Journal of Scientific Research and Reports

Volume 30, Issue 10, Page 981-989, 2024; Article no.JSRR.125395
ISSN: 2320-0227

Effect of Bio-Agents on Enzymatic Activity and Nematode Management in Tomato Plants Infected with *Meloidogyne javanica*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jsrr/2024/v30i102519

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/125395

Original Research Article

Received: 14/08/2024
Accepted: 16/10/2024
Published: 21/10/2024

ABSTRACT

The tomato (*Solanum esculentum* Mill.) is one of the most widely cultivated vegetable crops worldwide. It serves as a favorable host for plant-parasitic nematodes, particularly the root-knot nematode (*Meloidogyne javanica*). Investigations were carried out to determine the effectiveness of bio-agents against root-knot nematodes in pot conditions. The bio-agents included *Metarhizium anisopliae*, *Bacillus subtilis*, *Verticillium lecanii*, *Trichoderma harzianum and, Trichoderma*

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Cite as: *Dhayal, Raju, B.S. Chandrawat, Kavita Choudhary, G.K. Mittal, and Basant Kumar Dadrwal. 2024. "Effect of Bio-Agents on Enzymatic Activity and Nematode Management in Tomato Plants Infected With Meloidogyne Javanica". Journal of Scientific Research and Reports 30 (10):981-89. https://doi.org/10.9734/jsrr/2024/v30i102519.*

asperellum) against root-knot nematode in pot condition. Effect of bio-agents was also estimated for accumulation of PPO and PAL and Root-knot nematode-infected tomato roots containing phenol. The results of the experiment showed that all the bio-agents significantly increased the levels of PPO, PAL, and phenol in tomato roots, improved plant growth parameters, and reduced nematode reproduction compared to the untreated control. The best treatment with the highest PPO, PAL, and phenol activity among the investigated bio-agents was determined to be *Trichoderma harzianum* at 3 gm/kg soil, followed by *Bacillus subtilis* and *Metarhizium anisopliae*. It improves the plant growth parameter and lowers the number of nematodes in the pot.

Keywords: Tomato; root-knot nematode; enzyme activity; bio-agents; Meloidogyne spp.

1. INTRODUCTION

Globally grown, tomatoes (*Solanum esculentum* Mill.) are a vegetable crop valued for their economic significance and high nutritional content. It is grown in temperate and tropical areas worldwide. The USA, China, India, and other countries are the main producers of tomatoes. India is the second-largest producer of tomatoes in the world, after China. In India tomato are grown in area of 789.2 thousand hectare with production of 205.72 thousand million tons NHB, [1] and productivity of 25.0 tons per hectare [2].

At every stage of growth, from the nursery to maturity, tomato crops are vulnerable to a wide range of diseases caused by bacteria, fungi, viruses, and nematodes. Nematodes are particularly problematic pests for tomatoes, with root-knot nematodes (*Meloidogyne* spp.) being the most destructive, leading to significant financial losses [3,4]. Plants infected by root-knot nematodes exhibit an untidy appearance, along with symptoms such as yellowing, rotting, wilting, premature leaf shedding, and severe stunting, all of which result in major crop losses [5]. According to Jain [6],there was a 27.21% yield loss in tomatoes and a monetary loss of up to Rs. 2204 million. The "All India Coordinated Research Project on Nematodes in Agriculture" centers reported output losses ranging from 5 to 37 percent for various tomato cultivars [7]. Nematicides' efficacy is diminished in nematodes due to their ability to withstand the penetration of their cuticle, cyst wall, and eggshell, among other protective mechanisms that shield them from unfavorable environmental conditions. Nematicides are currently used extensively for the treatment of root-knot nematodes because they are efficient, quick, reliable, affordable, and widely available. Nematicides are more effective against nematodes in the soil phase than they are once they received inside plants. Nematicides are frequently applied at higher doses to achieve effective control, which can be expensive, unfeasible, phytotoxic, and result in residue issues that might disrupt the natural ecology. Consequently, applying higher dose of nematicides arrive at the desired control may not be practical or economical due to the nematicides' numerous side effects. Since nematodes are not safe for the environment, alternative plant protection strategies such introducing systemic acquired resistances are becoming more and more popular.

In order to ascertain their impact on plant growth and the biochemical changes induced in tomato plants grown in pots after application, the present study set out to monitor the in vivo nematicidal potential of five bio-agents (namely, *Trichoderma harzianum, Metarhizium anisopliae, Verticillium lecanii, Trichoderma asperellum* and *Bacillus subtilis*) against *M. javanica*. Stress-related enzymes such as phenylalanine ammonia lyase (PAL), phenol and polyphenol oxidase (PPO) have generated special attention.

2. MATERIALS AND METHODS

The research was conducted in pots to control tomato root-knot nematodes using bio-agents.

2.1 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 main field for experiment.

2.2 Preparation and Maintenance of Pure Culture of *M. javanica*

The *Meloidogyne javanica* infected tomato plants were removed from the pure culture plots and brought to the laboratory for further analysis. Initially, any adhering dirt particles were carefully removed from the roots by thoroughly washing them with water. To aid in hatching, egg masses extracted from the contaminated roots were

stored at room temperature in watch glasses filled with distilled water. To establish a sufficiently pure population of *Meloidogyne javanica* on the plants and in the soil for further studies, freshly hatched second-stage juveniles (J_2) were paired with one-month-old tomato plants grown in earthen clay pots filled with steam-sterilized soil.

2.3 Testing the Effects of Bio-Agents on the Induction of Defence Enzymes against the Root-Knot Nematode in Tomato Roots

An experiment was carried out under cage house condition in pots filled with naturally infested soil having 2 J_2/g of soil of RKN, *M. javanica* for estimation of the initiation of defence enzymes PAL, phenoland PPO by bio-agents in tomato roots. Bio-agents (*Metarhizium anisopliae, Trichoderma asperellum, Verticillium lecanii*, *Trichoderma harzianum* and *Bacillus subtilis*) were applied to tomato seedlings at a rate of 3g/kg soil per treatment.Untreated check was also maintained for comparison. Four replication of each treatment were conducted. After 14 days of transplanting, the plants were taken out to evaluate the initiation of the defence enzymes PPO, PAL, and phenol. Sixty days following transplantation, the observations on nematode reproduction and plant growth characteristics were made.

2.4 Estimation of Polyphenol Oxidase (PPO) Enzymes in Tomato Roots

The procedure outlined Mayer, et al*.,* [8] was used to determine the undertaking of polyphenol oxidase (EC 1.10.3.1). 0.5 g of plant root tissue were homogenised in 2 ml of the extraction medium, which contained 0.1M sodium phosphate buffer (pH 6.5), to create the enzyme extract. The supernatant from the homogenate was used for the experiment after centrifugation at 16,000 rpm for 15 minutes at 4 °C. The reaction mixture consisted of 200 µL of the enzyme extract and 1.5 mL of 0.1 M sodium phosphate buffer (pH 6.5). To initiate the reaction, 200 µL of 0.01 M catechol was added, and the activity was measured as changes in absorbance at 495 nm per minute per milligram of protein.

2.5 Estimation of Phenylalanine Ammonia Lyase (PAL) Enzymes in Tomato Roots

The activity of phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) was measured according to the method of Dickerson et al*.* [9]. One gram of root samples was homogenized in three milliliters of ice-cold 0.1 M sodium borate buffer (pH 7.0), containing 0.1 g of insoluble polyvinylpyrrolidone and 1.4 mM 2-mercaptoethanol. The homogenate was filtered through cheesecloth, and the filtrate was centrifuged at 16,000 rpm for 15 minutes. The supernatant served as the enzyme source. PAL activity was calculated based on the rate of conversion of L-phenylalanine to trans-cinnamic acid, measured at 290 nm. For the assay, 0.5 mL of 0.1 M borate buffer (pH 8.8) and 0.5 mL of 12 mM L-phenylalanine were added to 0.4 mL of enzyme extract, and the mixture was incubated at 30°C for 30 minutes. The amount of trans-cinnamic acid produced was determined using an extinction coefficient of 9630 M^{-1} cm^{-1}. Enzyme activity was expressed as nmol of trans-cinnamic acid produced per minute per milligram of protein (nmol trans-cinnamic acid min^{-1} mg^{-1} protein).

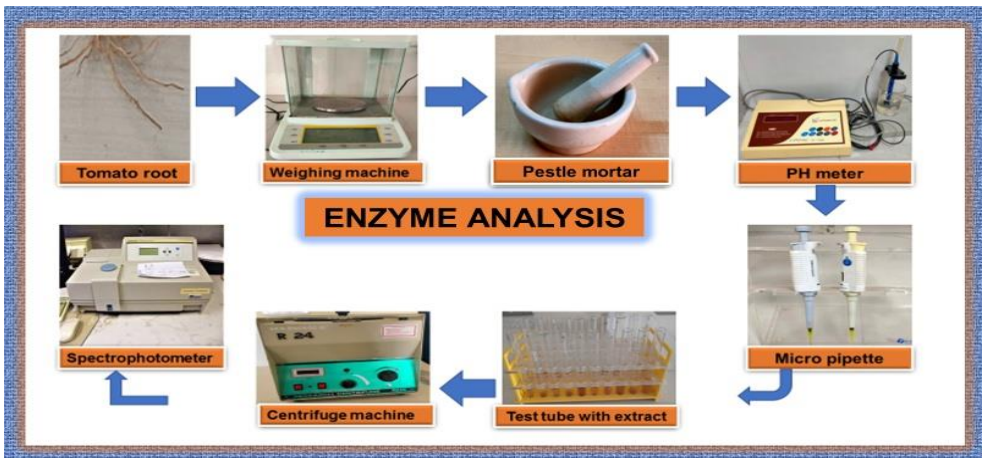

Fig. 1. Enzyme analysis process in tomato roots infected with root- knot nematode

2.6 Estimation of Phenol Content in Tomato Roots

The procedure Malick, [10] was used to measure the phenol's activity. One gram of root material was crushed using a pestle and mortar in ten milliliters of 80% ethanol. The homogenate was then centrifuged at 10,000 rpm for 20 minutes. The supernatant was dissolved and dried using 5 cc of distilled water. Two millilitre aliquots were placed in test tubes and filled with three millilitres of water and half a millilitre of Folin-Ciocalteau reagent. Each tube received two millilitres of 20% Na2CO3 after three minutes, which was followed by a minute in boiling water and cooling. At 650 nm, the absorbance was measured.

2.7 Statistical Analysis

Following the experiment's conclusion, data were statistically examined to evaluate the results. For a meaningful treatment comparison at the 5% level of significance, the crucial deference was computed.

3. RESULTS

3.1 Effect of Bio-Agents on Estimation of Enzymatic (PAL and PPO) Activities and Phenol Content in Tomato Roots

The effect of bio-agents was tested on estimation of PAL, PPO and Phenol content in root-knot nematode diseased tomato plants. The extracts of tomato plant roots were used for estimation of the PPO, PAL and Phenol. The presence of the PPO, PAL and Phenol were confirmed using UV-visible Spectrophotometer applying standard methods. After 14 days of transplanting in tomato roots, all of the investigated bio-agents significantly improved PPO, PAL, and Phenol activity over the untreated control. Experimental results set out in Table 1 revealed that among all the bio-agents maximum (0.12umol/min/gm) PPO activity was recorded with *Trichoderma harzianum* @ 3g/kg soil followed by *Bacillus subtilis* (0.11umol/min/gm) and *Metarhizium anisopliae* (0.10umol/min/gm) @ 3g/kg soil. The highest PAL activity was recorded with *Trichoderma harzianum* @ 3/kg soil (0.48umol/min/gm) followed by *Bacillus subtilis* (0.23umol/min/gm) and *Metarhizium anisopliae* 3g/kg soil (0.20umol/min/gm). However, minimum was observed in untreated Check.

Similarly, *Trichoderma harzianum* @ 3/kg soil was best treatment with maximum (2.41mg/gm) phenol activity in tomato roots followed by *Bacillus subtilis* (1.19mg/gm) and *Metarhizium anisopliae*@ 3g/kg soil (0.93mg/gm) after 14 days of transplanting.

On the other hand, the least successful treatment was the untreated tick, which showed the lowest activity of phenol (0.55 mg/gm), PAL (0.14 umol/min/gm), and PPO (0.05 umol/min/gm) in tomato roots (Table 1).

Table 1. Impact of bio-agents on tomato roots infected with root-knot nematode on PPO, PAL, and Phenol estimates

Treatment	PPO (umol/min/gm)	PAL (umol/min/gm)	Phenol (mg/gm)
T. asperellum	0.09 (1.68)	0.17 (2.39)	0.91 (5.48)
T. harzianum	0.12 (2.01)	0.48 (3.95)	2.41 (8.94)
V. lecanii	0.06 (1.37)	0.16 (2.30)	0.71 (4.82)
M. anisopliae	0.10 (1.85)	0.20 (2.58)	0.93 (5.54)
B. subtilis	0.11 (1.93)	0.23 (2.76)	1.19 (6.26)
Control	0.05 (1.33)	0.14 (2.18)	0.55 (4.26)
SEm±	0.002	0.001	0.001
CD 5%	**0.005**	**0.002**	**0.002**
CV	**3.08**	**0.43**	**0.10**

** Average of four replications;*
** Dose =@ 3 gm/kg soil*

Table 2. Effect of bio-agents on plant growth and nematode reproduction in pot condition

Treatment	Shoot length (cm)	Shoot weight (gm)	Root length (gm)	Root weight (gm)	No. of galls/plant	No. of egg masses/ Plant	Number of eggs and larvae / egg mass	Nematode juvenile/ 200cc soil	Final nematode population
T. asperellum	34.50	28.35	30.00	3.51	176.50	152.00	204.25	956.25	32003.25
T. harzianum	48.75	57.74	41.75	6.42	71.25	52.25	102.00	511.25	5840.00
V. lecanii	32.00	13.38	24.25	2.56	196.25	181.75	236.50	1201.75	44184.75
M. anisopliae	38.50	34.55	33.75	4.56	128.25	115.00	179.50	852.00	21495.25
B. subtilis	41.50	42.84	39.50	5.57	99.00	76.00	136.00	637.75	10974.00
Control	21.00	8.59	18.25	1.61	255.75	210.25	297.75	1452.75	64054.50
SEm±	**0.662**	**0.275**	**0.437**	**0.137**	**0.698**	**0.628**	**0.769**	**2.398**	**158.664**
CD 5%	**1.953**	**0.812**	**1.290**	**0.403**	**2.060**	**1.853**	**2.268**	**7.074**	**468.058**
CV	**6.12**	**3.14**	**4.66**	**11.29**	**1.51**	**1.60**	**1.33**	**0.85**	**1.78**

** Average of four replications*

**Dose = @3 gm/ kg soil at the time of transplanting followed by 20 DAT and 40 DAT (Day After Transplanting)*

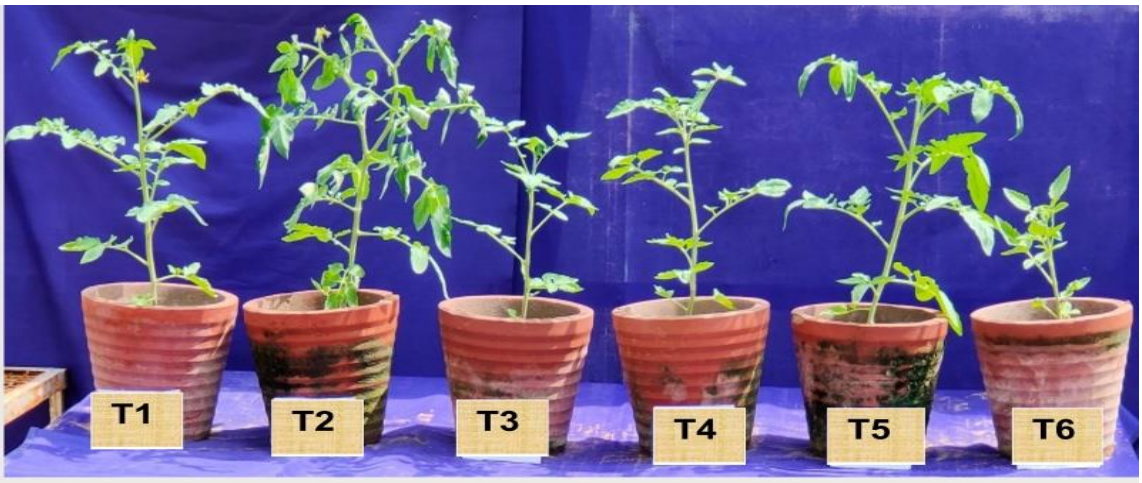

Fig. 2. Effect of bio-agents on plant growth and development in tomato

3.2 Management of Root-Knot Nematode on Tomato

The results showed that, under pot conditions, all of the bio-agents significantly enhanced the growth of tomato plants and reduced nematode reproduction. The dosage of the bio-agents used was 3 g/kg of soil. The findings indicated that the application of *Trichoderma harzianum* was the most effective treatment for improving the growth characteristics of the plants. Maximum shoot length was recorded with *Trichoderma harzianum* (48.75cm) followed by *Bacillus subtilis* (41.50cm) and *Metarhizium anisopliae* (38.50cm). Maximum shoot weight was recorded with *Trichoderma harzianum* (57.74gm) come after *Bacillus subtilis* (42.84gm) and *Metarhizium anisopliae* (34.55gm), maximum root length was recorded with *Trichoderma harzianum* (41.75cm) followed by *Bacillus subtilis* (39.50cm) and *Metarhizium anisopliae* (33.75cm) and maximum root weight showed that *Trichoderma harzianum* (6.42gm) followed by *Bacillus subtilis* (5.57gm) and *Metarhizium anisopliae* (4.56gm).

Regarding nematode reproduction, every bio-agent greatly decreased nematode reproduction; nonetheless, *Trichoderma harzianum* once again showed to be the most suitable treatment. The number of galls per plant recorded with *Trichoderma harzianum* (71.25)followed by *Bacillus subtilis* (99.00) and *Metarhizium anisopliae* (128.25), minimum number of egg masses per plant were recorded with *Trichoderma harzianum* (52.25) followed by *Bacillus subtilis* (76.00) and *Metarhizium anisopliae* (115.00), minimum eggs per egg mass was recorded with *Trichoderma harzianum* (102.00) followed by *Bacillus subtilis* (136.00) and *Metarhizium anisopliae* (179.50), minimum larval population per 200cc soil were recorded with *Trichoderma harzianum* (511.25) followed by *Bacillus subtilis* (637.75) and *Metarhizium anisopliae* (852.00) minimum nematode population were recorded with *Trichoderma harzianum* (5840.00) followed by *Bacillus subtilis* (10974.00) and *Metarhizium anisopliae*(21495.25) Table 2.

In contrast to other treatments, the untreated check was discovered to be the least successful in terms of both reducing the population of nematodes and increasing plant development characteristics.

4. DISCUSSION

The use of several bio-control agents promoted plant growth, provided a variety of nutritional components, and made plants more resistant to external stresses [11]. Tomato root tissue treated with the *Pseudomonas fluorescens* isolate Pfl exhibited an accumulation of defensive enzymes, including peroxidase, polyphenol oxidase, chitinase, phenylalanine ammonia-lyase, and catalase, in response to invasion by the root-knot nematode *Meloidogyne incognita*. According to Anita et al. [12], bacterised tomato root tissues injected with nematodes exhibited significantly increased activities of all the enzymes. The application of various biocontrol agents can enhance the accumulation of defense enzymes such as peroxidase (PO), polyphenol oxidase (PPO), and phenylalanine ammonia lyase (PAL) to induce systemic resistance against the lesion nematode *Pratylenchus coffeae*. In treated

plants, the nematode population decreased, and coffee yield increased [13]. *Trichoderma harzianum* exhibited the highest levels of total phenol content and biochemical activities of PAL, PPO, and PO. Additionally, it demonstrated a reduction in nematode multiplication both on tomato plants and in the soil, along with improvements in tomato plant growth parameters [14]. It was found that *Pochonia chlamydosporia* at a concentration of 4 percent was the most effective in enhancing maize plant growth and reducing infection by *Heterodera zeae* [15]. The best treatments were found to be *P. fluorescens* and *T. viride* applied at a rate of 4g/kg soil in order to maximise plant growth characteristics, minimise nematode populations, and raise the levels of PO, PPO, PAL, and SOD in tomato roots as well as in chilli roots [16]. Systemic resistance activity against the nematode infection was established by *Pseudomonas* spp. and *Bacillus* spp. Vigila et al*.,* [17]. To induce systemic resistance in tomato and cucumber against the avirulent *Meloidogyne incognita*, combine *Trichoderma asperellum* and *Trichoderma harzianum* [18]. According to Kiewnick and Sikora [19], *P. lilacinus* production of acetic acid, chitinases, proteases, and leucinotoxin has been linked to the infection process. According to Duan et al*.* [20], *Aspergillus niger* can reduce the root-knot index and nematode populations while enhancing the activities of defense enzymes in tomato plants. The biocontrol agent *Trichoderma viride* (seed treatment at 4 g/kg seed plus soil application at 4 g/kg soil) was found to be significantly more effective in reducing *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *Lycopersici*, along with improving plant growth parameters, compared to *Paecilomyces lilacinus* [16]. Additionally, when hot water, organic amendments (such as tea waste, tobacco churi, poultry manure, water hyacinth leaf powder, lantana leaf powder, and neem cake), and bio-agents (*Paecilomyces lilacinus* and *Trichoderma harzianum*) were applied together, plant growth parameters improved, and nematode reproduction was significantly reduced [21]. According to Kumari et al*.* (2021) *T. viride* and *T. asperellum* were shown to be equally and considerably effective in inhibiting hatching and larval mortality of *M. incognita*. With *Trichoderma viride* at 5.0g per plant, the greatest reductions in nematode population, root galls, egg mass contents, and egg masses were observed on cucumber plants. *Paecilomyces Lilacinus* and *Trichoderma harzianum* were next in line [22],[23-25].

5. CONCLUSION

To study the effect of bio-agents in assessment of PPO, PAL and phenol in tomato roots infested with root-knot nematode, *M. javanica*. Results showed that application of *Trichoderma harzianum* was found to be the best treatment to enhance PPO, PAL and phenol activity, improve plant growth characters and minimum nematode reproduction @ 3gm/kg soil.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ACKNOWLEDGEMENT

The author sincerely thanks the Department of Plant Pathology, the Department of Nematology, the Department of Biochemistry, and the Division of Nematology at RARI, Durgapura, Jaipur (SKNAU, Jobner) for their support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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