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Study on the biological control of fusarium wilt of tomato

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Abstract
In present study, the effect of a root colonizing fungus *Penicillium* sp. EU0013_90S as biocontrol agent (BCA) against Fusarium wilt pathogens was observed in dual culture experiment as well as under screen house conditions. Dual culture experiment was conducted to see whether EU0013_90S has an inhibitory effect on different fungal pathogens especially Fusarium wilt pathogens e.g. *Fusarium oxysporum* f. sp. *lycopersici* race 1 CU1, race 1 MAFF 305121, and race 2 JCM. The radial colony developments of the pathogens were inhibited by zone of different lengths. In screen house experiment, different conidial concentrations (1×10^4, 1×10^5, 1×10^6 ml^{-1}) of *Penicillium* sp. EU0013_90S were treated with tomato seedling followed by Fusarium wilt pathogens of tomato (JCM, MAFF) having different conidial concentrations (1×10^5, 1×10^6, 1×10^7 ml^{-1}) A week after the treatment, tomato plants were observed for external symptoms. Stunting, yellowing and wilting of leaves were caused by *Fusarium oxysporum* isolates (JCM, MAFF). Disease severity was assessed through visual observation. The data was taken with two days interval for 24 days. Treatment of tomato plants with EU0013_90S resulted in significant disease reduction. Disease reduction varied with the different conidial concentrations of BCA and the Fusarium wilt pathogens (JCM, MAFF) used. The highest disease reduction (95.24% and 80.58%) occurred in the treatment where BCA was applied at lowest conidial concentrations (1×10^4 conidia ml^{-1}) with JCM and MAFF respectively.

Keywords: *Penicillium* spp. *Fusarium oxysporum*, biocontrol agent, pathogens

1. Introduction
Tomato is economically the second most important vegetable produced worldwide at around 115.95 million tons per year [4]. In 2008 the production of tomato in Pakistan was 536,217 tonnes. In Pakistan per hectare yield of tomato is very low. Many factors effect field losses e.g. susceptibility of tomato to many plant pathogens and pests, as well as post harvest losses due to improper handling practices.

One of the most destructive and economically damaging diseases of tomato is tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*. The characteristic symptoms include wilting, chlorosis, stunted seedlings [13]. As a result the infected plant dies. It is difficult to control due to various constraints e.g. crop rotation is not effective as the pathogen is soil inhabiting and polyphagus in nature. The most economical and environmental friendly method of disease control is the use of resistant cultivars but new races of pathogens break the resistance of tomato. The use of chemicals is neither economical nor environmental friendly [21]. So the difficulty in controlling Fusarium wilt has stimulated researchers for biological control.

A large number of biological control agents have been used for protecting tomato plants against wilt diseases including various fungal and bacterial species [5, 14]. Among the biocontrol agents non-pathogenic isolates of *F. oxysporum* and *F. solani* are reported to be effective antagonists and provide significant disease reduction [15]. 'biological control or biocontrol is the use of living organisms to suppress the density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be. Aim of the present study is to analyse whether *Penicillium* sp. EU0013_90S can be used as a biocontrol agent against Fusarium wilt of tomato in Malakander (KPK, Agricultural University Peshawar) under screen house conditions.

2. Materials and Methods

In this study *Penicillium* sp. EU0013_90S was used as biocontrol agent against Fusarium wilt pathogens are listed below.

a) *Fusarium oxysporum* f. sp. *lycopersici* race 1 CU1,
b) *Fusarium oxysporum* f. sp. *lycopersici* race 1 MAFF 305121
c) *Fusarium oxysporum* f. sp. *lycopersici* race 2 JCM.

2.1 Sub culturing

Inoculum of EU0013_90S and *Fusarium oxysporum* isolates were produced using PDA. Inoculum of fungus were placed in the centre of Petri dishes aseptically and incubated at 25ºC in the dark.

2.2 Dual culture experiment

In dual culture test EU0013_90S and the pathogen of Fusarium wilt of tomato were allowed to grow in the same Petri dish. The biocontrol agent was placed in the centre of Petri dish and the pathogens were placed at equal distances apart from each other (Fig.1). Apart from the Fusarium isolates (JCM, MAFF and CUI) some other fungal species were also included in this experiment e.g. *Curvularia* sp. and a local isolate of *Fusarium oxysporum* f. sp. *lycopersici* isolated from Charsadda.

The interaction was observed by either formation of inhibition zone between them or over growth by the pathogens. The plates were examined on daily basis and the inhibition zones were measured in mm.

2.3 Inoculum production of *Penicillium* sp. EU0013_90S for screen house experiment

For conidial production the fungal cultures were kept in incubator in darkness for 14 days. For inoculum preparation the two week old cultures were taken. 30 ml sterilized distilled water was put on plate containing the inoculum and disturbed with bent rod to make spore suspension. Then the suspension was poured in 500 ml flask. The volume of the suspension was adjusted to 250 ml by adding sterilized distilled water. Then it was kept on stirrer for three hours at 120 rpm. After stirring, the suspension was filtered through muslin cloth and the spore concentration was measured with the help of Haemocytometer. The concentrations of EU0013_90S were adjusted as 1×10^4, 1×10^5 and 1×10^6 conidia ml^{-1}.

2.4 Inoculum production of *Fusarium oxysporum* isolates

The same procedure as stated above was followed for the inoculum production of *Fusarium oxysporum* isolates (JCM, MAFF). The conidial concentrations of these isolates prepared were 1×10^5, 1×10^6, 1×10^7 conidia ml^{-1}.

2.5 *In vivo* treatment of tomato roots with EU0013_90S.

To study the effect of biocontrol agent EU0013_90S on the severity of Fusarium wilt of tomato, the following experiment was performed in screen house (fig.2).

A French variety of tomato named as “Piogranda” was sown in sterilized soil for growing nursery in screen house at Malakander. The soil was sterilized at 80 ºC for two hours. Three week old tomato plants were uprooted and the roots were washed with sterilized distilled water (SDS). Seedling roots were treated with different conidial concentrations of EU0013_90S (1×10^4, 1×10^5, 1×10^6 conidia ml^{-1}) for ten minutes followed by treatment with *Fusarium oxysporum* isolates (MAFF, JCM) (1×10^5, 1×10^6, 1×10^7 conidia ml^{-1}). For comparison control plants were also used which were treated with either BCA or pathogen. Ten days after inoculation, the tomato plants started showing disease symptoms. Characteristic symptoms observed were stunting, choloris, wilting that ultimately resulted in the death of tomato plant. Disease severity was assessed through visual observation.

3. Results and Discussion

3.1 Effect of *Penicillium* sp. EU0013_90S on the growth of *Fusarium oxysporum* isolates (JCM, MAFF, and CUI) in dual culture experiment.

In dual culture test, inhibition zones were formed by EU0013_90S against the pathogens (Fig.1). This shows that some antifungal compounds are produced by EU0013_90S that inhibit the growth of these pathogens. However the nature of these compounds is not fully understood [1]. The inhibitory effect of EU0013_90S varied with the pathogen (Table 1). The highest zone of inhibition was recorded against JCM and *Curvularia* that was 2.4mm.

The mechanism of biocontrol by non-pathogenic *Fusarium oxysporum* is due to the colonization and competition for nutrients [5]. But the dual culture experiment proved that competition for space is not involved. It may be due to the production of antibiotics by EU0013_90S.

3.2 Effect of root treatment with *Penicillium* sp. EU0013_90S on the severity of Fusarium wilt.

Root treatment with different conidial concentrations of EU0013_90S resulted in disease reduction caused by Fusarium wilt pathogens (JCM, MAFF). The percent disease reduction (Table 2 and 3) was different at different inoculum concentrations used. It was observed that increased conidial concentration of BCA did not show much disease reduction. It was also noted that with increasing conidial concentration of Fusarium wilt pathogens (JCM, MAFF), disease severity increased. The highest disease severity (41.7%) was recorded in the treatment where 1×10^7 ml^{-1} conidial concentration of MAFF was applied with 1×10^6 conidial ml^{-1} of EU0013_90S and the lowest disease severity (8.4%) was recorded in the treatment where 1×10^5 ml^{-1} conidial concentration of MAFF was applied with 1×10^4 ml^{-1} conidial concentration of BCA. Similarly treatment of highest (1×10^6 conidia ml^{-1}) concentration of JCM with 1×10^5 conidia ml^{-1} of BCA resulted in highest disease severity (Fig.3) that was 62.06% (Table 3). It was observed that the lower concentration of BCA (1×10^4 conidia ml^{-1}) was more effective (Fig.5 and 6) against every concentration of *Fusarium* isolates applied. e.g the highest disease reduction (95.24%) was observed when 1×10^4 conidia ml^{-1} BCA was applied with JCM having 1×10^5 conidia ml^{-1} concentration. Plants treated with only pathogens (Fig. 4) showed severe symptoms and those treated with only BCA (Fig.7) showed more reduction in disease incidence.

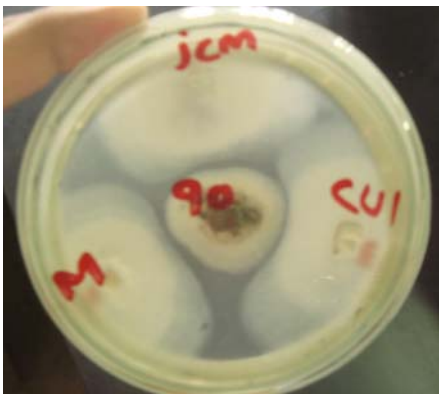


Fig 1: Inhibition zones formed by EU0013_90S against tomato wilt

pathogens in dual culture.

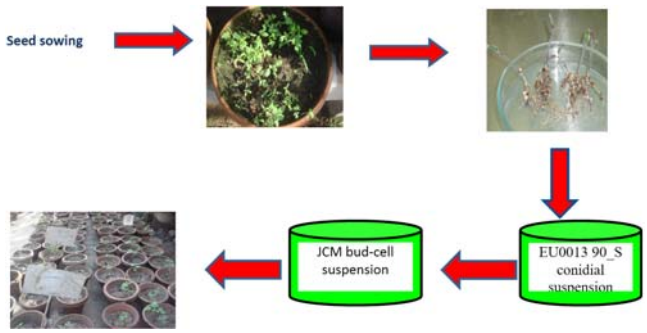


Fig 2: Procedure for Screen house experiment

Fig 3: EU0013_90S and JCM treated seedlings

Fig 4: Only JCM treated seedling

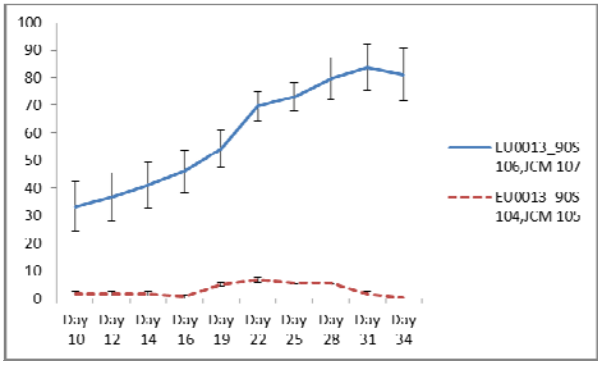


Fig 5: Effect of *Penicillium* sp. EU0013_90S and JCM application on the development of Fusarium wilt disease.

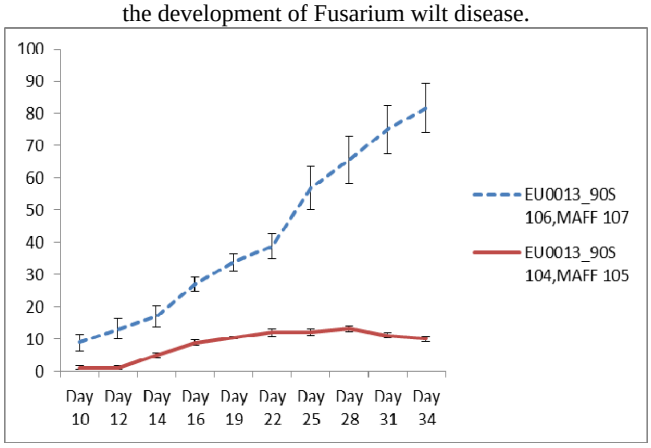
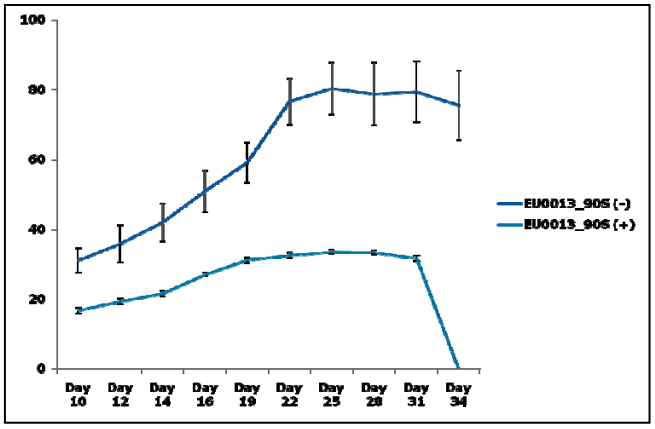


Fig 6: Effect of *Penicillium* sp. EU0013_90S and MAFF application on the development of Fusarium wilt disease.



(- sign denotes no BCA and + denotes treated with BCA

Fig 7: Effect of *Penicillium* sp. EU0013_90S application on the development of Fusarium wilt disease.

Table 1: Effect of EU0013_90S on the growth of Fusarium wilt pathogens on Potato Dextrose Agar (PDA) after 12 days of incubation.

Pathogens	Inhibition zone (mm)
Fusarium sp.(local isolate)	*2.1±0.12
Fusarium oxysporum f. sp *lycopersici* race 2 JCM	*2.4±0.41
Fusarium oxysporum f. sp *lycopersici* race 1 MAFF 305121	*2.1±0.35
Fusarium oxysporum f. sp *lycopersici* race 1 CU1	*1.3±0.5
Curvularia	*2.4±0.28

*Mean±SE

Table 2: Effect of different conidial concentrations of *Penicillium* sp. EU0013_90S on disease severity of tomato wilt caused by *Fusarium oxysporum* f.sp *lycopersici* race 1 MAFF 305121 under screen house conditions.

MAFF conidia ml^{-1}	EU0013_90S conidia ml^{-1}	Disease severity (Disease reduction)
1×10^7	1×10^6	41.7 * (3.62)
1×10^7	1×10^5	39.58 (8.52)
1×10^7	1×10^4	36.61 (15.39)
1×10^6	0	43.270
1×10^6	1×10^5	39.33 (16.03)
1×10^6	1×10^4	35.02 (19.06)
1×10^5	1×10^6	31.32 (27.61)
1×10^5	1×10^5	36.3 (16.10)
1×10^5	1×10^4	8.4 (80.58)

*letters in parenthesis indicate percent disease reduction relative to the pathogen inoculated plants only.

Table 3: Effect of different conidial concentrations of *Penicillium* sp. EU0013_90S on disease severity of tomato wilt caused by *Fusarium oxysporum* f.sp *lycopersici* race 2 JCM under screen house conditions.

JCM conidia ml^{-1}	EU0013_90S conidia ml^{-1}	Disease severity (Disease reduction)
1×10^7	1×10^6	59.92 *(4.93)
1×10^7	1×10^5	61.02 (3.18)
1×10^7	1×10^4	26.14 (58.52)
1×10^6	0	63.030
1×10^6	1×10^5	62.06 (1.60)
1×10^6	1×10^4	38.71 (38.58)
1×10^5	1×10^6	37.83 (39.98)
1×10^5	1×10^5	33.95 (46.13)
1×10^5	1×10^4	3.0 (95.24)

*letters in parenthesis indicate percent disease reduction relative to the pathogen inoculated plants only.

Conclusion and Recommendation

EU0013_90S application effectively reduced the severity of Fusarium wilt races in screen-house conditions. Application of EU0013_90S in the beginning of growing tomato seedlings before transplantation to the greenhouse or field will enable the early establishment of EU0013_90S in tomato roots, therefore decreasing the losses from the future arrival of Fusarium wilt pathogens.

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