Eco. Env. & Cons. 30 (October Suppl. Issue) : 2024; pp. (S133-S136)
Copyright@ EM International
ISSN 0971–765X

DOI No. http://doi.org/10.53550/EEC.2024.v30i06s.020

Impact of Different Fungal Bio-agents on Biochemical Alteration of Phenylalanine Ammonia Lyase (PAL) on Tomato (*Lycopersicon esculentum* L.)

Ramavath Abhi*, M.K. Sharma, Manisha, B.L. Baheti, Deepak Kumar, R. N. Kumhar, C. P. Nama and Parwati

Department of Nematology, Rajasthan College of Agriculture, MPUAT, Udaipure 313 001, Rajasthan, India

(Received 4 March, 2024; Accepted 10 May, 2024)

ABSTRACT

A study was conducted in two consecutive years, 2021 and 2022, in a cage house condition in soil infested with root-knot nematodes (2 J2/g soil), to evaluate the induction of the defense enzyme phenylalanine ammonia lyase (PAL) by various fungal bio-agents against *Meloidogyne incognita* infecting tomato. *Trichoderma harzianum, T. viride, Purpureocillium lilacinum, Metarhizium anisopliae*, and *Beauveria bassiana* were the bioagents used as soil application (SA) and seedling treatment (ST). Two control treatments were also maintained for comparison: *M. incognita* alone and un-inoculated and untreated control. Each treatment was replicated four times. When bioagents were applied, increased the level of PAL in tomato leaf, shoot and root sample. Application of *T. harzianum* was shown to be the most effective treatment among all of them to increase PAL activity at 15, 30, and 45 days after inoculation (DAI) followed by *T. viride* and *P. lilacinum*.

Key words: *Phenylalanine ammonia lyase, Fungal bio-agents, Root-knot nematode, Tomato.*

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is an important crop grown throughout the world (Agrios, 2005). The tomato originated in South America, more precisely in Peru, Ecuador, and Bolivia (Nonneoke, 1989). Fruits and vegetables are vital parts of our diets because they supply vitamins and minerals that the body needs for development and growth. The top tomato-growing countries in the world are China, Egypt, India, Italy, The United States and Turkey. India is the second-largest producer of veggies in the world, after China.

Poor seed quality, unfavorable environmental factors, and the existence of diseases brought on by pests and pathogens, particularly plant-parasitic nematodes (PPNs) have all been connected to low tomato yields. Overall, PPNs cause 21.3 per cent crop losses amounting to Rs. 102,039.79 million (1.58 billion USD) annually. *Meloidogyne incognita* was economically most important root-knot nematode (RKN) causing yield losses of Rs. 6035.2 million in tomato (Kumar *et al.*, 2020). *Meloidogyne* spp. are polyphagous in nature infecting over 3000 host species including vegetables, fruits, oil, fiber, cereals and leguminous crops, next to weeds that are considered secondary hosts to nematodes (Khalil, 2013).

One of the main tomato pests that reduces fruit yield globally is RKN (Shoresh *et al.*, 2010). The formation of galls by root-knot nematode grossly affect nutrient partitioning and water uptake in the host thus, affecting yield of the crop (Anwar and

Mckenry 2012). In order to obtain effective control, nematicides are often applied at higher doses, which may be costly, phyto-toxic and may cause residue problems which may create ecological disturbance in the nature. However, the use of biocontrol agents remains the most viable option.

Biocontrol agents is one of the environmentally friendly strategy for the management of nematode diseases. A plant's response to a root-knot nematode infection involves a sequence of biochemical and physical processes. Certain compounds that are poisonous to root-knot nematodes are synthesized by plants. Reactive oxygen species (ROS) are crucial for plant defense, and in resistant plants, ROS detoxifying enzymes like CAT, PO, PPO and PAL are frequently inhibited during pathogen attack (Klessing *et al.,* 2000).

Much research has been done on *M. incognita* on tomatoes thus far, but little is known about the function of bioagents in identifying defense enzymes like PAL against root-knot nematodes. Consequently, the current study was started.

Materials and Methods

Collection and maintenance of *Meloidogyne incognita* and fungal bioagents

M. incognita was obtained from naturally infested tomato fields of Department of Nematology, MPUAT, Udaipur (Rajasthan), and maintained as a pure culture on potted tomato plants in a net-house. Fungal bioagents *i.e., Trichoderma harzianum* and *T. viride* was obtained from Department of Plant Pathology, *Purpureocillium lilacinum* was obtained from Department of Nematology, and *Metarhizium anisopilae* and *Beauveria bassiana* was obtained from Department of Entomology, RCA, Udaipur. Pure culture of these fungal bio-agents was maintained on Potato Dextrose Agar media in laboratory for further studies.

Preparation of culture filtrates of fungal bioagents

In order to prepare the fungal culture filtrates, 250ml Erlenmeyer flasks containing 100 ml of potato dextrose broth were filled and seeded with fungal bio-agents that were already evaluated. The inoculated flasks were incubated in a BOD incubator for 15 days at 25± 2 °C. Later that, Whatman filter paper no. 1 was used to filter the fungal culture filtrates. After that, the filtrates were centrifuged again at 2000 rpm to get rid of any remaining mycelia and spores. Then, supernatants were gathered and utilized for the in vitro research.

Treatment details

The experiment was conducted in the net house of the Department of Nematology, RCA, Udaipur during in the year of 2021 and 2022. Pots were arranged in a CRD with four replications for each treatment. All the pots were transplanted with 25 days old seedlings of tomato. The pots receiving the treatments of bio-agents (*i.e., T. harzianum, T. viride*, *P. lilacinus*, *M. anisopliae* and *B. bassiana* were added to soil each @ 5g per kg soil and seedling treatment (ST) @ 5g per liter of water) were inoculated with *M. incognita* @ 2 J_2/cc soil as also fifteen days old culture of bio-agents enriched vermicompost. Two control treatments *viz., M. incognita* alone (2 J2/cc soil) and un-inoculated and untreated control was also maintained for comparison.

Enzymatic assay

Phenylalanine ammonia lyase (PAL)

The PAL (EC 4.31.5) activity assessment was conducted according to with the guidelines protocol established by Dickerson *et al.* (1984). To begin, 1 g of plant samples were homogenized in 3 ml of ice-cold, 0.1 M sodium borate buffer with a pH of 7, which also contained 1.4 mM of 2-mercaptoethanol and 0.1 g of insoluble polyvinyl pyrrolidine. Following homogenization, the extract was centrifuged for 15 minutes at 16000 rpm, and the supernatant was used as the source of enzymes. The PAL activity was quantified by monitoring the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm. Specifically, 0.4 ml of enzyme extract was incubated with 0.5 ml of 0.1 M borate buffer at pH 8.8 and 0.5 ml of 12 M ml L-phenylalanine in the same buffer for 30 min at 30!. Enzyme activity was expressed as trans-cinnamic acid min^{-1} μg^{-1} protien.

Results and Discussion

All the bioagents showed greater influence for induction of defense enzymatic activities against *M. incognita* in tomato (Table 1-3). The phenylalanine ammonia lyase (PAL) activity in the leaf, shoot and roots of tomato were found to be significantly increased in all the treatments after 15, 30 and 45 DAI as compared to the controls (Figure 1-3), the maximum being recorded in T1 *i.e.,* SA and ST with *T. harzianum*. In this treatment the pooled data of (2021 and 2022 year) PAL activity was recorded to be (20.58, 22.58 and 25.62 µg in leaf, shoot and root respectively at 15 DAI), (22.04, 24.90 and 27.22 µg in leaf, shoot and root respectively at 30 DAI) and (25.50, 26.03 and 29.14 µg in leaf, shoot and root respectively at 45 DAI) (Table 1-3). In respect of other bio-agents increased PAL activity were recorded in *T. viride* followed by *P. lilacinum* and *B. bassiana.* However, all the treatments were found to be significantly different from each other.

Deepa *et al.* (2014) observed the biochemical mechanism of bio-control agents like, *T. harzianum, T. viride* and *P. chalmydopsoria* against *Tylenchulus semipenetrans* on *Citrus limonia* and they recorded that, *T. harzianum* show highest enzymatic activities as compared to other fungal bio-agents thus confirming the results of the present investigation. Naserinasab *et al.* (2012) studied that application of *Trichoderma* spp observed to be increases in the enzymatic activities in treated *Lycopersicon esculentum* (tomato) which ultimately decrease the biotic potentiality of root-knot nematode, *M. incognita* and support the result of the present investigation. The level of enzymatic activity (PO, PPO, PAL and SOD) was increased in the tomato roots by application of bio-agents (*T. viride, T. harzianum, P. chlamydosporia, P. lilacinus* and *P. fluorescens*). However, the similar result also studied by Annapurna *et al.*, (2018) reported that all the bioagents *viz., T. viride, T. harzianum, P. chlamydosporia and P. lilacinum* recorded greater influence for induction of defense enzymatic activities against *M. incognita* in tomato. The PO, PPO, PAL activities and total phenol content in the roots of tomato were recorded to be sig-

Table 1. Effect of fungal bio-agents on Phenylalanine ammonia lyase (PAL) activity in tomato leaf infected with root-knot nematode, *Meloidogyne incognita* under pot condition (2021, 2022 and pooled data)

Treatments	Specific activity of PAL in tomato leaf sample (nmol trans-cinnamic acid min^{-1} μg^{-1}protein)								
	15 DAI			30 DAI			45 DAI		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
T1	20.59	20.57	20.58	22.05	22.04	22.04	25.51	25.50	25.50
T2	18.13	18.12	18.12	20.91	20.90	20.90	21.98	21.96	21.97
T3	17.98	17.95	17.96	19.15	19.14	19.14	20.86	20.84	20.85
T4	10.32	10.30	10.31	12.98	12.97	12.97	14.32	14.31	14.31
T5	13.59	13.57	13.58	15.51	15.50	15.50	17.02	17.01	17.01
T6	4.83	4.82	4.825	6.01	6.00	6.00	7.21	7.20	7.20
T7	6.68	6.65	6.66	8.13	8.12	8.12	10.12	10.11	10.11
SEm±	0.19	0.18	0.11	0.26	0.26	0.15	0.27	0.23	0.14
CD at 5%	0.57	0.55	0.31	0.77	0.78	0.43	0.80	0.67	0.41
CV(%)	2.96	2.85	2.90	3.51	3.53	3.53	3.26	2.73	3.00

Table 2. Effect of fungal bio-agents on Phenylalanine ammonia lyase (PAL) activity in tomato shoot infected with root-knot nematode, *Meloidogyne incognita* under pot condition (2021, 2022 and pooled data)

Treatments	Specific activity of PAL in tomato shoot sample (nmol trans-cinnamic acid min^{-1} μg^{-1} protein)								
	15 DAI			30 DAI			45 DAI		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
T1	22.59	22.58	22.58	24.91	24.90	24.90	26.05	26.02	26.03
T2	20.91	20.90	20.90	22.11	22.09	22.10	24.51	24.50	24.50
T3	18.05	18.03	18.04	20.91	20.88	20.89	21.56	21.50	21.53
T4	11.92	11.90	11.91	13.13	13.13	13.13	15.96	15.95	15.95
T5	15.93	15.91	15.92	17.03	17.01	17.02	19.57	19.53	19.55
T6	5.83	5.81	5.82	7.05	7.04	7.04	9.15	9.14	9.14
T7	8.95	8.94	8.94	10.03	10.01	10.02	12.81	12.80	12.80
SEm±	0.21	0.21	0.12	0.29	0.28	0.16	0.31	0.27	0.17
CD at 5%	0.62	0.63	0.35	0.85	0.81	0.46	0.91	0.78	0.48
CV(%)	2.83	2.88	2.86	3.50	3.36	3.43	3.34	2.88	3.12

Table 3. Effect of fungal bio-agents on Phenylalanine ammonia lyase (PAL) activity in tomato root infected with root-knot nematode, *Meloidogyne incognita* under pot condition (2021, 2022 and pooled data)

Treatments	Specific activity of PAL in tomato root sample (nmol trans-cinnamic acid min^{-1} μg^{-1}protein)								
	15 DAI			30 DAI			45 DAI		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
T1	25.63	25.62	25.62	27.23	27.21	27.22	29.15	29.13	29.14
T2	22.56	22.53	22.54	24.31	24.30	24.30	26.09	26.07	26.08
T3	20.18	20.15	20.16	22.15	22.13	22.14	25.19	25.17	25.18
T4	13.83	13.81	13.82	15.98	15.97	15.97	18.01	18.00	18.00
T5	16.78	16.73	16.75	19.31	19.30	19.30	21.11	21.10	21.10
T6	6.07	6.05	6.06	9.12	9.11	9.11	11.98	11.95	11.96
T7	10.95	10.93	10.94	12.65	12.63	12.64	14.01	14.00	14.00
SEm±	0.46	3.37	0.24	0.28	0.27	0.16	0.34	0.37	0.21
CD at 5%	1.36	1.09	0.69	0.83	0.79	0.45	1.01	1.08	0.59
CV(%)	5.58	4.47	5.06	3.03	2.86	2.95	3.30	3.53	3.42

Treatment details: $\mathbf{T_1}$-SA and ST with *T. harzianum* at 5g/kg soil & 5g /lit of water, $\mathbf{T_2}$- SA and ST with *T. viride* at 5g/kg soil & 5g /lit of water, $\mathbf{T_3}$- SA and ST with *P. lilacinum* at 5g/kg soil & 5g /lit of water, $\mathbf{T_4}$- SA and ST with *M. anisopliae* at 5g/kg soil & 5g /lit of water, $\mathbf{T_5}$- SA and ST with *B. bassiana* at 5g/kg soil & 5g /lit of water, $\mathbf{T_6}$-*M. incognita* @ 3000 J2/kg of soil alone, $\mathbf{T_7}$-Uninoculated and Untreated control.

nificantly increased in all the treatments after 15, 30 and 45 DAI as compared to the untreated and uninoculated controls, the maximum being showed in *T. harzianum* followed by *T. viride*, *P. chlamydosporia* and *P. lilacinum* thus, confirming the results of the present investigation. The tested bio-agents have ability to show increased in the PAL activity after 15, 30 and 45 DAI and it indicates that all the tested bioagents have capacity to induce resistance mechanism through release of such bio chemicals which showed antagonistic activity toward pathogen *M. incognita*. Among the tested bio-agents, *T. harzianum* was recorded to be more virulence in term of release of biochemical *viz*., PAL content in inoculated tomato plant. Hence, the study revealed that the tested fungal bioagents like *T. harzianum*, *T. viride*, *P. lilacinum*, *M. anisopliae* and *B. bassiana* has ability in the increases of defense enzymes like PAL content in tomato against *M. incognita*.

References

Agrios, G.N. 2005. *Plant Pathology*, 5th Edition, Academic press, New York, USA.

Annapurna, M., Bhagawati, B. and Kurulkar Uday, 2018. Biochemical Mechanism of Native Fungal Bioagents in the Management of Root-Knot Nematode *Meloidogyne incognita* on Tomato. *International Journal of Current Microbiology and Applied Sciences*. 7(11): 380-395.

Anwar, S.A. and Mckenry, M.V. 2012. Incidence and population density of plant patrasitic nematodes infecting vegetable crops and associated yield losses. *Pakistan Journal of Zoology*. 44: 327-333.

Deepa, S. P., Subramanian S. and Ramakrishnan S. 2014. Biochemical mechanism of biocontrol agents in the management of citrus nematode, *Tylenchulus semipenetrans* on Lemon, *Citrus limonia* L. *Indian Journal of Nematology*. 44 (1): 1-5.

Dickerson, D.P., Pascholati, S., Hagerman, A.E., Butler, L.G. and Nicholson, R.L. 1984. Phenylalanine ammonia lyase and hydroxycinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *H. carbonum*. *Physiological Plant Pathology*. 25: 111-123.

Khalil MS. 2013. Alternative Approaches to Manage PlantParasitic Nematodes. *Journal of Plant Pathology Microbiology*. 4:e105.

Klessing, D.F., Dumer, J. and Noad, R. 2000. Nitric oxide and salicylic acid signaling in plant defense. *Proceedings of Nature Academic Sciences*, USA. 97: 8849-8855.

Kumar, V., Khan, R.M. and Walia, R.K. 2020. Crop loss estimations due to plant parasitic nematode in major crops in India. *National Academy Science Letters*.

Naserinasab, F., Sahebani, N. and Etebarian, H.R. 2012. Biological control of *Meloidogyne javanica* by *Trichoderma harzianum* BI and salicylic acid on tomato. *African Journal of Food Science*. 5(3): 276 – 280.

Nonneoke, I.L. 1989. Vegetable production. Van Nostrand Reinhold, New York, p. 657.

Shoresh, M., Harman, G. E and Mastouri, F. 2010. Induced systemic resistance and plant responses to fungal biocontrol agents. *Annual Review of Phytopathology*. 48: 21–43.

Tisserat N. 2006. Root-knot nematodes of tomato. Fact sheets tomato. Extension plant pathology. Kansas State University, Manhattan.