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Screening of Bioagents against Wilt Complex Pathogens of Tomato

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ABSTRACT

Bioagents like Tv-1, Th-1, Pl-1 and Pc-1 and two bacterial bioagents (Pf-1, and Bs-1) isolated from healthy rhizosphere of tomato from North Eastern parts of Karnataka (NEK) were maintained and tested their efficacy against wilt complex pathogens of tomato (Fusarium oxysporum f. sp. lycopersici and Meloidogyne incognita). Bioagents are evaluated against Fusarium for their mycelial inhibition under dual culture technique. The per cent inhibition varied from 16.66 to 58.00 with a mean of 32.41. The results obtained were highly significant between the different bioagents tested and also over control. Maximum inhibition of 58.00 per cent was observed in Trichoderma viride followed by T. harzianum (57.77) and Paecilomyces lilacinus (35.55). Least inhibition of 16.66 per cent was observed in Bacillus subtilis followed by Pseudomonas fluorescens (25.55). Efficacy of different bioagents against root knot nematode by using culture filtrates of bioagents at 100, 75, 50 and 25 per cent significantly inhibited the hatching of eggs. The greatest decrease in egg hatching was recorded (8.67) with the bioagent P. lilacinus, T. viride (10.33) and Pochonia chlamydospora (11.00) in treatment concentration interaction (82.66, 79.34 and 78.00 per cent inhibition) over control respectively. Maximum number of juveniles were hatched in control (50.00) followed by 45.03 in Bacillus subtilis at 25 per cent concentration.

Key words: *Bioagent, Trichoderma, Pseudomonas, Wilt complex, Tomato*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) belonging to the family *Solanaceae* is an extremely popular and widely grown vegetable in the world. Tomato is grown in kitchen gardens, commercial fields and also economically exploited in green houses or controlled environmental facilities. In India, it ranks second among vegetables next only to potato in area and production. It occupies an area of - 880.00 thousand ha with an annual production of 18227.00 thousand metric tons accounting to an average productivity of 20.70 metric tons per ha. In Karnataka, tomato ranks second among the vegetables with an area of 57.80 thousand ha and annual production of about 1916.60 thousand metric tons with an average productivity of 33.20 metric tons per ha[8].

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Tomato is susceptible to a number of diseases incited by fungi, bacteria, nematodes, mycoplasmas and viruses of all these diseases, root-knot caused by *Meloidogyne* spp. is most destructive and a serious constraint in the successful cultivation of this crop. Yield loss of tomato due to root-knot nematode *Meloidogyne* spp. in India ranges from 40 to 46 per cent[2&10]. Roots infected with *Meloidogyne* spp. were severely galled. Moreover, plants show poor in growth with symptoms of chlorosis. Many effective pesticides have been used against soil borne pathogens but not considered as long term solution because of concerns about exposure risks, health and environmental hazards, expensiveness, residue persistence, development of resistance to pesticides and elimination of natural enemies. Biocontrol strategy has become the alternative method in recent years due to the indiscriminate and frequent use of chemical agents and its ill effects to soil and environment. Several antagonistic organisms have been used successfully as biocontrol agents for controlling soil borne pathogens. Soil-borne plant pathogens affecting agricultural plants can be controlled by the use of species of *Trichoderma, Bacillus subtilis, Pseudomonas fluorescens*. Currently, cost-effective fungicide is also not available that gives guaranteed control against wilt-root knot complex disease. Thus, considering the seriousness of the loss and ineffectiveness of the available chemicals to control the disease, the present investigation was undertaken to evaluate some promising fungicides and formulations (neem cake and talc) of bioagents against wilt-root knot complex in tomato crop.

MATERIAL AND METHODS

Screening of bioagents

In vitro screening of bioagents against *Fusarium oxysporum* f. sp. *lycopersici.*

Bioagents like *Tricoderma viride* (Tv-1), *T. harzianum* (Th-1), *Paecilomyces lilacinus* (Pl-1*:* from IIHR, Bangalore) and *Pochonia chlamydospora* (Pc-1 from IIHR, Bangalore) and two bacterial bioagents like, *Pseudomonas fluorescens* (Pf-1) and *Bacilus subtilis* (Bs-1) were isolated from healthy rhizosphere of tomato from different parts of North Eastern parts of Karnataka were tested for their inhibitory activity against mycelial growth of *Fusarium* by following the dual culture technique. Mycelial discs of 5 mm diameter of seven days-old culture of *Fusarium* was placed in one side of the Petri plate containing 20 ml PDA medium. Seven days old cultures of each fungal bioagents (Tv-1, Th1, Pl-1, and Pc-1) discs were placed on one side of the Petri plate opposite to the *Fusarium* disc in an equal distance.

For bacterial bioagents (Ps1 and Bs1) mycelial discs of 5 mm diameter of seven days-old culture of *Fusarium* was placed in the middle of the Petri plate containing 20 ml PDA medium. Twenty four hour old cultures of each bacterial bioagents was streaked parallelly on either side of the *Fusarium* disc (3 cm away from the disc). The plates were incubated at room temperature (28 ± 2°C) for 8-10 days, until the plate covered completely by the *Fusarium* in control. The plates with only *Fusarium* disc without bacterial streaks served as control. Each treatment was replicated five times. After incubation, *i.e.* when control plate reached 90 mm/ 9 cm diameter, the radial growth of the pathogen was measured. Per cent inhibition over control was calculated by using the formula of Vincent (1947) as follows;

$$I = \frac{(C-T)}{C} \times 100$$

I = Per cent inhibition of mycelium
C = Growth of mycelium in control
T = Growth of mycelium in treatment

In vitro screening of bioagents against *Meloidogyne incognita*

Preparation of cell free extract

A single colony of each bioagents were cultured in a screw-capped test tubes containing 10 ml of sterilized broth (PDB/NB), incubated at 28^0 C with mechanical shaker at 150 rpm for sufficient time. The culture was subsequently passed

through sterilized Whatman No.1filter papers no.1 and 42, concentrated by centrifugation at 10,000 rpm for 10 minute and the supernatant was collected and finally passed through Millipore filter of 0.22 µm, this was designated as undiluted standard cell free filtrate of bioagents (100 %). The cell free extract was further diluted to 75, 50 and 25 percent respectively and these dilutions were used to study their effect on nematodes. *In vitro* evaluation of antagonists against root-knot nematode was carried out on egg hatching and on juvenile's mortality.

***In vitro* egg hatching test**

Egg masses were collected from culture plants maintained in glasshouse at Department of Plant Pathology, Agricultural College, Bheemarayanagudi. Egg masses were picked up and treated with NaOCl (1%) to dissolve the egg matrix and to separate the individual eggs. A known number of eggs (50) were carefully transferred to each vial containing 5 ml of cell free culture filtrate of bio-agents of 100, 75, 50 and 25 per cent concentrations. Inoculated vials are incubated at room temperature (28±2ºC) for 36 hours; one control was maintained by transforming 50 eggs to a vial containing distilled water. After 48hours, the number of juveniles hatched was counted under stereo binocular microscope and per cent inhibition of egg hatching in different dilutions in each vial was calculated.

***In vitro* mortality test of juveniles**

Freshly hatched, 50 active juveniles were counted in a counting dish using a stereo binocular microscope and were carefully transferred to individual vials containing 5 ml of each of the bioagents cell free filtrates of different concentrations (100, 75, 50 and 25 per cent). Each treatment was replicated three times and arranged in completely randomized design and incubated at 28±2ºC. Observations were recorded at 12h, 24h and 48h after exposure period and per cent mortality was calculated.

Efficacy of bioagents on plant growth promoting activity under in vitro (Roll towel method)

Seeds of tomato were surface sterilized with one per cent sodium hypochlorite for 30 seconds rinsed in sterile distilled water and dried overnight under sterile stream of air in a laminar air flow. Bioagents culture filtrates taken in Petri plates. Known number of seeds (50) was soaked in 10 ml culture filtrates for 12h. Then the seeds were dried under sterile stream of air.

Roll towel method

Plant growth promoting activity of bioagents were assessed based on the seedling vigour index using the standard roll towel method. Twenty tomato seeds were kept over the presoaked germination paper. The seeds were held in position by placing another presoaked germination paper strip and gently pressed. The polythene sheet along with seeds were then rolled and incubated in growth chamber for 15 days. Three replications were maintained for each treatment. Seeds soaked in sterile water served as control. Root length and shoot length of individual seedlings were measured and the germination percentage of seeds was recorded. The vigour index was calculated by using the formula[1].

Vigour index (VI) = Seedling length (mm) x Germination percentage.

Five seedlings were taken randomly from each treatment and their fresh weight was recorded. Later, the seedlings were kept in the hot air oven for 4 days at 60ºC for complete desication and dry weight of the seedlings was recorded.

RESULTS AND DISCUSSION

Screening of bioagents against the pathogens Fusarium oxysporum

Bio agents like Tv-1, Th-1, Pl-1 and Pc-1 and two bacterial bioagents (Pf-1 and Bs-1) isolated from healthy rhizosphere of tomato from different parts of Karnataka were maintained and screened against *Fusarium* for mycelial inhibition by dual culture technique *in vitro* as explained in 'Material and Methods'. Inhibition zone (in cm) was recorded and the per cent inhibition was calculated the results thus obtained are presented in the Table 1.

The per cent inhibition varied from 16.66 to 58.00, with a mean of 32.41. The

results obtained were highly significant between the different bioagents tested and also over control. Maximum per cent inhibition of 58.00 was observed in *Trichoderma viride* followed by *T. harzianum* (57.77) and *P. lilacinus* (35.55). Least inhibition of 16.66 per cent was observed in *B. subtilis* followed by *P. fluorescens* (25.55) (Table 1).

In vitro screening of bio-agents against nematode

Effect of culture filtrates of bioagents on hatching of M. incognita

Hatching of *M. incognita* juveniles increased with decrease in concentration of bioagents. However, hatching was significantly reduced at all concentrations of bioagents. The interaction between treatment and concentration was significant which indicating that an increase in concentration tended to modify the effect of other in a significant manner.

The culture filtrates of bioagents at 100, 75, 50 and 25 per cent significantly inhibited the hatching of eggs. The greatest decrease in egg hatching was recorded (8.67) with the bioagent *P. lilacinus*, *T. viride* (10.33) and *Pochonia chlamydospora* (11.00) in treatment concentration interaction (82.66, 79.34 and 78.00 per cent inhibition over control respectively). Maximum number of juveniles were hatched in control (50.00) followed by 45.03 in *Bacilus subtilis* at 25 per cent concentration (Table 2).

Effect of cell free culture filtrates of bioagents on juvenile mortality of M. incognita in vitro

Cell free culture filtrates of six bioagents were tested *in vitro* for their nematicidal activity against *M. incognita*. Data indicated that various bioagents and their different concentrations were highly deleterious to the nematode. In general, juvenile mortality increased with increase in exposure period and concentration of bioagents. No nematode mortality was recorded in control treated with (distilled water).

A maximum nematode mortality (86%, 84% and 82%) was observed in 100 per cent concentration of bioagents, *P lilacinus*, *P chlamydospora* and *T viride* respectively at 48 hour exposer. Similar results were observed with 75, 50 and 25 per cent concentration of the culture filtrates of the same bioagents. The lowest juvenile mortality was recorded (16.00) at 25 per cent concentration.

The immobile/inactive nematodes were randomly picked up and placed in sterile water. None of the juveniles regained their activity, indicating that the nematicidal action of the bioagents was long lasting. (Table 3).

Plant growth promoting activity of bioagents in vitro

The growth promoting activities of six selected bioagents were tested for seed germination and seedling vigour by treating tomato seeds in roll towel method. Tomato seeds treated with the different bioagents showed improved plant growth parameters than untreated seeds.

Roll towel method

In roll towel method, the different bioagents showed more than 85 per cent seed germination and produced higher shoot and root length as well as fresh and dry weight of tomato seedlings with enhanced vigour index after 15 days. The maximum vigour index of 1482.00 was recorded in *T. viride* treated seedlings followed by 1396.50 and 1125.00 vigour index in *P. fluorescens and T. harzianum* treated seedlings respectively. Least germination (70 %), less shoot and root length as well as less fresh and dry weight of seedlings with less vigour index of 581.00 was observed in untreated control (Table 4).

Selection of efficient bioagent effective against both the pathogens of tomato.

Among the bioagents screened against *F. oxysporum* in dual culture technique, the results revealed that Tv-1 was highly effective in inhibiting the mycelial growth of the test pathogen to an extent of 58.00 percent. Similarly Pl-1 was proved to be highly larvicidal and ovicidal activity against *M. incognita*. In roll towel method (*in vitro*) the Tv-1 showed 95 percent seed germination and produced higher shoot and root length as well as recorded maximum vigour index of 1482. Based on the *in vitro* performance of the bioagents selected for their efficiency on

individual pathogen and plant parameters in tomato Tv-1 and Pl-1 showed high efficacy against both the pathogen were selected and used for further studies.

Bioagents like Tv-1, Th-1, Pl-1, Pc-1, Pf-1 and Bs-1 screened against *F. oxysporum.* Among six bioagents Tv-1 was found to grow and sporulate faster than the other fungi and exhibited significant growth inhibition of *F. oxysporum* f.sp. *lycopersici*. Similar results were also observed by the previous authors[7].

Various research workers also demonstrated the role of Tv-1 in the inhibition of *Fusarium.* Some species of *Trichoderma* produce toxic enzymes like, chitinase, gluconase, peptides and metabolites such as trichodermin, dermin, trichobruchin, which kill soil borne fungal pathogens and nematodes[3].

Effect of culture filtrates of bioagents against M. incognita

In the present study, *in vitro* bioassay with cell free culture filtrates of six bioagents at different concentrations revealed an egg hatching inhibition and increased juvenile mortality with increase in exposure period as well as concentration of culture filtrates. *M. incognita* eggs and juveniles were highly vulnerable to the cell free culture filtrates of bioagents. Among the six bioagents tested, four showed (Pl-1, Pc-1, Tv-1 and Th-1) significantly higher ovicidal and larvicidal action on *M. incognita* eggs and juveniles respectively. These results are in conformity with PGPR strains against *M. incognita* of Coleus[6].

Reduction in viability and mobility of eggs and juveniles of *M. incognita* is induced by secondary metabolites such as trichodermin, dermin, 2,4-diacetylphlorglucinol (DAPG), pyrolnitrin, tropolone, pyocyanin, phenazines and lytic enzymes which are produced in culture filtrates of *P. fluorescens*[4]. Similar toxic property in culture filtrate of *P. fluorescens* was also reported on the juvenile mortality of *M. incognita* and *Heterodera cajani*. The juvenile mortality and egg hatching inhibition of *M. incognita* was also observed in the present study might be due to antibiosis.

Plant growth promoting activity of bioagents in vitro

In the present study, all six bioagents treated seeds were found to increase the seed germination and the vigour index of tomato significantly when compared to untreated control under roll towel method. The observations made in the present study corroborate with that with other native bioagents[9&5], where enhanced germination and seedling vigour were recorded in tomato and hot pepper respectively by seed treatment with *P. fluorescens* and *B. subtilis*.

Table 1: *In vitro* screening of bioagents against *F. oxysporum* f. sp. *lycopersici* under dual culture

Bioagent	Radial mycelial growth(cm)	Per cent mycelial inhibition
T. viride (Tv-1)	3.78	58.00 (49.60)*
T. harzianum(Th-1)	3.8	57.77 (49.43)
P. lilacinus (Pl-1)	5.8	35.55 (36.57)
P. chlamydospora (Pc-1)	6	33.33 (35.24)
P. fluorescens (Pf-1)	6.7	25.55 (30.33)
B. subtilis (Bs-1)	7.5	16.66 (24.04)
Control	9	0
SEm ±		0.2
CD@1%		0.8

*Figures in the parenthesis are arc sine transformed values

Table 2: Effect of culture filtrates of different bioagents on egg hatching of *M. incognita*

Bioagent	Concentration								Mean (J_2hatched)
	100%		75%		50%		25%		
	No. of J_2 hatched	% IOC*	No. of J_2 hatched	% IOC	No. of J_2 hatched	% IOC	No. of J_2 hatched	% IOC	
Tv-1	10	80 (63.44)**	11	78 (62.03)	14	72 (58.05)	38	24 (29.33)	18.25
Th-1	14	72.09 (58.12)	20	60 (50.77)	21	58 (49.60)	40	20 (26.56)	23.75
Pl-1	8	84 (66.42)	9	82 (64.90)	11	78 (62.03)	35	30 (33.21)	15.75
Pc-1	11	78 (62.03)	18	64 (53.13)	20	60 (50.77)	39	22 (27.97)	22
Pf-1	13	74 (59.34)	20	60 (50.77)	30	40 (39.23)	43	14 (21.97)	26.5
Bs-1	20	60 (50.77)	24	52 (46.15)	29	42 (40.40)	45	10 (18.44)	29.5
Distilled water	50	0 (0)	50	0 (0)	50	0 (0)	50	0 (0)	50
Mean	18	64 (53.13)	21.7	56.5 (48.73)	25	50 (45.00)	41.4	17.1 (24.43)	26.5
SEm±	**0.53**		**0.53**		**0.53**		**0.53**		
CD@1%	**2.25**		**2.25**		**2.25**		**2.25**		

*IOC= Inhibition over control; **Figures in the parenthesis are $\sqrt{X + 0.5}$ transformations

Table 3: Effect of culture filtrates of different bioagents on juvenile mortality of *M. incognita*

BIOAGENT	Mortality of *M. incognita*																Total Mean
	100% concentration				75% concentration				50% concentration				25% concentration				
	12 hr	24 hr	48 hr	Mean	12 hr	24 hr	48 hr	Mean	12 hr	24 hr	48 hr	Mean	12 hr	24 hr	48 hr	Mean	
Tv-1	40.00 (39.23)*	56.00 (48.45)	82.00 (64.90)	59.33 (50.36)	28.00 (31.95)	40.00 (39.23)	62.00 (51.94)	43.33 (41.15)	16.00 (23.58)	26.00 (30.66)	42.00 (40.40)	28.00 (31.95)	8.00 (16.43)	16.00 (23.58)	30.00 (33.21)	18.00 (25.10)	37.16 (37.52)
Th-1	36.00 (36.87)	44.00 (41.55)	78.00 (62.03)	52.67 (46.49)	24.00 (29.33)	36.00 (36.87)	56.00 (48.45)	38.66 (38.41)	10.00 (18.44)	20.00 (26.56)	38.00 (38.06)	22.66 (28.38)	4.00 (11.54)	12.00 (20.27)	26.00 (30.66)	14.00 (21.97)	31.99 (34.39)
Pl-1	42.00 (40.40)	60.00 (50.77)	86.00 (68.03)	62.67 (52.30)	36.00 (36.87)	52.00 (46.15)	70.00 (56.79)	52.66 (46.49)	22.00 (27.97)	30.00 (33.21)	52.00 (46.15)	34.66 (36.03)	12.00 (20.27)	22.00 (27.97)	38.00 (38.06)	24.00 (29.33)	57.99 (49.54)
Pc-1	40.00 (39.23)	58.00 (49.60)	84.00 (66.42)	60.67 (51.12)	32.00 (34.45)	46.00 (42.71)	68.00 (55.55)	48.66 (44.20)	18.00 (25.10)	30.00 (33.21)	48.00 (43.85)	32.00 (34.45)	10.00 (18.44)	20.00 (26.56)	34.00 (35.67)	21.33 (27.49)	40.66 (39.58)
Pf-1	16.00 (23.58)	20.00 (26.56)	28.00 (31.95)	21.33 (27.49)	12.00 (20.27)	16.00 (23.58)	30.00 (33.21)	19.33 (26.06)	11.00 (19.37)	14.00 (21.97)	26.00 (30.66)	17.33 (24.58)	4.00 (11.54)	8.00 (16.43)	18.00 (25.10)	10.00 (18.44)	17.49 (24.65)
Bs-1	18.00 (25.10)	24.00 (29.33)	34.00 (35.67)	25.33 (30.20)	14.00 (21.97)	22.00 (27.97)	32.00 (34.45)	22.66 (28.38)	16.00 (23.58)	20.00 (26.56)	26.00 (30.66)	20.66 (26.99)	6.00 (14.18)	10.00 (18.44)	16.00 (23.58)	10.66 (19.00)	19.82 (26.42)
Distilled water	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)	00.00 (0)
SEm±	**1.05**	**0.53**	**0.53**		**0.53**	**0.53**	**0.53**		**0.53**	**0.53**	**0.53**		**0.53**	**0.53**	**0.53**		
CD@ 1%	**4.41**	**2.25**	**2.25**		**2.25**	**2.25**	**2.25**		**2.25**	**2.25**	**2.25**		**2.25**	**2.25**	**2.25**		

*Figures in the parenthesis are arc sine transformed values

Table 4: Plant growth promoting activity of different bioagents in tomato seedlings under *in vitro* conditions (Roll towel method)

Bioagent	Germination (%)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	SVI**
Tv-1	95 (77.08)*	9.4	6.2	1.1	0.3	1482
Th-1	90 (71.56)	7.2	5.3	0.8	0.2	1125
Pl-1	80 (63.44)	8.0	4.4	0.9	0.1	992
Pc-1	90 (71.56)	5.4	3.8	0.6	0.07	763
Pf-1	95 (77.08)	8.5	6.2	1.00	0.2	1397
Bs-1	85 (67.21)	6.6	4.4	0.75	0.09	935
Distilled water	70 (56.79)	5.1	3.2	0.5	0.05	581
SEm±	**0.58**	**0.58**	**0.58**	**0.05**	**0.04**	
CD@1%	**2.43**	**2.43**	**2.43**	**0.23**	**0.17**	

*Figures in the parenthesis are arc sine transformed values

**SVI- Seedling vigour index

CONCLUSION

Trichoderma viride caused maximum inhibition of 58.00 per cent followed by *T. harzianum* (57.77) and *Paecilomyces lilacinus* (35.55). Whereas, least inhibition of 16.66 per cent was due to *Bacillus subtilis* followed by *Pseudomonas fluorescens* (25.55). Efficacy of different bioagents against root knot nematode by using culture filtrates of bioagents at 100, 75, 50 and 25 per cent significantly inhibited the hatching of eggs. The greatest decrease in egg hatching was recorded (8.67) with the bioagent *P. lilacinus*, *T. viride* (10.33) and *Pochonia chlamydospora* (11.00) in treatment concentration interaction (82.66, 79.34 and 78.00 per cent inhibition) over control respectively. Maximum number of juveniles were hatched in control (50.00) followed by 45.03 in *Bacillus subtilis* at 25 per cent concentration.

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