Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; SP2: 749-752

V Vigila
Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

S Subramanian
Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

K Devrajan
Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
V Vigila
Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

PGPR induced systemic resistance in tomato plants against root knot nematode, *Meloidogyne incognita*

V Vigila, S Subramanian and K Devrajan

Abstract
The present study was aimed to find out the efficacy of *Pseudomonas* spp. and *Bacillus* spp. against root knot nematode, *M. incognita* in tomato plants under field condition. Induced activity of the defense enzymes were observed in the plants treated with the PGPR. Increased activity of the defense enzymes, *viz*., PO, PPO, PAL and phenolic compounds observed during the study revealed the induced systemic resistant activity of the bioagents against the nematode infestation. Higher amount of accumulation of these defense related compounds in the PGPR treated plants rendered protection against the nematodes by decreasing the level of infestation of the nematodes in the plants.

Keywords: Tomato, *M. incognita* and induced systemic resistance

Introduction
The root knot nematodes (*Meloidogyne* spp.) are among the most destructive agricultural pests globally. They have a wide host range of plants, causing yield losses especially in tropical and sub-tropical agriculture (Sikora and Fernandez 2005) [14]. Recently, *Meloidogyne* has a vital role as limitation factor for several crop cultivation **(**Baker *et al.*, 2011) [1]. The control of root knot nematodes is very challenging (Karssen *et al*., 2013) [7]. Multiple control methods such as regulatory, cultural, physical, biological and chemical methods were used for nematode control on host plants with different advantages and disadvantages. Currently, the use of nematicides is being limited, which are expensive, given the increasing concern for human health as well as the environment. Biocontrol appears to offer an environmentally safe and ecologically feasible option for plant protection with great potential for promoting sustainable agriculture. With this background, we intend to study the induction of systemic resistance in tomato by *Pseudomonas* spp. and *Bacillus* spp. against root knot nematode, *M. incognita*.

Materials and Methods
Biochemical changes induced by *Pseudomonas* spp. and *Bacillus* spp. in tomato challenged with *M. incognita*
Biochemical analysis was made with tomato root samples collected from the evaluation of liquid formulation of bacterial isolates under field conditions. The samples were collected at 1, 7, 13, 30, 90 DAT.

Treatments
T_1-*P. fluorescens* (Pfpv1), T_2-*Pseudomonas* spp. (Pfks 23), T_3-*Pseudomonas* spp. (Pfpv12), T_4-*P. fluorescens* (Pf1), T_5- *B. subtilis* (Bsvn11), T_6 - *B. pumilus* (Bsvn12), T_7-*B.cereus* (Bsks2), T_8 - *B. subtilis* (Bbv57) T_9- Control

Estimation of total phenols
The procedure developed by Malik and Singh (1980) was followed for the estimation of total phenol from the plant samples collected in the present study.
One g 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 Na_2Co_3 was added to each tube and placed in boiling water for a min and cooled. The absorbance was measured at 650 nm.

Estimation of Peroxidase (PO)
The peroxidase activity was assayed spectrophotometrically (Hartee, 1995) [5]. Reaction mixture consists of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1%

hydrogen peroxide. The reaction mixture was incubated at room temperature (28 ± 2ºC). The change in absorbance at 420 nm was recorded at 30 sec intervals for three min. Boiled enzyme preparation served as blank. The enzyme activity was expressed as change in the absorbance of the reaction mixture per min on fresh weight basis (Hammerschmidt *et al.,* 1982) [4].

Estimation of polyphenoloxidase (PPO)

The polyphenoloxidase activity was determined following the procedure as described by Bryant and Forrest (1979) [2]. The enzyme extract was prepared by homogenizing one g root tissue in 100 ml aliquots of cold acetone. The homogenate was filtered through Whatman No.1 filter paper and air dried. The resulting dry powder was used for the estimation of activity of PPO.

One g dry powder prepared was ground with two successive 20 ml aliquots of 25 mm phosphate buffer (pH 6.2) in a mortar chilled in ice bath. Then filtered through Whatman No.1 filter paper and diluted to 50 ml with phosphate buffer. Each two ml of phosphate buffer and enzyme extract was taken in a test tube and to this 1 ml of paracoumaric acid and 1 ml of manganese choride were added and incubated in dark at 30 ºC. Before and after 50 min of incubation 2 ml of the mixture, were taken and 5.2 ml of perchloric acid and 0.5 ml of ferric nitrate solution were added and diluted to 10 ml with water. After incubation period of 60 min in dark, the absorbance was measured at 535 nm.

Estimation of Phenylammonialyase (PAL)

One gram of plant sample was homogenized in 3 ml of ice cold 0.1 M sodium borate buffer, pH 7.0, containing 1.4 mm of 2-mercaptoethanol and 50 mg of insoluble polyvinylpyrrolidone (PVP). The resultant extract was filtered through cheese cloth and the filtrate was centrifuged at 15,000 rpm for 15 min at 4ºC and the supernatant was used as the enzyme source. The PAL activity was determined as the rate of conversion of L-Phenylalanine to trans-cinnamic acid at 290 nm. Sample containing 0.4 ml of enzyme extract was incubated with 0.5 ml. of 0.1 M borate buffer (pH 8.8) and 0.5 ml of 12mm L-phenylalanine in the same buffer for 30 min at 30ºC. The amount of transcinnamic acid synthesized was calculated using its extinction coefficient of 9630 M^{-1} cm^{-1} (Dickerson *et al.,* 1984) [3]. The enzyme activity was expressed in fresh weight basis as nmol trans-cinnamic acid min^{-1} mg^{-1} of sample.

Statistical analysis

The data generated in the present study were subjected to analysis by using the statistical software Design Expert version 7.1.6, AGRESS and Excel-2000. The data were analysed and the effect of treatments were compared at $p \leq 0.05$ level of significance using the critical difference (CD) test which was performed by Excel-2000.

Results and discussion

Biochemical changes in *Pseudomonas* spp. and *Bacillus* spp. treated tomato plants infested with *M. incognita* by spectrophotometric assay

Total phenols

The highest quantity of total phenols was noticed in plants treated with liquid formulation of bacterial isolate Pfpv1 (8.02 mg g^{-1} fresh root) after 7 days of application of the treatment (Table 1). The lowest phenol activity was recorded in the untreated control. The use of many biocontrol agents including PGPR resulted in the accumulation of phenols as biochemical changes in favour of plants and against invading pathogens like nematodes (Pitcher *et al.,* 1989) [10].

Activity of Peroxidase

The highest quantity of PO activity of 2.431 $min^{-1}g^{-1}$ root was noticed in plants treated with liquid formulation of bacterial isolate (Pfpv1) at 7 days after application which recorded 56.51 per cent increase over control (Table 2). PO activity started to decline gradually from 13 days after treatment. The lowest PO activity was recorded in the untreated control. The invasion of nematodes into plant roots appears to increase the activity of peroxidase in infested roots of host (Ibrahim, 1991) [6].

Activity of Polypheonal oxidase

The highest PPO activity of 2.238 $min^{-1}g^{-1}$ root was observed in the treatment of liquid formulation of bacterial isolate (Pfpv1) at 7 days after application (Table 3). PPO activity started to decline gradually from 13 days after treatment. The lowest PPO activity was recorded in the untreated control. Ramanujam *et al*. (2012) [11] reported that three isolates of *B. subtilis viz.,* EXB-123, ENB-24 and S-9 proved to be promising bacterial antagonists against chilli anthracnose pathogen, *Colletotrichum capsici* by increasing the activities of polyphenoloxidase.

Activity of Phenylalanine ammonia lyase (PAL)

The highest PAL activity was recorded in liquid formulation of Pfpv1 treated plants which showed the PAL activity of 7.228 $min^{-1}g^{-1}$ root which was observed after 7 days of application (Table 4). PAL activity started to decline gradually from 13 days after treatment. The lowest PAL activity was recorded in the untreated control. Similar studies which resulted in the increase in PO, PPO and PAL activity were reported by Sandeep (2004) [13] in banana against *M. incognita*, Kavitha (2005) [8] and Samuthiravalli (2006) [12] in tomato against *M. incognita*.

Table 1: Effect of liquid formulation of bacterial isolates on total phenols content in tomato infested with *M. incognita* under field condition

Treatments	Total phenols (mg/g fresh weight)				
	1 DAT	7 DAT	13 DAT	30 DAT	90 DAT
	Root	Root	Root	Root	Root
T_1-Pfpv 1	7.59 (42.29)	8.02 (34.28)	5.87 (49.23)	3.09 (56.63)	1.60 (46.25)
T_2-Pfks23	7.46 (41.28)	7.79 (32.34)	5.49 (45.71)	2.75 (51.27)	1.45 (40.68)
T_3-Pfpv12	7.24 (39.50)	7.65 (31.11)	5.22 (42.91)	2.42 (44.62)	1.41 (39.00)
T_4-Pf1	7.35 (40.40)	7.42 (28.97)	5.36 (44.40)	2.59 (48.26)	1.43 (39.86)
T_5-Bsvn11	7.51 (41.67)	7.93 (33.54)	5.59 (46.69)	2.87 (53.31)	1.56 (44.87)
T_6-Bsvn12	7.09 (38.22)	7.51 (29.82)	5.19 (42.58)	2.35 (42.97)	1.35 (36.29)
T_7-Bsks2	5.55 (21.08)	6.98 (24.49)	4.82 (38.17)	2.16 (37.96)	1.15 (25.21)
T_8-Bbv 57	6.85 (36.05)	7.29 (27.70)	5.17 (42.35)	2.31(41.99)	1.27 (32.28)
T_9-Control	4.38	5.27	2.98	1.34	0.86
SEd	0.017	0.013	0.0136	0.0082	0.0038
CD(P=0.05)	0.037	0.029	0.0288	00174	0.0080

Values are mean of three replications, Figures in parentheses are per cent increase over control

Table 2: Effect of liquid formulation of bacterial isolates on peroxidase activity in tomato infested with *M. incognita* under field condition

Treatments	Peroxidase activity (change in absorbance min $^{-1}$ g $^{-1}$)				
	1 DAT	7 DAT	13 DAT	30 DAT	90 DAT
	Root	Root	Root\	Root	Root
T_1-Pfpv 1	1.546 (33.95)	2.431 (56.51)	2.417 (58.54)	0.984 (57.82)	0.078 (66.66)
T_2-Pfks23	1.453 (29.73)	2.367 (55.34)	2.243 (55.32)	0.799 (48.06)	0.059 (55.93)
T_3-Pfpv12	1.342 (23.91)	1.923 (45.03)	1.395 (28.17)	0.723 (42.60)	0.047 (44.68)
T_4-Pf1	1.427 (28.45)	2.198 (51.91)	2.000 (49.90)	0.784 (47.06)	0.051 (49.01)
T_5-Bsvn11	1.537 (33.57)	2.324 (54.51)	2.312 (56.66)	0.813 (48.95)	0.065 (60.00)
T_6-Bsvn12	1.329 (23.17)	1.826 (42.11)	1.654 (39.41)	0.634 (34.54)	0.042 (38.09)
T_7-Bsks2	1.212 (15.75)	1.567 (32.54)	1.289 (22.26)	0.542 (23.43)	0.034 (23.52)
T_8-Bbv 57	1.319 (22.59)	1.714 (38.33)	1.459 (31.32)	0.621 (33.17)	0.037 (29.72)
T_9-Control	1.021	1.057	1.002	0.415	0.026
SEd	0.0031	0.0074	0.0082	0.0029	0.0003
CD(P=0.05)	0.0067	0.0157	0.0174	0.0061	0.0006

Values are mean of three replications, Figures in parentheses are per cent increase over control

Table 3: Effect of liquid formulation of bacterial isolates on polyphenol oxidase activity in tomato infested with *M. incognita* under field condition

Treatments	Polyphenol oxidase activity (change in absorbance min $^{-1}$ g $^{-1}$)				
	1 DAT	7 DAT	13 DAT	30 DAT	90 DAT
	Root	Root	Root	Root	Root
T_1-Pfpv 1	1.754 (59.00)	2.238 (55.58)	1.995 (52.93)	0.257 (43.96)	0.079 (68.35)
T_2-Pfks23	1.654 (56.52)	2.112 (52.93)	1.587 (40.83)	0.232 (37.93)	0.057 (56.14)
T_3-Pfpv12	1.587 (54.69)	1.926 (48.39)	1.352 (30.54)	0.221 (34.84)	0.046 (45.65)
T_4-Pf1	1.613 (55.42)	2.004 (50.39)	1.478 (36.46)	0.227 (36.56)	0.051 (50.98)
T_5-Bsvn11	1.722 (58.24)	2.197 (54.75)	1.877 (49.97)	0.245 (41.22)	0.065 (61.53)
T_6-Bsvn12	1.498 (52.00)	1.854 (46.38)	1.254 (25.11)	0.198 (27.27)	0.039 (35.89)
T_7-Bsks2	0.985 (27.00)	1.525 (34.81)	1.129 (16.82)	0.177 (18.64)	0.028 (10.71)
T_8-Bbv 57	1.342 (46.42)	1.638 (39.31)	1.167 (19.53)	0.185 (22.16)	0.031 (19.35)
T_9-Control	0.719	0.994	0.939	0.144	0.025
SEd	0.0058	0.0064	0.0058	0.0006	0.0003
CD(P=0.05)	0.0123	0.0137	0.0123	0.0012	0.0007

Values are mean of three replications, Figures in parentheses are per cent increase over control

Table 4: Effect of liquid formulation of bacterial isolates on phenyl alanine ammonia lyase activity in tomato infested with *M. incognita* under field condition

Treatments	Phenyl alanine ammonia lyase activity (change in absorbance min $^{-1}$ g $^{-1}$)				
	1 DAT	7 DAT	13 DAT	30 DAT	90 DAT
	Root	Root	Root	Root	Root
T_1-Pfpv 1	6.960 (35.01)	7.228 (35.74)	6.856 (43.48)	0.567 (50.97)	0.095 (58.94)
T_2-Pfks23	6.837 (33.84)	6.956 (33.23)	5.848 (33.73)	0.489 (43.14)	0.081 (51.85)
T_3-Pfpv12	6.632 (31.80)	6.729 (30.98)	5.452 (28.92)	0.412 (32.52)	0.064 (39.06)
T_4-Pf1	6.723 (32.72)	6.820 (31.90)	5.781 (32.97)	0.423 (34.27)	0.073 (46.57)
T_5-Bsvn11	6.914 (34.58)	7.112 (34.70)	6.321 (38.69)	0.553 (49.72)	0.086 (54.65)
T_6-Bsvn12	5.812 (22.17)	5.912 (21.44)	4.849 (20.08)	0.399 (30.32)	0.057 (31.57)
T_7-Bsks2	5.624 (19.57)	5.721 (18.82)	4.561 (15.04)	0.341 (18.47)	0.041 (04.87)
T_8-Bbv 57	5.749 (21.32)	5.815 (20.13)	4.623 (16.17)	0.354 (21.46)	0.049 (20.40)
T_9-Control	4.523	4.644	3.875	0.278	0.039
SEd	0.0136	0.0140	0.0155	0.0015	0.0004
CD(P=0.05)	0.0288	0.0297	0.0329	0.0031	0.0009

Values are mean of three replications, Figures in parentheses are per cent increase over control.

References

1. Baker R, Mahdy M, Mousa E. A survey of root knot and citrus nematodes in some new reclaimed lands in Egypt. Paki J Nematol. 2011; 29:165-170.
2. Bryant SD, Forest EL. Indole-3-acetic acid oxidase from Peas. I: Occurrence and distribution of peroxidative and non- peroxidative forms. Plant Physiol. 1979; 63:696-699.
3. Dickerson DP, Pascholati SF, Hagerman AE, Butler LG, Nicholson RL. Phenylalanine ammonia-lyase and hydroxyl cinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. Physiological Plant Pathol. 1984; 25:111-123.
4. Hammerschmidt R, Nuckles EM, Kuc J. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium.* Physiol. Plant Pathol. 1982; 20:73-82.
5. Hartee EF. Catalase, peroxidase and metmyoglobin as catalysts of coupled peroxidatic reactions. Biochem. J. 1995; 60:310-325.
6. Ibrahim SK. Peroxidase isoenzymes from *Meloidogyne* spp. cultured on different hosts. Revue de Nematologie. 1991; 14:335-344.
7. Karssen G, Wesemael W, Moens M. Root knot nematodes. Plant Nematology. 2013, 73-108.

8. Kavitha PG. Management of Root knot nematode *Meloidogyne incognita* with plant growth promoting rhizobacteria in Tomato. Unpublished M.Sc. (Ag) Thesis, Department of Nematology, Tamil Nadu Agricultural University, Tamil Nadu, India. 2005, 75.
9. Malick CP, Singh MB. Plant enzymology and histoenzymology. Kalyani Publications. New Delhi. 1980, 286.
10. Pitcher DG, Saunders NA, Owen RJ. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett. Appl. Microbiol. 1989; 8:151-156.
11. Ramanujam B, Basha H, Hemannavar V, Chowdappa P, Rangeshwaran R. Induction of defence related enzymes and phenols in chilli plants by *Bacillus subtilis* against anthracnose pathogen, *Colletotrichum capsici*. Indian Phytopathol. 2012; 65(4):382-385.
12. Samuthiravalli M. Manageent of root knot nematode, *Meloidogyne incognita* and wilt disease *Fusarium oxysporum* f.sp. *lycopersicion* tomato by endophytic bacteria. M.sc., (Ag.,) Thesis, TNAU, Coimbatore-3, India, 2006, 103.
13. Sandeep A. Bioefficacy of *Pseudomonas fluorescens* (Native Isolates) on *Meliodogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 in banana (*Musa* spp.) Unpublished M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, 2004, 87.
14. Sikora RA, Fernandez E. Nematode parasites of vegetables. Plant parasitic nematodes in subtropical and tropical agriculture. 2005; 2:319-392.