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Effect of Actinobacteria and *Glomus fasciculatum* against *Fusarium oxysporum* f. sp. *lycopersici* in Tomato Plant

Anusha Suresh Gadag^{1*} and P.U. Krishnaraj²

¹Molecular Biology and Biotechnology, University of Agricultural Sciences,
Dharwad, Karnataka, India

²Department of Agriculture Microbiology, College of Agriculture, Vijayapur, University
Agricultural Sciences, Dharwad, India

*Corresponding author

ABSTRACT

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Fusarium oxysporum f. sp. *lycopersici* is a soil-borne plant pathogen responsible for the development of *Fusarium* wilt which is one of the most important and destructive fungal plant diseases worldwide. The pathogenic strain of *Fusarium oxysporum* f. sp. *lycopersici* is isolated from the infested plant samples of tomato stem. The identification and confirmation of the isolate were done based on cultural characteristics, pathogenicity test. Out of eighty, Actinobacteria isolates which were collected from Agricultural College, Dharwad, 3 isolates (AUDT 626, AUDT 699 and AUDT 693) were showed a significant zone of inhibition 30.99 mm against *Fusarium oxysporum* f. sp. *lycopersici*. *Glomus fasciculatum* culture applied to the tomato plant showed 58.8% root colonization along with AUDT 622. Under challenge inoculation with a pathogen, AUDT 626 and both AUDT 626 and *G. fasciculatum* showed significant control of the wilt disease of tomato. In the present study AUDT 626 with *G. fasciculatum* showed 100% disease control with root length of 3.18 cm, shoot length 16.9 cm and total dry weight of 80 mg compared to disease control plant.

Introduction

Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* is a most important fungal disease of tomato growing areas in worldwide. The pathogen is soil borne and causes wilt which leads to root and basal stem deterioration and results in wilting of the plant. Browning of the vascular tissue is proof of *Fusarium* wilt. This pathogen persists in soil without a host for many years.

As a result of extensive use of chemical pesticide, several insects and pathogens have developed the resistance to these chemical

pesticides (Ntalli and Spiroudi, 2011). Therefore usage of microbial inoculant as disease suppressive or resistant agents and as plant growth promoting agents are the best alternate solution to the use of chemical pesticides and fertilisers. Arbuscular mycorrhiza fungi are an essential component of the rhizosphere, and they cause beneficial effects on growth and yield of different crops. AM fungi (*G. fasciculatum*) improve nutrients uptake example nitrogen, phosphorus, micronutrient and others. It also plays an important role in disease tolerance and to

decrease soil pathogen incidence from genera such as *Fusarium*, *Macrophomina*, *Phytophthora*, *Pythium*, and *Rhizoctonia* (Cornado *et al.*, 2013). Actinobacteria is one of the important sources of many biologically active components such as metabolites, alkaloids, enzymes, antibiotics, etc. In many areas, actinobacteria act as biocontrol tool against many plant pathogen such as *Alternaria solani*, *Alternaria alternata*, *Fusarium solani*, *Phytophthora megasperma*, *Verticillium dahlia* and *saccharomyces cervisiae* etc. (Aghighi *et al.*, 2004) and can colonize plant surface, antibiotics against plant pathogens, synthesis of extracellular enzymes and the degradation of phytotoxins. Nawar, (2016) used the arbuscular mycorrhiza and actinobacteria isolates as bio-inoculum for growth promotion and biocontrol of damping off disease caused by *Rhizoctonia solani*. Therefore, the present study on the biocontrol activity of actinobacteria and arbuscular mycorrhiza against *Fusarium oxysporum* f. sp. *lycopersici* on tomato plant growth parameter and disease resistance were done.our study further provide insight in biocontrol research.

Material and Methods

Sample collection

The collection of tomato plants which are infected by *Fusarium* wilt is done from fields of University of Agriculture Science, Dharwad, India. A soil sample from the rhizosphere of tomato plant showing typical wilt symptoms is collected from the field. A part of them is labelled and packed in polyethene bags and stored in a cold room till they are used for the isolation of the fungus.

Isolation of *Fusarium* and pathogenicity

Fusarium is isolated from stem and root tissues of infected tomato plants. Sections of the lateral stem are surface sterilised with 1 %

NaOCl solution for 2 min and then stems washed three times in sterile distilled water and blotted on sterile filter paper to remove excess water. Tissue pieces are inoculated on PDA plates and incubated at 28 ± 2 °C for 7-10 days (Yamauchi, *et al.*, 2004). The potato dextrose broth (PDB) is used to prepare the primary inocula. The concentration of the *Fusarium* spore suspension concentration is determined with a haemocytometer and adjusted with sterile distilled water to a final concentration of 1×10^6 spores per ml. The tomato seeds of DMT-2 varieties are obtained from the Department of Horticulture, University of Agricultural Sciences, Dharwad. The seeds are sown in seedling trays filled with coconut pith compost and watered regularly. 30 days old healthy seedlings are inoculated with the pathogen by root dip method.

In vitro* Selection of antagonistic actinobacteria against *Fusarium oxysporum* f. sp. *lycopersici

In primary screening a total of 80 actinobacteria are screened for their antagonistic activity against *Fusarium oxysporum* f. sp. *lycopersici* by cross streak method as per the protocols of Rahman *et al.*, (2011). Actinobacterial isolates are streaked at the centre of SCBA media plates, and then plates are incubated at 28 °C for seven days after that fungal disc of 6 mm diameter is bored at 90° angles on both sides at defined length. From the primary screening, potent isolates are subjected to the secondary screening on SCA plates. Media plate with pathogen alone is maintained as a control. All these plates are incubated at room temperature and observed for the inhibition of the growth of the pathogen. Observations are taken six days after pathogen inoculation. The zone of inhibition is measured (Biratu *et al.*, 2013). and colony growth inhibition (%) is calculated by using the formula: $PI = (C - T)/C \times 100$, where PI is the percent inhibition,

C is the colony growth of the pathogen in control, and *T* is the colony growth of the pathogen in dual culture. All isolates are tested in triplicate.

Application of *G. fasciculatum* culture

The plastic pots are filled with soil (500 g per pot), and tomato seeds treated with the actinobacteria are sown (as indicated in section 3.9.2.1). *G. fasciculatum* inocula @ 25 g pot⁻¹ (125 spores/50 g of soil) is mixed thoroughly with the top 10 to 15 cm of the soil (Habte and Osorio, 2002). There are four treatments with eight replications. Plants are maintained in a greenhouse and analyzed for per cent root colonization.

Per cent root colonization

The mycorrhizal root colonization is determined as per the procedure proposed by Philips and Hayman (1970) and the percentage of roots colonized by mycorrhizae is calculated by the formula given by Nicolson (1960):

$$\text{Per cent root Colonization} = \frac{\text{Number of root segments positive for colonization}}{\text{Total number of root segments}} \times 100$$

In vivo screening of actinobacterial and *G. fasciculatum* for inhibitory activity against *F. oxysporum* f. sp. *lycopersici* MB6-1

Actinobacteria isolates which showed significant inhibition of *F. oxysporum* f. sp. *lycopersici* MB6-1 *in vitro* are selected for *in vivo* screening. Three selected actinobacteria isolates viz, AUDT 699, AUDT 693 and AUDT 626 and *G. fasciculatum* are used for the greenhouse experiment.

Raising of tomato plants

For *in vivo* study, tomato variety of DMT-2, which is known to be susceptible to *F.*

oxysporum f. sp. *lycopersici* MB6-1 is used. Tomato seeds are sown in small pots containing sterilized soil and sand mixture in 3:1 proportion. Completely randomised block design (CRBD) is used as a statistical design.

Method of application of actinobacteria isolates

Two methods are followed for the application of actinobacteria.

Seed priming

Tomato seeds are surface sterilized with 0.02 per cent mercuric chloride for 5 min and rinsed thoroughly in sterile water. The priming of the seed is done by soaking seeds in culture broth of actinobacterial isolates with 1 per cent sterilized carboxy methyl cellulose (CMC) as a sticker.

The suspensions are incubated for 10 min to facilitate attachment of bacterial cells to the seed coat. Later, the seeds are allowed to dry and then sown (Ramanathan *et al.*, 2000).

Preparation and application of the lignite based culture

The lignite based culture is prepared by mixing the culture growth of actinomycetes isolates with sterile lignite at 1:3 ratios and soil application is done at a rate of 100 mg of lignite based culture/kg of soil (Ramamoorthy *et al.*, 2002).

The foliar application is performed with 2 per cent lignite based culture at fifteen days interval *i.e.* 15 and 30 days after sowing (DAS) (Bahadur *et al.*, 2007).

Method of application of *G. fasciculatum* inoculum

The plastic pots are filled with soil (500 g pot⁻¹), *G. fasciculatum* inoculum @ 25 g pot⁻¹

(125 spores/50 g of soil) mixed thoroughly with the top 10 to 15 cm of the soil and then tomato seeds treated with the actinobacteria are sown. There are eight treatments with eight replications. Plants are maintained in a green house.

Challenge inoculation of *F. oxysporum* f. sp. *lycopersici* MB6-1

Tomato seedlings are inoculated with *F. oxysporum* f. sp. *lycopersici* MB6-1 by using spore suspension with conidial concentration of 1×10^6 conidia/ml by root dip method (Anil and Garampalli, 2013).

Thirty days old healthy seedlings are uprooted carefully preserving root integrity, shaken to remove the adhering soil particles and washed gently under tap water. The root tip (1 cm) is trimmed with a sterile scissor and submerged for 30 min in the conidial suspension. The inoculated seedlings and control are individually planted in their respective pots (Nirmaladevi and Shrinivas, 2012).

Monitoring of the disease

The monitoring of wilt disease is done by taking the observation for symptom on 5th and 6th days after inoculation of the pathogen. The per cent disease incidence is calculated using the formula given by Shelly *et al.*, (2005):

$$\text{Disease incidence} = \frac{\text{Number of plants infected}}{\text{Total numbers of plants}} \times 100$$

Results and Discussion

Isolation of *Fusarium* from infested samples and pathogenicity

The pathogen is first confirmed by observing the typical colony morphology on PDA, *Fusarium* isolated from the stem of diseased

tomato plants formed a hyaline, branching mycelium that is white to grey (Fig. 1). Isolates formed macroconidia as elliptical, gradually pointed or curved edges and the septation is of 3 to 5. The microconidia are usually aseptate or single septate (Fig. 2). The virulent *Fusarium* isolates are inoculated to 30 days old tomato seedlings by root injury inoculation technique. Initially, drooping down of youngest leaves followed by wilting of the whole plant after five to six days is observed (Fig. 3). Among the five isolates, *Fusarium* (MB6-1, MB6-2, MB6-3, MB6-4 and MB6-5), MB6-1 isolate showed high disease severity. Therefore, MB6-1 *Fusarium* isolate is used for further work.

Antimicrobial activity of the actinobacterial isolates against *Fusarium oxysporum* f. sp. *lycopersici* MB6-1

During primary screening six actinobacteria isolates showed inhibition to *Fusarium oxysporum* f. sp. *lycopersici* MB6-1. These isolates are used for secondary screening. Six isolates that showed inhibitory activity in primary screening are selected for secondary screening. Both the diameter and annular radius of the inhibition zone shown by isolates are measured and analyzed. All the isolates showed a significant difference in their inhibition against the pathogen. Isolate AUDT 626 is the most potent isolate followed by AUDT 699 and AUDT 693 (Table 1). The three isolate are selected further for cultural analysis.

Influence of the actinobacteria on the percent colonization *G. fasciculatum* in tomato

The observations on *G. fasciculatum* spore count are carried out at 30 days after the sowing (DAS). The percent root colonization is recorded at 30 DAS and presented in table 3. Plants treated with only *G. fasciculatum* showed 52.5 % of root colonization, whereas

plants treated with *G. fasciculatum* along with actinobacteria AUDT 626 showed 58.8 % root colonisation. Hence, AUDT 626 isolate showed resistance to disease compared to other two isolates (AUDT 699, AUDT 693) alone. Further, AUDT 626 + *G. fasciculatum* induced high disease resistance as compared to other two isolates in association with *G. fasciculatum*. Therefore, actinobacterial isolate AUDT 626 individually and in association with *G. fasciculatum* is used for transcript analysis (Table 2).

The role of actinobacteria in association with *G. fasciculatum* in tomato *Fusarium* wilt disease resistance

When three actinobacteria isolates AUDT 626, AUDT 699 and AUDT 693 separately and along with *G. fasciculatum* are screened against wilt disease in tomato, it is found that plants treated with actinobacterial isolates showed low diseased symptoms whereas plants treated with actinobacterial isolates in association with the *G. fasciculatum* lower disease symptoms compared to uninoculated one. All the three isolates alone and in association with *G. fasciculatum* showed significant disease control over pathogen control. Though AUDT 626 (Fig. 3) alone is found effective (25 % disease incidence), AUDT 626 in association with *G. fasciculatum* (Fig. 4) showed higher efficiency (no disease incidence). The plants treated with the only pathogen as expected showed 100 % disease symptom (Table 3).

Role of actinobacteria in association with *G. fasciculatum* in tomato *Fusarium* wilt disease resistance and plant growth promotion

Root length

Individual inoculation of actinobacteria and *G. fasciculatum* has improved the root growth

significantly (Table 4). However, in the presence of the pathogen, the interaction is insignificant in term of the length. AUDT 626 caused the highest increase (12.6 cm) in root length compared to control (3.1 cm) and only pathogen infected plant (1.7 cm). The inoculation of *G. fasciculatum* has increased the root length (14.7 cm) significantly over the control (3.1 cm), only pathogen (1.7 cm) inoculated and actinobacteria (7-12.6 cm) inoculated treatments.

The interaction of actinobacteria and *G. fasciculatum* to the pathogen infected plants did not show any significant change in the root length over pathogen plants. However, in the presence of AUDT 626 + pathogen (2.1 cm) and *G. fasciculatum* + pathogen (1.96 cm), there is no significant difference over the uninoculated control (3.1 cm).

Co-inoculation of actinobacteria (AUDT 626, AUDT 699) and *G. fasciculatum* improved the plant root length over individual inoculation which is not significantly different from those of the pathogen inoculated.

Shoot length

Individual inoculation of actinobacteria and *G. fasciculatum* has increased the shoot length significantly over the uninoculated control and pathogen infected control. Amongst the actinobacteria, AUDT 626 has caused the highest increase (14.0 cm). Inoculation of *G. fasciculatum* showed significantly higher shoot length (15.3 cm) than the actinobacteria AUDT 626 (14.0 cm). The inoculation of actinobacteria to AUDT 626, AUDT 693, AUDT 699 and *G. fasciculatum* has improved the shoot length (11.70 cm, 8.00 cm, 7.86 and 10.54 cm, respectively) significantly over pathogen inoculated plants (6.6 cm).

The co-inoculation of the *G. fasciculatum* and actinobacteria (AUDT 626, AUDT 693 and

AUDT 699) also resulted in significant increase in shoot length (16.9 cm, 10.88 cm, 11.34 cm, respectively) over individual inoculation of actinobacteria AUDT 6262, AUDT 693, AUDT 699 and *G. fasciculatum* (11.70 cm, 8.00 cm, 7.86 and 10.54 cm, respectively).

Total dry weight of the plant

Individual inoculation of actinobacteria AUDT 626 and *G. fasciculatum* has increased the total dry weight significantly over the uninoculated control and pathogen inoculated control. AUDT 626 caused the highest increase (90 mg) in total dry weight compared to control (30 mg) and only pathogen infected (20 mg).

The inoculation of *G. fasciculatum* has significantly increased the total dry weight (96 mg) over the uninoculated control (30 mg) and only pathogen (20 mg) inoculated.

The interaction of AUDT 626 + *G. fasciculatum* with that of the pathogen infected plants showed a significant increase in the total dry weight (80 mg) over the uninoculated control and only pathogen inoculated plants.

Co-inoculation of actinobacteria AUDT 626, AUDT 699 and AUDT 693 and *G. fasciculatum* high dry weight (132 mg, 110 mg, 70 mg, respectively) significantly over their respective individual inoculation of actinobacteria which is significantly different from those of the uninoculated control, and the pathogen inoculated plants.

Tomato is infected by a number of pathogens, including *F. oxysporum* f. sp. *lycopersici* (Sacc.), the causal agent of *Fusarium* wilt of tomato (Ignjatov *et al.*, 2015), which is one of the most important pathogen species on tomato (Sahi and Khalid, 2007).

Table.1 Zone of inhibition of *F. oxysporum* f. sp. *lycopersici* MB6-1 by actinobacterial isolates

Sl. No.	Actinobacteria isolate	Zone of inhibition (mm)
1	AUDT 699	30.59
2	AUDT 697	20.24
3	AUDT 726	29.5
4	AUDT 693	30.21
5	AUDT 626	30.99
6	AUDT 701	19.70
	S. Em. +	0.93
	C.D. at 5%	2.93

Table.2 Percent of root colonization by *G. fasciculatum* in tomato plant

Treatments	% Root colonization
Control	00.0
<i>G. fasciculatum</i>	52.5
AUDT 626 + <i>G. fasciculatum</i>	58.8
AUDT 699 + <i>G. fasciculatum</i>	56.3
AUDT 693 + <i>G. fasciculatum</i>	53.0
S. Em. +	0.16
C.D. at 5 %	0.46

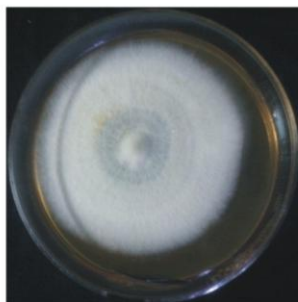


Fig.1: Growth of the *Fusarium oxysporum* f. sp. *lycopersici* MB6-1 on PDA

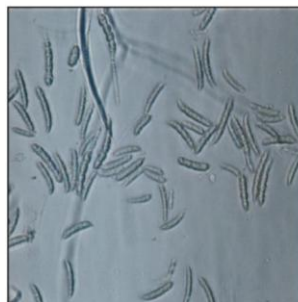


Fig.2: Microconidia and macroconidia of *Fusarium oxysporum* f. sp. *lycopersici* MB6-1

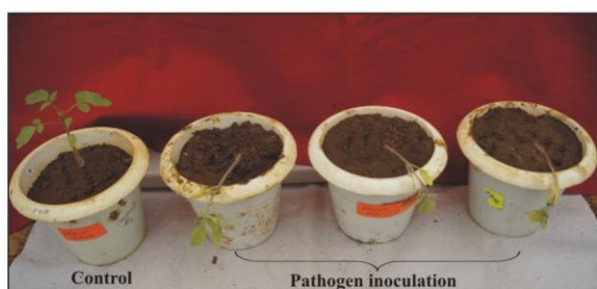


Fig.3: Tomato plant showing sever wilt symptoms when inoculated with the *Fusarium*, uninoculated control.

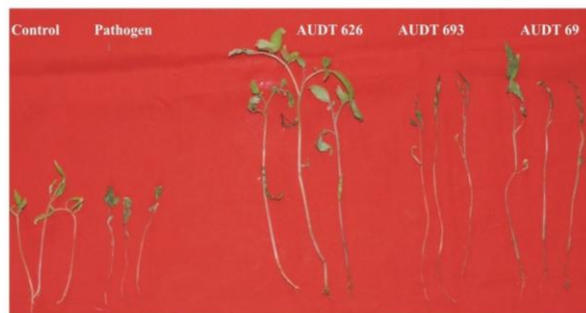


Fig. 4: Comparison of control, pathogen treated, AUDT 626, AUDT 699 and AUDT 693 in challenge inoculation of *Fusarium* in tomato plant

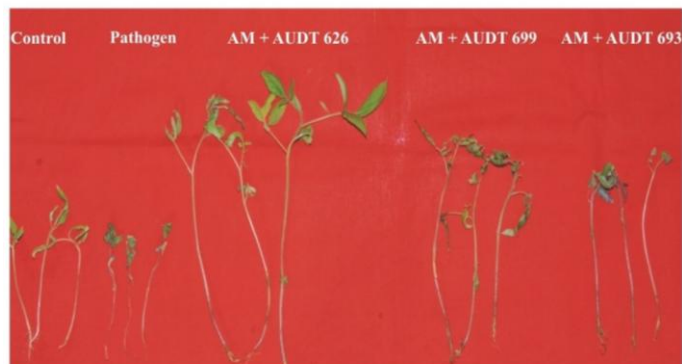


Fig. 5: Comparison of control, pathogen treated, AM + AUDT 626, AM + AUDT 699 and AM + AUDT 693 in challenge inoculation of *Fusarium* in tomato plant

Table.3 Percent disease incidence in tomato plants inoculated with actinobacteria, *G. fasciculatum* and *F. oxysporum* f. sp. lycopersici MB6-1 tomato

Sl. No.	Treatments	Percent disease incidence
1	<i>G. fasciculatum</i> + AUDT 626+ Pathogen	0
2	<i>G. fasciculatum</i> + AUDT 699 + Pathogen	55
3	<i>G. fasciculatum</i> + AUDT 693 + Pathogen	80
4	<i>G. fasciculatum</i> + Pathogen	70
5	AUDT 626 + Pathogen	25
6	AUDT 699 + Pathogen	85
7	AUDT 693 + Pathogen	92
8	Pathogen only (disease control)	100
9	Control	0

Table.4 Plant growth parameters as influenced by actinobacteria and *G. fasciculatum* with *Fusarium oxysporum* f.sp. lycopersici MB6-1 inoculation in tomato 36 DAI under greenhouse condition

Treatments	Root length (cm)	Shoot length (cm)	Total dry weight (mg)
Control	3.1	6.82	30
Pathogen	1.7	6.6	20
AUDT 626	12.6	14	90
AUDT 693	7	9.5	26
AUDT 699	8.8	10.9	20
<i>G. fasciculatum</i>	14.7	15.3	96
AUDT 626 + Pathogen	2.1	11.7	40
AUDT 693 + Pathogen	1.5	8	14
AUDT 699+ Pathogen	1.9	7.86	16
<i>G. fasciculatum</i> + Pathogen	1.96	10.54	50
AUDT 626+ <i>G. fasciculatum</i>	22	18.5	132
AUDT 693+ <i>G. fasciculatum</i>	11.8	12.4	70
AUDT 699+ <i>G. fasciculatum</i>	21.30	12.8	110
AUDT 626 + <i>G. fasciculatum</i> + Pathogen	3.18	16.9	80
AUDT 693 + <i>G. fasciculatum</i> + Pathogen	1.86	10.88	3
AUDT 699 + <i>G. fasciculatum</i> + Pathogen	2.58	11.34	50
S. Em±	0.39	0.28	0.37
C. D. at 5%	1.10	0.79	1.06

The result of the experiments done to understand the interaction of actinobacteria and *Glomus fasciculatum* in the presence of *Fusarium oxysporum* f. sp. lycopersici MB6-1 is discussed herein.

In the present study, the pathogen is isolated from the infested plant sample collected from the fields of University of Agriculture Sciences, Dharwad. Isolate samples are

surface sterilized and incubated on PDA plate at 28 °C for three days. Colonies are identified as *F. oxysporum* based on morphological characteristics of the macroconidia, microconidia, chlamydospores and colony growth traits (Nirmaladevi and Shrinivas, 2012). To check the virulence of the *Fusarium* isolates, pathogenesis test is carried out using five isolates. After four to five days of inoculation, it is observed that five isolates

showed wilting symptoms, but the degree of infection is variable. The isolated, MB6-1 showed the highest virulence and is selected as the most virulent isolate for further studies.

In the present study, primary screening is done using cross streak method for all 80 actinobacterial isolates against the test pathogen, *F. oxysporum* f. sp. *lycopersici* MB6-1. Of all the tested isolates, six isolates showed inhibition effect on *F. oxysporum* f. sp. *lycopersici* MB6-1. In secondary screening, six isolates AUDT 701, AUDT 699, AUDT 697, AUDT 726, AUDT 693 and AUDT 626 showed inhibitory activity against the target pathogen. Inhibition zone up to 30.99 mM diameter is observed in AUDT626 which is the highest of all the six. Thus it indicates that the actinobacterial isolates possessed antibacterial activity against the target pathogen and can be used as a component of integrated disease management practices. The antibacterial activity exhibited by actinobacteria may be due to the secretion of extracellular antimicrobial compounds. Antibiotics are known to be produced in the SCA media plates (Rahman *et al.*, 2011).

Some of the naturally antagonistic microorganisms, *Bacillus* species and *Pseudomonas fluorescens* (Anuratha and Gnanamanickam, 1990, Xue *et al.*, 2009), *Stenotrophomonas maltophilia* (Messiha *et al.*, 2007), *Streptomyces griseus* (Palaniyandi *et al.*, 2013) is shown to exhibit antagonistic activity against *Fusarium* and have been used as biocontrol agent against the wilt disease. Other bacteria including *Bacillus mesenteriacus*, *B. megaterium*, *B. subtilis*, *B. mycoides* and *Erwinia* have been reported to be active biological control agents (Kelman, 1953). In the present study, a wide range of response of tomato plants inoculated with test isolates alone and in association with *G. faiculatum* against tomato wilt disease pathogen is noticed. Among the test isolates,

inoculation of three actinobacterial isolates (AUDT 626 and AUDT 699) showed a reduction in disease symptoms. The development of disease symptoms in AUDT 626 individual inoculated plants showed 25 per cent at 36 DAI; in AUDT 626 + *G. faiculatum* showed no disease symptoms. Whereas it is 100 per cent in disease control and 85 and 92 per cent in other test isolates alone (AUDT 693 and AUDT 699 respectively) whereas 55 per cent in *G. faiculatum* + AUDT 699 and 80 per cent in *G. faiculatum* + AUDT 699 treated plant.

It has been reported that *G. faiculatum*, Plant Growth Promoting Rhizobacteria (PGPR) and actinomycetes, as rhizospheric microorganisms play an important role in promoting plant growth and protection against plant pathogens. These have participated in the direct or indirect enhancement of plant growth through their individual effects if any. However, the detrimental effect of the entire three groups with each other depends upon the species involved (Kamal *et al.*, 2015). The inoculation of *G. faiculatum* exhibited a reduction in root necrosis in tomato due to *F. oxysporum* f. sp. *lycopersici* MB6-1. The affect is more pronounced when *G. faiculatum* is inoculated four weeks before *F. oxysporum* f. sp. *lycopersici* MB6-1. *G. faiculatum* also is known to reduce the number of propagules of pathogenic fungi (Mukerji *et al.*, 1999). *G. faiculatum* and *G. macrocarpum* have been reported to suppress apple replant disease caused by phytotoxic myxomycetes (Catska, 1994).

Three actinobacterial isolates AUDT 626, AUDT 699 and AUDT 697 alone and actinobacterial isolates in association with *G. faiculatum* contributed to the improvement of plant growth parameters like, shoot length, root length, total dry weight in tomato.

The application of the test isolates of actinobacteria and *G. faiculatum* inocula

resulted in the observation of promotion of all tested plant growth parameters. The root length of plant inoculated with test isolates ranged from 1.50 to 12.6 cm whereas test isolates in association with *G. fasciculatum* ranged from 1.86 to 22 cm. It is however, 1.7 cm in disease control and 3.1 cm in healthy control. After 36 DAI, it is observed that, there are 123 per cent increase in root length of plant inoculated with AUDT 626 + pathogen, 187 % in *G. fasciculatum* + AUDT 626 + pathogen which is followed by (89.4 %) AUDT 699 + pathogen, (151.7 %) *G. fasciculatum* + AUDT 699 and (91 %) *G. fasciculatum* + AUDT 693 + pathogen inoculation over disease control.

The shoot length of plant inoculated with actinobacterial isolates alone ranged from 9.5 to 14 cm highest shoot length where as it ranged from 12.4 to 18.5 cm. It is however, 6.6 cm in disease control and 6.82 cm in healthy control. There is 177 per cent increase in shoot length in AUDT 626 + pathogen treated plant, 256 per cent increase in AUDT 626 + *G. fasciculatum*+ pathogen followed by (119 %) AUDT 699 + pathogen, (172 %) *G. fasciculatum* + AUDT 699 + pathogen, (121 %) AUDT 693 + pathogen and (164 %) AUDT 693 + *G. fasciculatum* + pathogen over disease control.

The total dry weight for plant inoculated with actinobacterial isolate ranged from 1.6 mg to 9 mg whereas it ranged from 3 mg to 13 mg in plants inoculated with *G. fasciculatum* + actinobacterial isolates. After 36 DAI, it is observed that, there is 300 per cent increase in dry weight of plant inoculated with AUDT 626 + pathogen alone, 400 per cent in AUDT 626 + *G. fasciculatum* + pathogen which is followed by (250 %) AUDT 699 + *G. fasciculatum* and AUDT 693 + *G. fasciculatum* (150 %) isolates over disease control. As observed among the three (AUDT 699, AUDT 626 and AUDT 693) isolates of actinobacteria alone treated with *Fusarium*

wilt, AUDT 626 alone showed high (75 %) disease resistance. The isolate AUDT 626 in association with *G. fasciculatum* is treated in challenged inoculation with *Fusarium oxysporum* f. sp. *lycopersici* MB6-1 showed 100 % of disease resistance where as AUDT 699 and AUDT 693 in association with *G. fasciculatum* treated with *Fusarium oxysporum* f. sp. *lycopersici* MB6-1 showed lesser resistance (45 % and 20 % respectively) in comparison with plants treated with AUDT 626 alone + *F. oxysporum* f. sp. *lycopersici* MB6-1 and also in the plants treated with AUDT 626 + *G. fasciculatum* (75 % and 100 % respectively) as mentioned in table 4. Finally, it is clear that, microbial inoculation with Streptomyces, mycorrhizal fungi or both decreased or prevent fungal infection or/and enhanced plant growth.

Consortia of *G. fasciculatum* and actinobacteria proved to be potent biocontrol agent apart from inducing disease resistance in plant also promoted the plant growth such as shoot length, root length and total dry weight.

The application of mixed cultures of actinobacteria along with arbuscular mycorrhiza is a simple methodology and ecofriendly to control wilt disease of tomato compared to harmful chemical pesticide and fertilizers.

Future line of work

Future work can be taken by identification and confirmation of the compound responsible for controlling the disease. Field trial can be taken out to help tomato.

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