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The Enzymatic Effect of Bio-agents on the Enzymatic Activity of Brinjal Infected with Root-knot Nematode (*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.

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ABSTRACT

Effect of culture filtrate (20 per cent concentrate) of bioagents (*viz., Trichoderma viride, Trichoderma harzianum, Purpureocillium lilacinum, Pochonia chlamydosporia* and *Pseudomonas fluorescens*) were tested on root-knot nematode (*M. javanica)*. The accumulation of PPO and PAL and Phenol in brinjal roots infected with root-knot nematode (*M. javanica)* were also evaluated. The

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results showed that application of bio-agents increased the level of PPO, PAL and phenol in brinjal roots. Among all the treatments, application of *T. viride* @ 3 gm/kg soil is best treatment enhancing PPO, PAL and phenol activity, improve plant growth characters as well as in reducing nematode population over other treatment.

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

1. INRODUCTION

"Brinjal (*Solanum melongena L*.) is a popular and widely consumed vegetable throughout the world. It is a good source of minerals and vitamins" [1,2].

"China, the largest producer of brinjal contributes about 68.7% of the world's brinjal production while India occupies second position in production and contributes 23.3%. Brinjal is infected by a number of diseases caused by fungi, bacteria, viruses and nematodes and these diseases cause extensive losses in yield" [3]. "Among them nematodes are hidden enemy of plant which is most harmful for vegetable production. Among the plant parasitic nematodes root-knot nematodes *Meloidogyne spp*. is an important polyphagous pest of vegetables all around the world" [4]. Which is causes severe damage to brinjal crop. The infected plant shows reduced root system with less feeder roots [5]. "Extensive galling and root damage is associated with nematode infection. Vegetable crops are among the most susceptible and worst affected by these nematodes. Nematode control is far more complex than any other kind of pathogens because nematodes mainly attack underground parts of plants" [6]. "Biological control and organic amendments appear the alternative strategies for management of plant parasitic nematodes in the soil" [7,8]." Organic amendments have consistently shown beneficial effects on soil nutrients, soil physical conditions, soil biological activity and thereby improving the health of plants and reducing populations of plant parasitic nematodes" [9].

The objective of the present study was monitor the *in vivo* nematicidal potential of bio-agents (*viz., Trichoderma viride, Trichoderma harzianum, Purpureocillium lilacinum, Pochonia chlamydosporia* and *Pseudomonas fluorescens*) against *M. javanica* in order to determine their effect on plant growth and the biochemical changes induced in brinjal plant in pots following application. Particular attention has been paid on stress enzymes including polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and phenol.

2. MATERIALS AND METHODS

The experiment was conducted for management of root-knot nematode in brinjal through bio-agents in pot condition.

Preparation and maintenance of pure culture of *M. javanica*: "Brinjal plants infected with *M. javanica* were uprooted from the pure culture plots brought to the laboratory. The roots were first rinsed carefully in water to remove adhering soil particles. Freshly hatched J2 were inoculated on one month old brinjal plants already grown and maintained in earthen clay pots filled with steam sterilized soil to obtained adequate pure population of *M. javanica* on the plants and in soil to carry out further experiments" [9].

2.1 Enzyme Analysis

Determination of polyphenol oxidase (PPO) enzymes in brinjal roots: "Polyphenol oxidase (EC 1.10.3.1) activity was estimated simultaneously by the method of Mayer et al. [10].

The enzyme extract was prepared by homogenizing 0.5 g of plant tissue in 2.0 ml of the extraction medium 0.1M sodium phosphate buffer (pH 6.5). The homogenate was centrifuged at 16,000 rpm for 15 min at 4°C and the supernatant was used for the assay. The reaction mixture consisted of 200µl of the enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5). To start the reaction 200µl of 0.01 M catechol was added and the activity was expressed as changes in absorbance at 495 nm min-1 mg-1 protein" [9].

Determination of phenylalanine ammonia lyase (PAL) enzymes in brinjal roots: The activity of phenylalanine ammonia lyase (EC 4.3.1.5) was measured following the method of Dickerson et al. [11].

"Root samples (1g) were homogenized in 3 ml of ice cold 0.1 M sodium borate buffer, pH 7.0 containing 1.4mM of 2-mercaptoethanol and 0.1 g of insoluble polyvinyl pyrrolidine. The extract was filtered through cheese cloth and the filtrate was centrifuged at 16,000 rpm for 15 min. The supernatant was used as enzyme source. Pal activity was determined as the rate of conversion of Lphenylalanine to trans-cinnamic acid at 290nM. A sample containing 0.4 ml of enzyme extract was incubated with 0.5 ml of 0.1M borate buffer, pH 8.8 and 0.5 ml of 12mM L-phenylalanine in the buffer for 30 min at 30°C. The amount of trans-cinnamic acid synthesized was calculated using its extinction coefficient of 9630 m-1. Enzyme activity was expressed as nmol trans-cinnamic acid min-1 mg-1 protein" [9].

Determination of phenol enzymes in brinjal roots: The activity of phenol was measured following the method of Malik and Singh (1980).

One gm root sample was ground in 10 ml of 80 per cent ethanol using pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant dried and dissolved in 5 ml distilled water. The aliquots (2 ml) taken in test tubes were made to the volume of 3 ml with water and 0.5 ml of Folin-Ciocalteau reagent. After three min, two ml of 20 per cent Na2Co3 was added to each tube and placed in boiling water for a min and cooled. The absorbance was measured at 650 nm.

2.2 Statical Analysis

After completion of experiment, data were statically analyzed for interpretation of finding. The critical deference was calculated for comparison of treatment for significant at 5% level of significance. Summary Table 1 along with SEm± and CD were worked out and presented in "Experimental Results".

3. RESULTS

Estimation of PPO and PAL and Phenol in brinjal roots infected with root-knot nematode: An experiment was conducted against root-knot nematode, *M. javanica* on brinjal. Experimental results showed that all the tested bio-agents significantly increased PPO activity in brinjal roots over untreated check after 14 days of transplanting. Maximum (0.22 umol/min/gm) PPO activity was recorded in plants treated with *T. viride* @ 3g/kg soil followed by *P. lilacinus* (0.14 umol/min/gm) and *P. fluorescens* (0.12 umol/min/gm) @ 3g/kg soil. While, minimum PPO activity (0.10 umol/min/gm) was observed in untreated control plant.

Results on accumulation of PAL revealed that all bio-agents significantly increased the PAL activity in brinjal as compared to untreated check after 14 days of transplanting. The highest PAL activity was recorded in plants treated with *T. viride* @ 3/kg soil (0.44 umol/min/gm) followed by *P. lilacinus* (0.40 umol/min/gm) and *P. fluorescens* @ 3g/kg soil (0.37 umol/min/gm). While, minimum PAL (0.11umol/min/gm) activity was observed in untreated control plant.

Results presented in Table 1 showed that maximum (0.50 mg/gm) phenol activity was recorded in plants treated with *T. viride* @ 3/kg soil followed by *P. lilacinus* (0.41 mg/gm) and *P. fluorescens* @ 3g/kg soil (0.39 mg/gm). While, minimum phenol activity (0.28mg/gm) was observed in untreated control plant. All tested bio-agents significantly increased the phenol activity in brinjal as compared to untreated check after 14 days of transplanting.

Table 1. Estimation of PPO and PAL and Phenol in brinjal roots infected with root-knot nematode using bio-agent

Treatment	PPO (umol/min/gm)	PAL (umol/min/gm)	Phenol (mg/gm)
T. viride	0.22(2.68)	0.44(3.82)	0.50(4.03)
T. harzianum	0.10(1.79)	0.20(2.56)	0.31 (3.21)
P. lilacinum	0.14(2.11)	0.40(3.61)	0.41(3.68)
P. chlamydosporia	0.11(1.89)	0.23(2.75)	0.36(3.42)
P. fluorescens	0.12(2.01)	0.37(3.51)	0.39(3.57)
Control	0.10(1.78)	0.11 (1.87)	0.28(3.01)
SEm±	0.001	0.001	0.002
CD 5%	0.002	0.004	0.005
CV %	0.97	0.95	0.84

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

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

Treatment	Shoot length (cm)	Shoot weight (gm)	Root length (cm)	Root weight (gm)	Number of galls/plant	Number of egg masses/ plant	Number of eggs/ egg mass	Nematode juvenile/ 200cc soil	Final nematode population
T. viride	48.75	44.28	35.70	8.43	111.00	70.25	268.75	416.75	19297.00
T. harzianum	21.50	16.15	15.53	2.50	209.50	132.50	291.75	667.75	39323.75
P. lilacinum	41.00	36.85	27.28	5.78	125.50	82.75	277.50	448.25	23410.75
P. chlamydosporia	26.00	21.98	17.08	3.33	190.25	105.50	286.50	609.00	30835.00
P. fluorescens	34.75	30.05	22.28	4.08	142.50	90.25	281.00	509.50	25868.75
Control	16.50	8.55	9.60	1.43	264.75	161.50	300.25	1245.75	49738.50
SEm±	1.140	1.014	0.788	0.331	0.901	0.922	1.215	3.578	322.311
CD 5%	3.386	3.012	2.340	0.984	2.678	2.740	3.609	10.632	957.635
CV %	7.25	7.71	7.42	15.57	1.04	1.72	0.85	1.10	2.05

* Average of four replications

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

Management of root-knot nematode on brinjal: The results showed that all the bio-agents significantly increase brinjal plant growth and reduced nematode reproduction in pot condition. The bio-agents were used @ 3g/kg soil.

Among the bio-agents the maximum shoot length was recorded with *Trichoderma viride* (48.75 cm) 3g/kg soil followed by *Purpureocillium lilacinum* (41.00cm) and *Pseudomonas fluorescens* (34.75 cm) @ 3g/kg soil. The maximum shoot weight was recorded with *Trichoderma viride* (44.28gm) followed by *Purpureocillium lilacinum* (36.85 gm) and *Pseudomonas fluorescens* (30.05 gm) @ 3g/kg soil. The highest root length was recorded with *Trichoderma viride* (35.70cm) followed by *Purpureocillium lilacinum* (27.28cm) and *Pseudomonas fluorescens* (22.28cm) @ 3g/kg soil. Maximum root weight was recorded with *Trichoderma viride* (8.43 gm) followed by *Purpureocillium lilacinum* (5.78 gm) and *Pseudomonas fluorescens* (4.08 gm) @ 3g/kg soil.

As regard to nematode reproduction, all the bio-agents significantly reduced the nematode reproduction but among them minimum number of galls per plant were recorded with *Trichoderma viride* (111.00) followed by *Purpureocillium lilacinum* (125.50) and *Pseudomonas fluorescens* (142.50) @ 3g/kg soil. The minimum number of egg masses per plant were recorded with *Trichoderma viride* (70.25) followed by *Purpureocillium lilacinum* (82.75) and *Pseudomonas fluorescens* (90.25) @ 3g/kg soil. The minimum eggs per egg mass were recorded with *Trichoderma viride* (268.75) followed by *Purpureocillium lilacinum* (277.50) and *Pseudomonas fluorescens* (281.00) @ 3g/kg soil. Whereas, the maximum number of eggs /egg mass (300.25) were recorded with untreated check. The minimum juvenile population per 200cc soil were recorded with *Trichoderma viride* (416.75) followed by *Purpureocillium lilacinum* (448.25) and *Pseudomonas fluorescens* (509.50) @ 3g/kg soil. The minimum nematode population were recorded with *Trichoderma viride* (19297) followed by *Purpureocillium lilacinum* (23410.75) and *Pseudomonas fluorescens* (25868.75) @ 3g/kg soil.

4. DISCUSSION

The present investigations were carried out the efficacy of bio-agents *in vitro* condition and to determine the changes in PPO, PAL and phenol in brinjal roots after 14 days of transplanting and to management of root-knot nematode, *M. javanica* using bio-agents. An experiment was carried out under cage house condition in pots filled with naturally infected soil with root-knot nematode @ 2 J2/g soil to assess the induction of defence enzymes PPO, PAL and Phenol by bioagents against *M. javanica* in a susceptible variety of brinjal. Application of bio-agents *T. viride, T. harzianum, P. lilacinum, P. chlamydosporia* and *P. fluorescens* were added to soil each @ 3g per kg soil. Application of a talc-based formulation of bio-agents as soil treatment significantly reduced the root-knot nematode population in brinjal roots. Root galling in brinjal roots due *to M. javanica* infection was also less in treated plants with bioagents as compared to untreated plants. Among all the treatments application of *T. viride* was found to be the best treatments to enhance enzymatic activity against *M. javanica* in brinjal roots 14 days after transplanting. Similar results were recorded in tomato [12] and in chilli [13] that application of bio-agents increased the level of PO, PPO, PAL and SOD in tomato and chilli roots. It was gradually increased after 7, 14, 21, 28 day of transplanting but drastically decreased after 60 days of transplanting. “Among all the bioagents application of *P. fluorescens* and *Trichoderma viride* @ 4g/kg soil was found to be the best treatments to enhance enzymatic activity, to improve plant growth and reduction in nematode reproduction. The application of *P. fluorescens* + *P. lilacinus* recorded lowest nematode population in soil, root and tuber over control” [14]. “Among the bio-agents application of *P. fluorescens* and *T.viride* @ 4g/kg soil was found to be the best treatments in regards to increase the level of PO, PPO, PAL and SOD in tomato roots” [15] as well as in chilli roots [16].

5. SUMMARY AND CONCLUSION

Efficacy of bio-agents *in vitro* condition and to determine the changes in PPO, PAL and phenol in brinjal roots after 14 days of transplanting and to management of root-knot nematode, *M. javanica* using bio-agents.

An experiment was carried out in pots filled with naturally infested soil of root-knot nematode 2 J2/g soil to assess the accumulation of PPO, PAL and phenol by bio-agents against *M. javanica* in brinjal. Bio-agents (viz., *T. viride, T. harzianum, P. lilacinum, P. chlamydosporia* and *P. fluorescens*) were added to soil each @ 3gm / kg soil. An untreated check was also maintained

for comparison. All the treatments replicated four times. Brinjal plants were harvested after 14 and 60 days of transplanting for estimation of PPO, PAL and phenol activity (14 DAT) as well as plant growth parameters and nematode reproduction (60 DAT). The effect of bioagents was assayed in brinjal roots infested with the root- knot nematode *M. javanica* showed that application of bio-agents increased the level of, PPO, PAL and phenol in brinjal roots. Among all the treatments, application of *T. viride* @ 3gm/kg soil was found to be the best treatment to enhance PPO, PAL and phenol activity followed by *P. lilacinum* and *P. fluorescens* @ 3gm/kg soil. However, untreated check was found inferior with lowest PPO, PAL and phenol activity. Among all the treatments application of *Trichoderma viride* @ 3g/kg soil was found to be the best treatments to improve plant growth characters and reducing nematode reproduction.

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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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