable, economical and widely in use at present time. But highest PPNs are present in the soil, some part of their life cycles. Soil itself act as a major barrier for the nematicide to reach at the target site in a lethal dose. In soil there are several biological micro-organisms which may reduce or inactivate the nematicide before reaching in sufficient quantity to kill the nematodes so a very high dose is applied to achieve the desired results. In nature, nematodes are protected from unfavourable environmental state by their cuticle, cyst wall and eggshell *etc*. which can withstand the penetration of nematicides and reduce their effectiveness. Phytonematodes spend some of their life stages in plants either endoparasitically or ectoparasitically. The efficacy of nematicides is more on the nematodes in soil phase as compared to when they have penetrated the plants. Effective management, nematicides are often put in at excessive doses, as the case may be costly, uneconomical or phytotoxic and may bring about residue problems which may create eco-friendly disturbance in the environment, so a very high dose to get the desired control which may not be economical and feasible due to several side effects of the nematicides. With this background view, the present investigations were under taken to assess the capacity of bio-agents against RKN *in vivo.*

Materials and Methods

The experiment on management of RKN in tomato for the duration of bio-agents was conducted in field condition.

I. Raising Nursery and Transplanting

Tomato variety pusa-gaurav was used in experiment. 4–5-week-old Uniform sized tomato seedlings thus grown were transplanted in experimental field.

II. Bed Preparation and Sampling from Beds

The beds of 1m length and 1 m width were prepared for the experimental purpose.The soil sampling was done before transplanting of the seedling for determine initial nematode population.

III. Testing of bio-agents on management of RKN in tomato

The experiment conducted in pure culture plot overspread with RKN. *M. javanica* (2 J_2/g of soil) at Jobner, Jaipur. Talc–based formulation of *Trichoderma asperellum, Trichoderma harzianum, Verticillium lecanii, Metarhizium anisopliae* and *Bacillus subtilis*were added to soil each @ 3 g per kg soil on different time interval *i.e.,* at the time of transplanting, 20 and 40 days after transplanting. Six plants of tomato were maintained in each plot. Every one treatment was replicated four times and untreated check used for comparison. Plants were uprooted after 60 days of transplanting and the recorded observations on plant growth characters and nematode reproduction.

Statistical Analysis

After completion of experiment, data were statistically study for interpretation of finding. The critical deference was calculated for comparison of treatment for significant at 5% level of significance.

Results and Discussion

Results in field trial appear that application of *Trichoderma harzianum* was create to be the best treatment to improve plant growth characters, come after the maximum shoot length was recorded with *Trichoderma harzianum* (70.75 cm) followed by *Bacillus subtilis* (61.50 cm) and *Metarhizium anisopliae* (53.00 cm), maximum shoot weight (45.76 gm) was recorded with *Trichoderma harzianum* followed by *Bacillus subtilis* (41.83 gm) and *Metarhizium anisopliae* (37.19 gm), maximum root length was recorded with *Trichoderma harzianum* (57.00 cm) followed by *Bacillus subtilis* (46.00 cm) and *Metarhizium anisopliae* (41.75 cm) and maximum root weight was recorded with *Trichoderma harzianum* (7.35 gm) followed by *Bacillus subtilis* (6.35 gm) and *Metarhizium anisopliae* (5.60 gm). While, untreated check was least effective treatment with minimum shoot length (38.00 cm), shoot weight (21.40 gm), root length (30.25 cm) and root weight (2.40 gm).

All the bio-agents considerable reduced nematode reproduction as compared to untreated check. Among all the bio-agents recorded the minimum number of galls per plant with *Trichoderma harzianum* (92.75*)* followed by *Bacillus subtilis* (120.00) and *Metarhizium anisopliae* (170.25), minimum number of egg masses per plant (27.00) recorded with *Trichoderma harzianum* followed by *Bacillus subtilis* (89.75) and *Metarhizium anisopliae*(132.25), were recorded minimum number of eggs per egg mass with *Trichoderma harzianum* (147.75) followed by *Bacillus subtilis* (190.00) and *Metarhizium anisopliae* (262.75). Minimum larval population per 200 cc soil were recorded with *Trichoderma harzianum* (574.75) followed by *Bacillus subtilis* (680.00) and *Metarhizium anisopliae* (904.75) and minimum final nematode population recorded with *Trichoderma harzianum* (4615.00) followed by *Bacillus subtilis* (17828.5) and *Metarhizium anisopliae* (35725.25). While, untreated check was observed least effective with maximum number of galls per plant (359.75), number of egg masses per plant (300.75), eggs per egg mass (405.5), larval population per 200 cc soil (1502.5) and final nematode population (123540.80). All the bio-agents significantly reduced FNP_s in treated plants as compared to untreated check. (Table: 1)

Table 1: Bio-agent effect on plant growth and nematode reproduction in open field

Treatment	Shoot length (cm)	Shoot weight (gm)	Root length (cm)	Root weight (gm)	No. of galls/ plant	No. of egg masses/ Plant	Number of eggs and larvae / egg mass	Nematode juvenile/ 200 cc soil	Final nematode population
T. asperellum	49.75	33.29	38.00	4.60	248.50	180.00	328.50	1055.75	60190.75
T. harzianum	70.75	45.76	57.00	7.35	92.75	27.00	147.75	574.75	4615.00
V. lecanii	42.00	29.11	32.25	3.62	302.25	218.50	360.25	1265.75	80025.50
M. anisopliae	53.00	37.19	41.75	5.60	170.25	132.25	262.75	904.75	35725.25
B. subtilis	61.50	41.83	46.00	6.35	120.00	89.75	190.00	680.00	17828.50
Control	38.00	21.40	30.25	2.40	359.75	300.75	405.50	1502.50	123540.80
SEm±	0.584	0.337	0.574	0.131	4.700	4.700	6.666	2.107	2338.395
CD 5%	1.761	1.016	1.730	0.395	15.600	14.166	20.089	6.351	7047.196
CV	2.23	1.94	2.81	5.26	4.80	5.95	4.72	0.42	8.72
* Average of four replications									
*Dose = @ 3 gm/ kg soil at the time of transplanting followed by 20 DAT and 40 DAT (Day After Transplanting)									

Fig 1: Overall view of bio-agents effect on plant growth and development in tomato under field condition

Fig 2: Bio-agents effect on plant growth and nematode reproduction in tomato

Bio-agents are capable to reduce nematode population either directly attack on nematode or indirectly production of toxins which harmful to nematodes. The bacterial strain Bs2 showed maximum growth of plant and nematode reduction and followed by Bs1, *Trichoderma harzianum* and *A. fumigates* (Chaubey, 2010) [6]. *Trichoderma asperellum* reduced the galling in hyacinth beans remarkably and had no effect on the analyse plant growth parameters (Kamau, 2010) [9]. *T. harzianum* BI reduced *M. javanica* damage in tomato. *Trichoderma harzianum* at 10 g per litre water was reported the best treatment as contrast with *Trichoderma viride* at 10 g per litre water and *Pseudomonas fluorescens* at 10 g per litre water in develop plant growth further more in reducing nematode population in other treatment (Sen 2015) [19]. *T. harzianum* @ 3 g per kg soil better over other bio-agents (34). *T. harzianum* at 1.5 g/kg soil + *Trichoderma harzianum at* 5 g/kg seed were found most valuable in enhance plant growth and reduction of nematode reproduction over control (Nama, 2015) [14]. *P. chlamydosporia* at 4 percent was found successful to improve maize plant growth and to reduced the infection of *H. zeae* on maize (Kumhar *et al.*, 2018) [12]. The total highest average percentage cowpea increase plant growth by *B. pumilus* (Bp1) (Abd-El-Khair, 2019) [1]. Bio-control agent, *Trichoderma viride* (seed treatment @ 4 g/kg seed + soil application @ 4 g/kg soil) was establish significantly superior in reduction of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *Lycopersici* along with increased in plant growth parameters followed by *Paecilomyces lilacinum* (Meena, 2020) [13]. Combined application of organic amendments (tea waste, churi of tobacco, poultry manure, leaf powder water hyacinth, leaf powder lantana and neem cake), hot water and bio-agents (*P. lilacinus* and *T. harzianum*) considerable reduced nematode reproduction and enhanced plant growth parameters (Bhati *et al*., 2021) [3]. *T. viride* and *T. asperellum* were establish at par and significantly valuable inhibition on hatching and larval mortality of *Meliodgyne incognita* (Kumari *et al.*, 2021) [11]. The highest reductions were recorded in nematode population, root galls, egg mass contents and egg masses on cucumber with *T. viride* at 5.0 g per plant followed by *Paecilomyces lilacinus* and *Trichoderma harzianum* (Bhati *et al*., 2022) [4]. The results of our findings also similar that application of *Trichoderma harzianum* @ 3 g/kg soil found to be the best treatments to increase plant growth characters as comared as reducing nematode reproduction in tomato.

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Conflict of Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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