

**BIOCHEMICAL CHANGES DUE TO BIOAGENTS USED FOR THE CONTROL
OF ROOT KNOT NEMATODE IN POMEGRANATE SAPPLINGS UNDER
NURSERY**

VARPE, S. N.; *WALUNJ, A. R. AND DAMAME, S. V.

**DEPARTMENT OF ENTOMOLOGY & BIOCHEMISTRY
MAHATMA PHULE KRISHI VIDYAPEETH
RAHURI - 413 722, MAHARASHTRA, INDIA**

***EMAIL: jaigurudeo63@gmail.com**

ABSTRACT

The investigation on effectiveness of different bio-agents against root-knot nematode infesting pomegranate saplings grown under nursery was carried out during 2014-15. It was evident from the results that the activities of peroxidase and polyphenol oxidase activity of enzymes and total polyphenols were found significantly higher at 30, 60 and 90 days after treatments in the roots of saplings treated with bioagent, Phule Trichoderma plus (2×10^6 /g) @ 10 g/sapling than the other treatments. The highest activity of peroxidase and polyphenol oxidase enzymes and total polyphenols in the roots of pomegranate saplings correspondingly showed lower number of population, root galls and egg masses of root-knot nematodes in pomegranate saplings.

KEY WORDS: Bioagents, root knot nematode, saplings, peroxidase and polyphenol oxidase enzymes, pomegranate

INTRODUCTION

In Maharashtra and in adjoining states like Gujarat, Karnataka, Madhya Pradesh and Rajasthan, the area under pomegranate is continuously increasing. Therefore, there is huge demand by farmers for the pomegranate saplings from Maharashtra. In Maharashtra state, nearly 100 governmental and about 1000 private nurseries registered under horticultural crops, which are engaged in supplying healthy, quality and disease free pomegranate saplings to the farmers. However, the local private nursery holders and farmers raised the nursery by using local available soil from their field, which might be source of nematode inoculum. Such saplings in nursery get attacked by root-knot

nematodes. Diseases like wilt complex caused by nematodes are of economic importance as light soil favours the buildup of nematode population as compared to medium to heavy soils. The root-knot nematode, *Meloidogyne incognita* causing serious damage to the pomegranate in field (Walunj and Mhase, 2015). The various bioagents play an important role in management of root-knot nematode due to its versatile mode of action like antibiosis, competition, tolerance to stress at sapling stage in nursery, with increasing the sapling growth parameters. The soil application of *Trichoderma viride* and *Pseudomonas fluorescens* for the management of root-knot nematodes in coconut, significantly increase in the activities

of defense related enzymes viz., peroxidase and polyphenol oxidase (Karthikeyan *et al.* 2006). The *Trichoderma spp.* managed the root-knot nematode population through two mechanism of action. First mechanisms of action is direct parasitism of eggs and larva through the increase in chitinase and protease activities, which would be indicators of eggs infection capability and second mechanisms of action is inducing plant defense mechanism leading to systemic resistance through different biochemical's (Sharon *et al.* 2001). Keeping in view to the above facts, the present study was undertaken with an objective to find out the effect of fungal bioagents in biochemical parameters in the roots of bioagent treated sapling of pomegranate.

MATERIALS AND METHODS

An investigation on effectiveness of six different bioagents and one chemical against root-knot nematode infesting pomegranate saplings was conducted in randomized block design. The application of bioagents in pomegranate sapling was carried out at the time of planting air layers in polybags in Glasshouse nursery, under the project AICRP on Nematodes, Department of Agricultural Entomology, Mahatma Phule Krishi Vidyapeeth, Rahuri. The soil population of nematodes was measured at fortnight interval. For this purpose, 200 g of soil from the root zone was collected, thoroughly mixed and packed in polythene bag, brought to the laboratory and extracted by Cobb's Decanting and Sieving Method (Cobb, 1918). The roots of pomegranate were uprooted, washed, egg masses removed in water. The count of J^2 /200cms³ nematode, root galls and egg masses were undertaken under microscope periodically. The biochemical analyses

were carried out in the Forage Research Laboratory, MPKV, Rahuri. The peroxidase and polyphenol oxidase assay in the roots of pomegranate saplings were carried out according to the method described by Kumar and Khan (1982). The fresh root samples in triplicate were extracted and phosphate buffer was used as source of enzyme. The change in absorbance was recorded at 420 nm on Specronic 20D. The total polyphenols from pomegranate sapling roots were determined according to the method described by Swain and Hillis (1959) for that one gram of fresh chilled roots were cut in to small pieces, macerated and boiled in 25 ml distilled water for 15 min. The contents were filtered and volume made to 25 ml and from this, 1 ml filtrate was used in triplicate for colour development. To 1 ml filtrate in 50 ml volumetric flask, 25 ml distilled water was added followed by addition of 1.25 ml Folin Dennis reagent and 2.5 ml of alkaline reagent. The contents were shaken and kept at room temperature for 20 min for colour development. After 20 min. the absorbance was recorded at 740 nm on spectronic-20D. The content of total polyphenols was calculated by preparing a standard curve of tannic acid and the results are expressed as g/100g of sample weigh.

RESULT AND DISCUSSION

The data presented in Table 1 revealed that application of different biocontrol agents in pomegranate sapling bags for the management of root-knot nematode, the treatment of the phule *Trichoderma* plus @ 10 g/sapling (T₄) showed reduction of root-knot nematode population (72.00 %) at 60 days after treatment. At this stage, the treatments of *Pochonia chlamydosporium* (T₅) was the next best treatment recorded 68.00 per cent reduction in root-knot nematode

population. At 90 days after treatment, it was the evident that, Phule *Trichoderma* plus @ 10 g/sapling (T₄) was found superior and recorded 37.97 per cent reduction in root-knot nematode population. Rest of the biological treatments recorded 25.33 to 35.43 per cent reduction in root-knot nematode population after 90 days of treatment. These results are in agreement with the earlier findings of Walunj and Mhase (2015).

In order to assess the biochemical changes, i.e. change in peroxidase, polyphenol oxidase activity and total polyphenols in the root samples of pomegranate saplings, the root samples were analyzed periodically in the laboratory. The results indicated that, the application of carbofuran 3 G @ 30 g/sapling (T₇) recorded significantly the highest activity of peroxidase, polyphenol oxidase enzymes and per cent total polyphenols with a value of 0.130 Δ OD /min/g fresh weight, 0.042 Δ OD /min/g fresh weight and 0.40 per cent, respectively, at 30 days after treatments (Table 1). Santhoshkumar *et al.* (2009) reported that insecticide carbofuran increased the activities of peroxidase and polyphenol oxidase in the roots of *Cucumis sativus* L. Among the bioagents studied, Phule *Trichoderma* plus (T₄) also showed higher enzymatic activities of both peroxidase and polyphenol oxidase and total polyphenol content with a value of 0.118 Δ OD /min/g fresh weight, 0.041 Δ OD /min/g fresh weight and 0.38 per cent, respectively at 30 days after treatment; 0.224 Δ OD /min/g fresh weight, 0.109 Δ OD /min/g fresh weight and 0.87 per cent, respectively at 60 days after treatments; and ; 0.063 Δ OD /min/g fresh weight, 0.042 Δ OD /min/g fresh weight and 0.90 per cent, respectively at 90 days after treatments. These results are in

conformity with Karthikeyan *et al.* (2006) and Singh *et al.* (1973). Karthikeyan *et al.* (2006) reported that application of *Pseudomonas fluorescens* and *Trichoderma viride* significantly increased the activity of peroxidase, polyphenol oxidase enzymes in coconut roots. Singh *et al.* (1973) reported that the application of different bioagents and amendments resulted in increase in polyphenol concentration in brinjal against root-knot nematodes.

CONCLUSION

From the results, it can be concluded that the saplings of pomegranate should be treated with bioagent, Phule *Trichoderma* plus (2 x 10⁶/g) @ 10 g/sapling at the time of planting air layers, as the activities of peroxidase and polyphenol oxidase activity of enzymes and total polyphenols were found significantly higher at 30, 60 and 90 days after treatments in the roots of saplings treated with bioagent, Phule *Trichoderma* plus (2 x 10⁶/g) @ 10 g/sapling than the other treatments, which may helps in the control of root-knot nematode.

REFERENCES

- Cobb, N. A. (1918). Estimating the nematode population of soil. Agric. Tech. Circ. Bur. Pl. Ind. U.S. Dep. Agric. No.1, 48 pp.
- Kumar, K. B. and Khan, P. A. (1982). Peroxidase and polyphenol oxidase in excised ragi (*Eleusine corocana* cv. PR-202) leaves during senescence. *Indian J. Expt. Biol.*, **20**: 412-416.
- Karthikeyan, M.; Radhika, K.; Mathiyazhagan, S.; Bhaskaran, R.; Samiyappan, R. and Velazhahan, R. (2006). Induction of phenolics and defense-related enzymes in coconut (Cocos

- nucifera* L.) roots treated with biocontrol agents. *Braz. J. Plant Physiol.* **18**(3): 367-377.
- Santhoshkumar, K.; Prakashkumar, B. and Mathew, M. M. (2009). Effect of carbofuran on defence related enzymes in the calli of *cucumis sativus* Linn. *Plant Cell Biotechnol. Mole. Biol.*, **10**(3/4): 101-106.
- Swain, T. and Hillis, W. E. (1959). The phenolic constituent of *Prunus domestica*. The quantitative analysis of phenolic constituents. *J. Sci. Food Agric.*, **10**: 63-68.
- Singh, B. and Choudhary, B. (1973). The chemical characteristics of tomato cultivar resistant to root-knot nematode. *Nematologica*, **19**: 443-448.
- Sharon, E.; Bar-eyal, M.; Chet, I.; Herrera-Estrella, A.; Kleifeld, O. and Spiegel, Y. (2001). Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. *Phytopath.*, **91**(7): 687-693.
- Walunj, A. R. and Mhase, N. L. (2015). Bioagents for biological management of root-knot nematode, *Meloidogyne incognita* infesting pomegranate. *Bioinfolet*, **12** (1A): 30-34.

Table 1: Effect of different treatments on the peroxidase activity in the roots of pomegranate saplings grown in nursery

Tr. No	Treatments Soil Application of	Per Cent Decline of Root-Knot Nematode (J ²) 200 cm ³ of Soil / Sapling		Peroxidase Activity (Δ OD /min/g Fresh weight)			Polyphenol Oxidase Activity (Δ OD min/g fresh weight)			Total Polyphenols (%)		
		60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
T ₁	<i>Trichoderma viride</i> (2 x 10 ⁶ /g) @10 g/sapling.	62.60	35.43	0.099	0.204	0.061	0.034	0.073	0.032	0.30	0.85	0.87
T ₂	<i>Paecilomyces lilacinus</i> (2x10 ⁶ /g) @10g/sapling.	60.00	29.13	0.092	0.158	0.032	0.029	0.062	0.013	0.26	0.57	0.57
T ₃	<i>Pseudomonas fluorescens</i> (2 x 10 ⁶ /g) @ 10 g/ sapling.	62.66	27.83	0.095	0.183	0.054	0.012	0.062	0.029	0.26	0.57	0.58
T ₄	<i>Phule Trichoderma plus</i> (2 x 10 ⁶ /g) @ 10 g/sapling.	72.00	37.97	0.118	0.224	0.063	0.041	0.109	0.042	0.38	0.87	0.90
T ₅	<i>P. chlamydosporium</i> (2 x 10 ⁶ /g) @ 10 g / sapling.	68.00	31.63	0.113	0.211	0.053	0.034	0.091	0.016	0.30	0.66	0.67
T ₆	VAM (100 spores/g) @ 10g /sapling	61.33	29.13	0.094	0.174	0.056	0.033	0.060	0.015	0.30	0.56	0.57
T ₇	Carbofuran 3 G @ 30g / sapling.	54.66	25.33	0.130	0.149	0.027	0.042	0.053	0.013	0.40	0.50	0.52
T ₈	Untreated control	-	—	0.082	0.1332	0.014	0.021	0.048	0.010	0.22	0.30	0.37
S.Em ±				0.001	0.002	0.0003	0.0001	0.0005	0.005	0.004	0.009	0.005
C.D. at 5 %				0.003	0.007	0.001	0.0004	0.001	0.01	0.01	0.02	0.01

DAT: Days After Treatment

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