



Research Article

Evaluation of non-pathogenic *Fusarium* for antagonistic activity against *Fusarium* wilt of tomato

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ABSTRACT: *Fusarium* spp. isolated from rhizosphere soils of tomato from Karnataka, Tamil Nadu and Gujarat states of India were tested for their pathogenicity. Among these isolates, six were found non-pathogenic to tomato, red gram, bengal gram, groundnut, red pepper, water melon, castor and banana. These non-pathogenic isolates protected tomato plants when co-inoculated with pathogen isolate under pot culture conditions. Treatment with non-pathogenic *Fusarium* isolates improved the plant growth parameters (length and weight of root and shoot). These non-pathogenic *Fusarium* isolates can be exploited for the biocontrol of wilt disease.

KEY WORDS: Biocontrol, non-pathogenic isolates, *Fusarium* spp., tomato, wilt

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INTRODUCTION

Members of the genus *Fusarium* are well known soil inhabitants with high saprophytic potential. Some of the species cause vascular wilts in crop plants and are specific to host plant species and sometimes to specific cultivars. The pathogenic species have been classified as *formae speciales* and races. Many fungal and bacterial antagonists like *Trichoderma* spp., *Pseudomonas fluorescens*, *Burkholderia cepacia*, non-pathogenic strains/isolates of *Fusarium* spp., etc. have been tested for their efficacy in controlling *Fusarium* wilt of crop plants. Though many antagonists have been reported to have potential to reduce *Fusarium* wilt, they were not as consistent and effective as non-pathogenic *Fusarium* isolates (Larkin and Fravel, 1998). Specific non-pathogenic isolates of *F. oxysporum* and *F. solani* collected from a *Fusarium* wilt-suppressive soil were the most effective antagonists, providing significant and consistent disease control (50-80% reduction) in several repeated tests (Larkin and Fravel, 1998). There are many reports on the role of non-pathogenic *Fusarium* spp. isolated from different agro-climatic zones (Schneider, 1984; Paulitz *et al.*, 1987; Larkin *et al.*, 1993, 1996). Strains of *F. oxysporum* and *F. solani* were much more efficient in establishing suppressiveness in soil than other species of *Fusarium*.

Moreover, effective biocontrol strains of non-pathogenic *F. oxysporum* have been isolated from the stems of healthy plants (Ogawa and Komada, 1984; Postma and Rattink, 1992). The modes of action of non-pathogenic *Fusarium* spp. include competition for food and space and induced systemic resistance. We report the isolation of non-pathogenic *Fusarium* spp., confirmation of their non-pathogenicity by bioassays and their bioefficacy in tomato fusarial wilt management under greenhouse conditions.

MATERIALS AND METHODS

Isolation and maintenance of *Fusarium*

Soil samples were collected from rhizosphere regions of tomato (*Lycopersicon esculentum* Mill.) plants for isolation of non-pathogenic *Fusarium* from different tomato growing regions in Karnataka, Tamil Nadu and Gujarat states of India. *Fusarium* was isolated by serial dilution technique using Nash and Snyder's *Fusarium* selective medium (Nash and Snyder, 1962). *Fusarium* cultures were identified based on morphological characters like micro-conidia and macro-conidia (Subramanyam, 1970; Booth 1971). These cultures were stored at 4°C on potato dextrose agar (PDA) for further investigation.

Tests for non-pathogenicity of *Fusarium*

Plant material and planting medium

Tomato cv. Pusa Ruby (Nunhemns pvt. Ltd.) was used in all experiments. The seeds were surface sterilized with 1% sodium hypochlorite for two minutes and subsequently washed in sterile water and air dried before sowing and 15-day-old seedlings were used. Bio-peat (SG) compost that contains cocopeat was used as planting medium in greenhouse experiments to test the non-pathogenicity of *Fusarium* isolates. Red sandy loam soil was used in the pot culture experiment to determine the biocontrol potential of selected isolates.

Seedling dip in broth

Fusarium isolates were grown on 125 ml of potato dextrose broth (PDB) for three days in an incubator shaker. Fifteen-day-old seedlings of tomato were dipped in a beaker containing *Fusarium* biomass for five minutes and then transplanted in seedling trays containing Bio-peat (SG) compost. Disease incidence was recorded at two-day interval based on external symptoms.

Broth drench

Fusarium isolates were grown on PDB (125 ml) for three days in an incubator shaker. The mycelial biomass was finely ground in a mixer grinder. Fifteen-day-old tomato seedlings were transplanted in seedling trays containing Biopeat (SG) compost. Collar region of each plant was inoculated with one ml fungal biomass. Observations were recorded at two-day interval for wilting symptoms.

Test tube method

Four-week-old seedlings were uprooted and gently washed with running tap water and finally with sterile water. Spore suspension of *Fusarium* was prepared by flooding water on seven-day-old cultures on PDA plates. To obtain 100 ml spore suspension, three Petri plate cultures were used and the spore suspension was adjusted to $4-6 \times 10^6$ spores ml^{-1} and filtered through muslin cloth. The spore suspension thus obtained was diluted ten times. Seedlings were transferred to test tubes with diluted spore suspension (50 ml) so that the roots were immersed in the suspension and cotton was placed around the plant at the mouth of test tube. The tubes were set on a test tube stand and incubated at ambient temperature. Observations were recorded for wilt symptoms.

Spore drench method

Fifteen-day-old tomato seedlings were washed with sterile water and transplanted in seedling trays with

autoclaved Bio-peat (SG) compost. Spore suspension of $4-6 \times 10^6$ spores ml^{-1} was used to drench the collar region of seedlings. Observations were recorded on wilt symptoms for up to five weeks.

Response of non-target crops to non-pathogenic isolates of *Fusarium* spp.

Chick pea, pigeon pea and safflower seeds were treated with talc formulations of non-pathogenic *Fusarium* spp. and sown in aluminium trays containing sand and tested for pre- and post-emergence wilt symptoms. Seeds of bold seeded crops like groundnut, castor and water melon were treated with formulations and then sown in plastic pots and tested for wilt.

Bioefficacy tests

Dual plate

Selected isolates of *Fusarium* were tested against the wilt pathogen, *F. oxysporum* f. sp. *lycopersici*, for their antagonistic potential on PDA at room temperature. Discs (6 mm) of *F. oxysporum* f. sp. *lycopersici* were placed on a PDA plate one cm away from the edge. Discs of the same size from *Fusarium* cultures to be tested were inoculated on the opposite side, one cm away from edge. Three replications were maintained for each isolate. The colony diameters of the pathogen and antagonists were recorded and percentage growth reduction in the pathogen was calculated.

Greenhouse experiment with cocopeat as planting medium in seedling trays

The spore drench method was consistent when repeated again and was followed for the greenhouse experiment. Biopeat (G) compost was used as planting medium. Seven selected isolates, viz. NPFu1, NPFu2, NPFu3, NPFu4, NPFu7, NPFu24 and NPFu25, were tested. Besides per cent wilt incidence, plant growth parameters such as length and weight of root and shoot were recorded. After the bioefficacy tests, colonization of roots by non-pathogenic *Fusarium* isolates was assessed by plating 25 root-bits (1cm) on *Fusarium* selective medium.

Pot culture experiment with soil as planting medium

Seven selected isolates of non-pathogenic *Fusarium* were tested for their efficacy by spore drenching method. Red sandy loam soil was used as planting medium. The plant growth parameters and per cent root colonization were recorded besides per cent wilt incidence.

RESULTS AND DISCUSSION

Twenty-five *Fusarium* isolates were obtained from the soil samples collected from different locations. Isolates Fu1 to Fu15 and Fu25 were obtained from samples from Karnataka state. Isolates Fu22, Fu23, Fu24 were obtained from Gujarat. Fu16 and Fu17 were from Tamilnadu. Fu19 was isolated from UP.

Different inoculation methods like seedling dip in broth culture, drenching with broth culture, test tube method and drenching with spore suspension were tested. Seedling dip in broth culture resulted in wilting with many of the isolates and no wilt symptoms were observed with non-pathogenic *Fusarium* isolates Fu1, Fu2, Fu3, Fu4, Fu7, Fu12, Fu24 and Fu25. Drenching the seedlings with broth at collar region did not result in wilting of plants with isolates Fu1, Fu2, Fu3, Fu4, Fu7, Fu12, Fu13, Fu23, Fu24 and Fu25. Drenching with spores of isolates Fu1, Fu2, Fu3, Fu4, Fu7, Fu8, Fu12, Fu24 and Fu25 did not result in wilt infection while with other isolates wilting symptoms could be observed. In rapid assay with test tube method, isolates Fu1, Fu2, Fu3, Fu4, Fu7, Fu12, Fu19, Fu24 and Fu25 were non-pathogenic to tomato. Based on these results, isolates Fu1, Fu2, Fu3, Fu4, NPFu7, Fu12, Fu24, and Fu25 were selected for further studies.

Non-pathogenicity of selected *Fusarium* isolates was tested on other crop plants (chickpea, pigeonpea, safflower, groundnut, castor and water melon) that are susceptible to *Fusarium* wilt. No wilt symptoms were observed for up to one month after inoculation with any of the seven selected non-pathogenic isolates while

in the plants inoculated with respective pathogens, the wilt symptoms could be observed.

Antagonistic potential of seven non-pathogenic isolates against tomato wilt pathogen (*Fusarium oxysporum* f.sp. *lycopersici*) was tested *in vitro* by dual plant method. On seventh day, NPFu4, NPFu3, NPFu24 and NPFu25 inhibited the pathogen growth by 32-40% and NPFu1, NPFu2, NPFu3 and NPFu 7 resulted in 24-27% inhibition of the pathogen growth (Table 1). Overgrowth on pathogen by non-pathogen isolates was observed with isolate NPFu 25.

Out of the nine isolates tested using spore suspension drenching, Fu12 and Fu19 caused 30-50% wilt incidence with poor plant growth compared to control. They were not considered for further experiments. Maximum shoot length was observed in NPFu7 inoculated plants while maximum shoot weight was observed in NPFu2 inoculated plants. Maximum root length was recorded in NPFu24 treated plants while maximum root weight was with NPFu3 treated plants (Table 2). After bioefficacy test, root colonization by the non-pathogenic isolates was confirmed by the growth of *Fusarium* isolates (100% for all the isolates) on the surface of root bits plated on *Fusarium* selective medium.

There was a significant increase in plant growth, especially shoot weight, due to inoculation with non-pathogenic *Fusarium* isolates compared to control. No wilting symptoms were observed in any plants treated with the selected *Fusarium* isolates while wilt incidence was 100% in pathogen inoculated plants. When the plants

Table 1. Effect of non-pathogenic isolates of *Fusarium* on *in vitro* growth of *F. oxysporum* f. sp. *lycopersici*

Isolate	Colony diameter in cm on 5th day		% inhibition	Colony diameter in cm on 7th day		% inhibition
	NPF	FOL		NPF	FOL	
NPFu1	6.13	4.88	33.85	7.38	5.88	24.19
NPFu2	3.63	4.63	37.25	4.25	5.63	27.42
NPFu3	5.5	4.75	35.55	6.25	4.75	38.71
NPFu4	5.00	4.25	42.33	6.25	5.25	32.26
NPFu7	6.00	5.00	32.16	7.00	5.88	24.19
NPFu24	5.00	5.5	32.16	5.65	4.88	37.10
NPFu25	4.33	4.13	44.03	5.75	4.63	40.32
Pathogen	-	7.37	-	-	7.75	-

C.D @ P= 0.05, Time – 5.00, Fungus – 9.3, Interaction: T * F – 13.23; NPF: non-pathogenic *Fusarium*; FOL: *F. oxysporum* f. sp. *lycopersici*

Table 2. Plant growth parameters and wilt incidence in tomato plants treated with *Fusarium* isolates by spore drenching method in seedling trays (greenhouse condition)

Isolate	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	% wilt incidence	Root colonization
NPFu1	23.45	15.3	70	278	0	0
NPFu2	21.05	17.32	126	18.75	0	100
NPFu3	21.05	17.32	68	316	0	100
NPFu4	21.05	18.35	51	301	0	100
NPFu7	23.7	16.675	59	191	0	100
NPFu12	24.60	16.12	42	251	30	100
NPFu19	18.14	15.15	64	224	50	100
NPFu24	21.30	19.35	66	270	0	100
NPFu25	23.00	18.28	114	292	0	100
Control	16.55	12.4	48	226	0	–
Pathogen	11.38	7.13	9	28	85.99	100
C.D @ 5%	4.46	3.61	1.27	2.65		

Table 3. Plant growth parameters and wilt incidence in tomato plants treated with talc formulation of *Fusarium* isolates under pot culture conditions

Isolate	Shoot length (cm)		Root length (cm)		Shoot weight (g)		Root weight (g)		Wilt incidence (%)		Colonization (%)	
	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P
NPFu1	13.3	13.8	51.7	43.2	219.0	165.7	55.3	37.7	–	0	100	100
NPFu2	12.5	16.3	51.3	58.2	199.0	227.7	65.0	67.0	0	33	23	100
NPFu3	10.2	15.5	58.2	52.3	96.7	188.7	99.0	63.7	0		100	100
NPFu4	12.3	12.5	53.0	59.8	157.3	227.0	44.7	67.0	0	0	100	100
NPFu7	11.8	15.2	60.7	62.2	76.7	196.0	200.3	65.7	0	0	100	100
NPFu24	16.5	14.5	57.3	48.7	211.3	192.3	113.0	64.0	0	0	100	100
NPFu25	12.7	13.7	66.0	56.3	237.5	125.0	232.5	44.7	0	0	100	100
Pathogen	–	16.7	–	44.2	–	194.7	–	54.7	0	100	00	100
Control	2.05	0.0	4.0	0.0	5.9	0	94.3	0	–	–	100	0
C.D@5%	2.05	6.6	4.0	11.4	5.9	15.9	94.3	36.3	–	–	–	–

-P = without pathogen inoculation, +P = with pathogen inoculation

were treated with non-pathogenic *Fusarium* isolates along with pathogen inoculation, NPFu25 treated plants showed maximum shoot length, shoot weight and root weight while NPFu24 treated plants had maximum root length (Table 3). Maximum shoot length was observed in NPFu24

treated plants (16.50cm) while in control it was only 13.33 cm. There were no significant changes in shoot length in plants treated with the other isolates. Root length was maximum with NPFu7 (62.17cm) while it was 43-59cm in plants treated with other isolates and co-inoculated with

the pathogen culture. Shoot weight was higher (220g) in plants treated with Fu2 and Fu4 while root weight was higher with NPFu24 and NPFu25 isolates.

Non-pathogenic isolates of plant pathogens that lost virulence after mutation may be employed for biological control of virulent isolates as described by Freeman and Rodriguez (1993) with non-pathogenic *Colletotrichum magna* for the protection of cucurbits from both *Colletotrichum* and *Fusarium*. However, Haridon *et al.* (2007) showed that rev. 157, a non-pathogenic mutant of the pathogenic strain of *F. oxysporum* f. sp. *melonis* (Form 24), could not protect flax plants from *F. oxysporum* f. sp. *lini* while soil borne biocontrol strain Fo47 could protect the same. With naturally available saprophytic *Fusarium* spp. that are not pathogenic to crop plants, much success has been obtained in the biological control of *Fusarium* wilt as indicated in the review by Fravel *et al.* (2003).

Endophytic non-pathogenic *F. oxysporum* have been demonstrated to control both banana weevils and *Radopholus similis* (Griesbach, 2000; Niere *et al.*, 2001) by means of induced resistance and antibiosis to eggs and larvae besides acting as plant growth promoters (Tam *et al.*, 2006). The non-pathogenic strain of *F. oxysporum* Fo47 controlled *Fusarium* wilt of carnation (Postma and Rattink, 1992), tomato and melon (Alabouvette *et al.*, 1993), asparagus (Blok *et al.*, 1997) and flax (Duijff *et al.*, 1999). Hoch and Abawi (1979) have reported mycoparasitism of oospores of *Pythium ultimum* by *F. merismoides*.

To test the non-pathogenic nature of these endophytic *Fusarium* isolates isolated from the root bits of tomato plants, different inoculation methods like drenching the pots with broth culture of the fungus, dipping the seedlings in the broth, keeping the seeding immersed in spore suspension of the fungus in test tube and drenching the seedling with spore suspension were tested of which drenching with spores gave consistent results. All the pathogenic isolates infected the tomato plants in this method and in plants inoculated with standard isolate of pathogen there was 100 per cent infection by this method. Based on *in vitro* screening (dual culture with pathogen isolate), screening in seedling trays and pot culture experiments, it was found that isolates NPFu1, NPFu2, NPFu3, NPFu4, NPFu7, NPFu24 and NPFu25 were non-pathogenic to tomato.

When tested on non-target crops, inoculation methods were changed as per the host plant tested. These isolates were non-pathogenic to chickpea, pigeonpea, safflower,

groundnut and castor on which fusarial wilt is a common disease.

The per cent reduction in growth of pathogenic isolate (F.o.I.) ranged from 24-40% in dual culture. In greenhouse conditions, in seedling tray and pot culture experiments, treatment with NPFu1, NPFu3, NPFu4, NPFu7, NPFu24 and NPFu25 protected the plants from wilt incidence. Root colonization by these non-pathogenic isolates was seen in all the root bits (from plants inoculated with or without pathogen) plated on selective medium indicating their good root colonizing capacity.

Use of saprophytic or non-pathogenic isolates of *Fusarium* for biological control of pathogenic *Fusarium* spp. in various crops has been extensively studied and applied (Schneider, 1984; Larkin *et al.*, 1996; Mandeel and Baker, 1991; Yamaguchi *et al.*, 1992; Nel, 2006).

Protection of host plants from challenge inoculation with pathogenic or virulent strains has been reported by several workers (Nel, 2006; Gessler and Kuc, 1982; Ogawa and Komada, 1986; Thangavelu and Jayanthi, 2009). Though mutants of pathogenic isolates have been attempted for biocontrol purposes, the mutants could not protect the plants while non-pathogenic saprophytic isolates of *Fusarium* spp. could protect the plants. The active growth of non-pathogenic *F. oxysporum* was limited to the root epidermis (Paparou *et al.*, 2009; Behamou and Garnard, 2001).

In the present study also, non-pathogenic isolates of *Fusarium* spp. were found to have good root colonization on tomato roots, thereby protecting the plants from infection by pathogenic isolate and also promoted plant growth. The mode of action of these isolates can be attributed to their good root colonization and induced systemic resistance (Patil *et al.*, 2011). It is concluded that use of these isolates as seedling dip or seed treatment will help in managing *Fusarium* wilt in tomato.

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