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Effect of *Trichoderma* species to suppress *Fusarium oxysporum* f. sp. *cucumerinum* in rhizosphere of cucumber (*Cucumis sativus* L.)

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Abstract
The present investigation was conducted in the pot nursery under the polyhouse conditions. Artificial inoculation of *Trichoderma* isolates through different treatment combinations into soil pre-inoculated with *Fusarium oxysporum* f. sp. *cucumerinum* under pot culture study favoured greater proliferation of *Trichoderma* population in rhizosphere that resulted in drastic reduction of *F. oxysporum* f. sp. *cucumerinum* population. After 60 days of sowing, the local isolate, *T. viride* (Tv_5) reduced cfu (1.50×10^4 g^{-1} of soil) of the pathogen with the treatment seed biopriming (*Trichoderma*) + soil application (FYM + *Trichoderma*) which proved superior to the rest of the isolates. Under pot study, all the treatments with *Trichoderma* species reduced pre and post emergence mortality and increased root and shoot length. Pre and post emergence mortality reduced maximum with *T. viride* (Tv_5, local isolate), also, root and shoot length increased to the maximum with *T. viride* (Tv_5, local isolate), irrespective of the treatment.

Keywords: *Cucumis sativus*, *Trichoderma* spp., *Fusarium oxysporum* f. sp. *cucumerinum*, population dynamics, pre and post emergence mortality, growth

Introduction
Fusarium wilt of cucumber, caused by *Fusarium oxysporum* (Schlechtend: Fr.) f. sp. *cucumerinum* (Owen) Synder and Hansen, is manifested in young and mature plants throughout cucumber-growing regions (Owen, 1956) [20]. The disease is one of the major limiting factors for stable production of cucumber, since highly resistant cultivars have not been developed and effective control methods are not available. Accurate analysis of the population structure of the causal fungus would be helpful in developing effective control methods for this disease (Ahn *et al*., 1998) [1]. Of the several fungal antagonists tested, *Trichoderma* spp. have been extensively explored for the control of soil borne plant pathogens. They are reported to be frequently associated with both biocontrol activity and promotion of plant and root growth (Howell, 2003; Harman *et al*., 2004) [11, 10]. *Trichoderma* species, either added to the soil or applied as seed treatments, grow readily along with the developing root system of the treated plant (Harman, 2000) [11]. Rhizosphere competence is an important mechanism because a biocontrol agent cannot compete for space and nutrients if it is unable to grow in the rhizosphere. Among the desirable attributes of successful antagonists is its ability to produce inoculums in excess amount with different spore forms (Jash *et al*., 2003) [13] and to survive, sporulate, grow and proliferate in soil and the plant rhizosphere (Baker and Cook, 1974) [4]. Most of the studies revealed that native strains of different *Trichoderma* species are better mycoparasites than the commercial available formulations or antagonists. The present investigation was undertaken in which different *Trichoderma* isolates from Jammu region were evaluated against *Fusarium oxysporum* f. sp. *cucumerinum* in rhizosphere to observe the influence of different treatments on growth of cucumber plants and population dynamics of both the test pathogen and *Trichoderma* isolates in the rhizosphere.

Materials and Methods
The study was conducted in the pots nursery under polyhouse conditions. The pots were filled with 15-cm diameter plastic pots with soil, FYM and sand in the ratio of 2:1:1 previously sterilized by autoclaving at 15 psi for one hour consecutively for two days. *F. oxysporum* f. sp. *cucumerinum* was mass multiplied on pre-boiled and sterilized sorghum seeds supplemented with thoroughly mixed 5 percent anhydrous dextrose (Khan and Sinha, 2005) [15].

After 21 days, the colonized grains were used as inoculum in the pots. Soil was inoculated with the prepared inoculum at the rate of 10 g/kg soil before sowing. Infested pots were irrigated for 7 days before sowing. Twenty five cucumber seeds were sown (as per treatment) in each pot. Cucumber seeds used were surface-sterilized with 0.1% mercuric chloride solution for 30 seconds and rinsed in four successive changes of sterilized distilled water.

For evaluation of *Trichoderma* spp. in the pots, three *Trichoderma* isolates *viz*., *T. aureoviride* (Tau_2), *T. viride* (Tv_2) and *T. harzianum* (Th_1) which were selected from *in vitro* studies and also had high growth rates and one local isolate *T. viride* (Tv_5) were selected. *Trichoderma* isolates were evaluated individually through different treatment combinations which were as follows: Seed treatment (ST) with *Trichoderma*, Seed biopriming (SB) with *Trichoderma*, Seed treatment (ST) with *Trichoderma* + Soil application (SA) with *Trichoderma*, Seed treatment with *Trichoderma* + Soil application with (FYM + *Trichoderma*), Seed biopriming with *Trichoderma* + Soil application with (FYM + *Trichoderma*). Control and uninoculated control were also maintained for comparison. For control, uninoculated control and soil application treatments the seeds were soaked in sterilized distilled water for 30 minutes before sowing. The pathogen (*F. oxysporum* f. sp. *cucumerinum*) was inoculated in the potted soil 7 days before application of the *Trichoderma* species. Seed selection was done on the basis of seed germination test through blotter paper. The effect of starch on seed germination was also observed simultaneously which was done by dipping the apparently healthy seeds in 2 percent starch. No difference was evidenced in control and starch treated seeds in the germination test. Seed lot showing more than 85 percent germination under blotter paper test were used in the experimental study.

For seed treatment, *Trichoderma* spp. grown on Potato Dextrose Agar were flooded with sterile water to prepare spore suspension. The spore suspension was then adjusted to 1×10^7 spore ml^{-1} with the aid of a haemocytometer and by serial dilution. Cucumber seeds were surface sterilized and soaked in spore suspension of *Trichoderma* spp. Five gram of seeds were soaked in 50 ml of conidial suspension for one hour, air dried and sown in the *Fusarium* infested soil (Prasad *et al*., 1999) [21]. The seeds were bio-primed according to Jensen *et al*. (2004) [14] and El-Mohamedy *et al*. (2006) [8] in which cucumber seeds (surface sterilized) were treated with the spore suspension of *Trichoderma* spp. previously adjusted to the concentration of 1×10^7 spore ml^{-1} containing 2% solution of starch. The seeds were treated by mixing 5 g of seeds with 50 ml of conidial suspension. After 46 hours of treatment, the seeds were air dried in shade and stored at 25±2ºC for 24 hours in a self-sealing plastic bag before being tested under field conditions. The treated seeds were sown in the *Fusarium* sick soil in the pots. For soil application, inoculum of *Trichoderma* spp. multiplied on dextrose and sorghum grains was added to the soil at the rate of 5 g/kg of soil in each pot. The inoculum was mixed thoroughly with top 2-3 cm soil of the pots and nursery polybags (Nawar, 2007) [18]. The pots were watered for four days before sowing. Untreated surface-sterilized seeds were sown in the pots. In case of soil application with FYM + *Trichoderma*, *Trichoderma* isolates were first multiplied on Farm Yard Manure. Five hundred gram of *Trichoderma* isolates grown on sorghum grains (1×10^7 cfu/g) was mixed with 50 kg of Farm Yard Manure. The moisture of the organic medium was adjusted at 40% and it was covered with polythene sheet for 7 days. This was mixed thoroughly by giving turnings at two day interval and sufficient moisture was added (Saju *et al*., 2002) [23]. The mixture was then added to the pot soil at the rate of 40 g/pot before sowing of seeds in the pots filled with sick soil. Surface–sterilized cucumber seeds treated as in seed treatment were sown in the respective sick pots.

To determine the population of *Trichoderma* spp. and *F. oxysporum* f. sp. *cucumerinum*, soil samples from cucumber rhizosphere were collected by gently uprooting the plant and brushing the soil adhered to roots after 30, 45 and 60 days of sowing. Trichoderma selective medium (Shrestha, 1992) [25] and Peptone PCNB Agar (Nash and Synder, 1962) [17] were used for *Trichoderma* spp. and *F. oxysporum* f. sp. *cucumerinum*, respectively. The rhizosphere soil collected was thoroughly mixed and the rhizosphere population of *Trichoderma* and *F. oxysporum* f. sp. *cucumerinum* were estimated by serial dilution method (Aneja, 2002) [3]. Ten gram of soil sample was taken separately and suspended in 90 ml sterile distilled water and stirred well to get 1:10 dilution (10^{-1}). One ml from this suspension was transferred to a test tube containing 9 ml of sterile distilled water to get 1:100 dilution (10^{-2}). One ml from this suspension was transferred to a test tube containing 9 ml of sterile distilled water to get 1:1000 dilution (10^{-3}). Similarly 10^{-4} dilution was made and used for isolation of *Trichoderma* and *F. oxysporum* f. sp. *cucumerinum*. One ml of 10^{-4} dilution was added into sterilized petriplates. Fifteen ml of respective sterilized media were poured and each treatment was replicated thrice. Number of colony forming units (cfu) present in one gram of soil was calculated for both *Trichoderma* and the test pathogen. Watering was done on alternate day to ensure proper germination and plant growth. Data on both root and shoot length (after 35 days) were recorded.

Results and Discussion

Effect of different treatments of *Trichoderma* isolates on the population dynamics of *Trichoderma* species and *F. oxysporum* f. sp. *cucumerinum*

Application of *T. viride* (Tv_2) through different treatments, the population of *F. oxysporum* f. sp. *cucumerinum* dropped to 11.50 to 15.34 × 10^4 cfu g^{-1} of soil at 30 DAS as against 21.84 × 10^4 cfu g^{-1} of soil in control. Decrease in the population of *F. oxysporum* f. sp. *cucumerinum* continued and it dropped in the range of 6.50 to 9.50 × 10^4 cfu g^{-1} of soil as against 33.00 × 10^4 cfu g^{-1} of soil in control at 45 DAS. At 60 DAS, the population of *F. oxysporum* f. sp. *cucumerinum* was at lowest and ranged between 4.17 to 6.84 × 10^4 cfu g^{-1} of soil as against 41.50 × 10^4 cfu g^{-1} of soil in control on the same day (Fig. 1). Here again, the treatment SB (Tv_2) + SA (FYM + Tv_2) was the best in suppressing the population of *F. oxysporum* f. sp. *cucumerinum* upto 4.17× 10^4 cfu g^{-1} of soil, followed by treatments SB (Tv_2), ST (Tv_2) + SA (FYM + TV_2), ST (Tv_2) + SA (Tv_2), ST (Tv_2) and SA (Tv_2) with decrease in *F. oxysporum* f. sp. *cucumerinum* population upto 4.84, 5.17, 5.84, 6.17 and 6.84 × 10^4 cfu g^{-1} of soil on 60 DAS, respectively, as against 41.50 × 10^4 cfu g^{-1} of soil in control on the same day.

With the increase in the population of *Trichoderma* isolate, *T. aureoviride* (Tau_2), there was corresponding decrease in the population of the pathogen *F. oxysporum* f. sp. *cucumerinum*. The treatment SB (Tau_2) + SA (FYM + Tau_2) is the best

treatment in reducing the rhizosphere population of *F. oxysporum* f. sp. *cucumerinum*. It reduced the pathogen population to 8.50 after 30 DAS which further went down to 4.50 on 45th DAS and finally to as low as 2.17 × 10^4 cfu g^{-1} of soil on 60th DAS (Fig. 2). The least effective treatment in reducing the rhizosphere population of *F. oxysporum* f. sp. *cucumerinum* was SA (Tau_2) that record the pathogen population of 10.50× 10^4 cfu g^{-1} of soil at 30 DAS which dropped to 7.17× 10^4 cfu g^{-1} of soil on 45th DAS and finally to 4.50× 10^4 cfu g^{-1} of soil on 60th DAS as against 21.84, 33.00 and 41.50 × 10^4 cfu g^{-1} of soil with control on 30, 45 and 60 DAS, respectively.

With the increase in the population of *Trichoderma* isolate, *T. harzianum* (Th_1), there was corresponding decrease in the population of pathogen *F. oxysporum* f. sp. *cucumerinum* (Fig. 3). The effect of treatments on suppressing pathogen population have maintained a similar trend as was in case with *T. aureoviride* treatments. Here again, treatment SB (Th_1) + SA (FYM + Th_1), proved to be the best treatment in reducing the rhizospheric population of *F. oxysporum* f. sp. *cucumerinum*. It reduced the pathogen population to 12.84 × 10^4 cfu g^{-1} of soil on 30th DAS that further dropped to 7.17 on 45th DAS and finally to as low as 5.17 × 10^4 cfu g^{-1} of soil on 60th DAS as against 21.84, 33.00 and 41.50 × 10^4 cfu g^{-1} of soil with control after 30th, 45th and 60th DAS, respectively. The least effective treatment was SA (Th_1), which recorded rhizosphere population of 16.17, 9.84 and 7.84 × 10^4 cfu g^{-1} of soil on 30th, 45th and 60th DAS, respectively.

As compared to other treatments of *Trichoderma* isolates there is no change in the behaviour of different treatments of *T. viride* (Tv_5), a local isolate in suppressing the population of *F. oxysporum* f. sp. *cucumerinum*. Again the treatment SB (Tv_5) + SA (FYM + Tv_5) proved best and gave pathogen population count as low as 7.17 × 10^4 cfu g^{-1} of soil on 30th DAS which dropped to 4.34 on 45th DAS and finally to 1.50 × 10^4 cfu g^{-1} of soil on 60th DAS. The treatment SB (Tv_5) + SA (FYM + Tv_5) was followed by SB (Tv_5), ST (Tv_5) + SA (FYM + Tv_5), ST (Tv_5) + SA (Tv_5), ST (Tv_5) and SA (Tv_5) which gave corresponding rhizosphere population of the pathogen as 8.17, 8.50, 9.17, 9.34 and 9.84 on 30th DAS which dropped to 4.17, 5.17, 5.50, 5.84 and 6.50 × 10^4 cfu g^{-1} of soil on 45th DAS and finally dropped to low of 2.17, 2.50, 2.50, 2.84, 2.84 and 4.17 × 10^4 cfu g^{-1} of soil, respectively, on 60th DAS (Fig. 4).

The treatments through which bioagents (*Trichoderma* isolates) were applied to the soil resulted in increased rhizospheric population of *Trichoderma* species and decreased population of test pathogen *F. oxysporum* f. sp. *cucumerinum* as compared to control. For the management of any soil borne pathogen through biocontrol agent, rhizosphere competence is important because a biocontrol agent cannot compete for space and nutrients if it is unable to grow in the rhizosphere (Howell, 2003) [12]. The ultimate objective of any potential bio-agent is to destroy the primary source of pathogen inoculum in the soil and at the same time to increase its own population. In the present study also, all the *Trichoderma* isolates increased its own population in the soil and reduced the population of test pathogen though with varied degree. The resident *T. viride* (Tv_5) isolate showing superiority over other isolates in increasing its own population and suppressing the population of test pathogen in the present study also owe support from Vyas and Mathur (2002) [28] who advocated the use of local isolate of biocontrol agent recovered from naturally disease suppressive soils for achieving better results as compared to alien ones. Our results supported the earlier findings of Devika Rani *et al.* (2009) [7] who found that *T. viride* (1), an indigenous isolate, was superior in increasing the propagules of *T. viride* and reducing the population of *F. solani* in the soil.

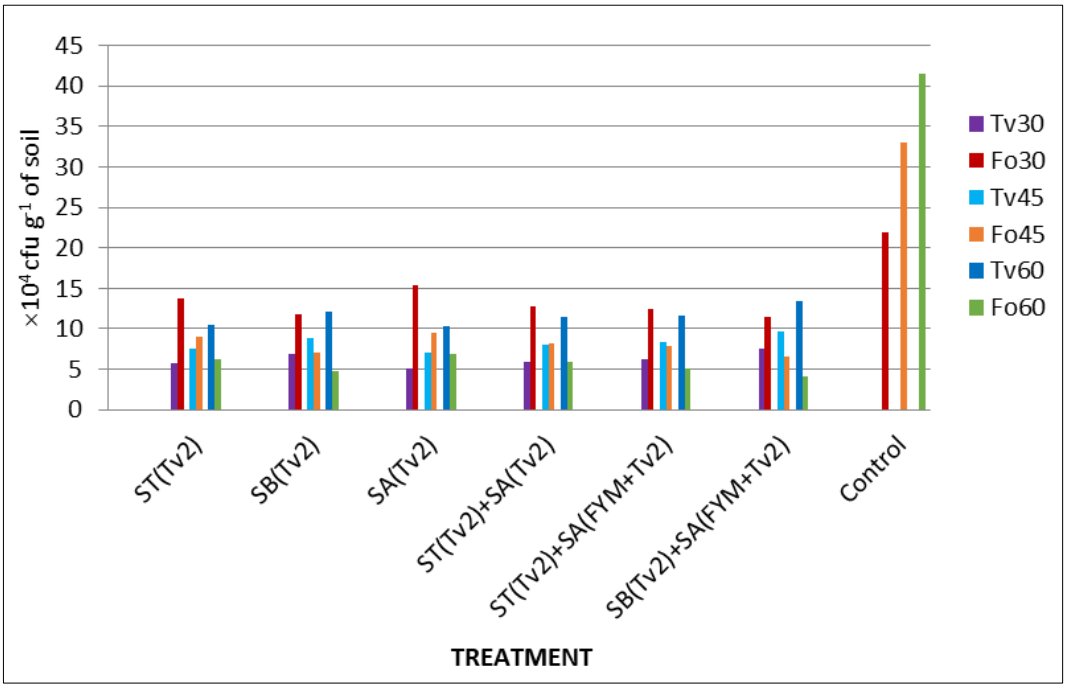

Fig 1: Population (×10^4 cfu g^{-1} of soil) of *T. viride* (Tv_2) and *F. oxysporum* f. sp. *cucumerinum* in cucumber rhizosphere at 30, 45 and 60 days after sowing

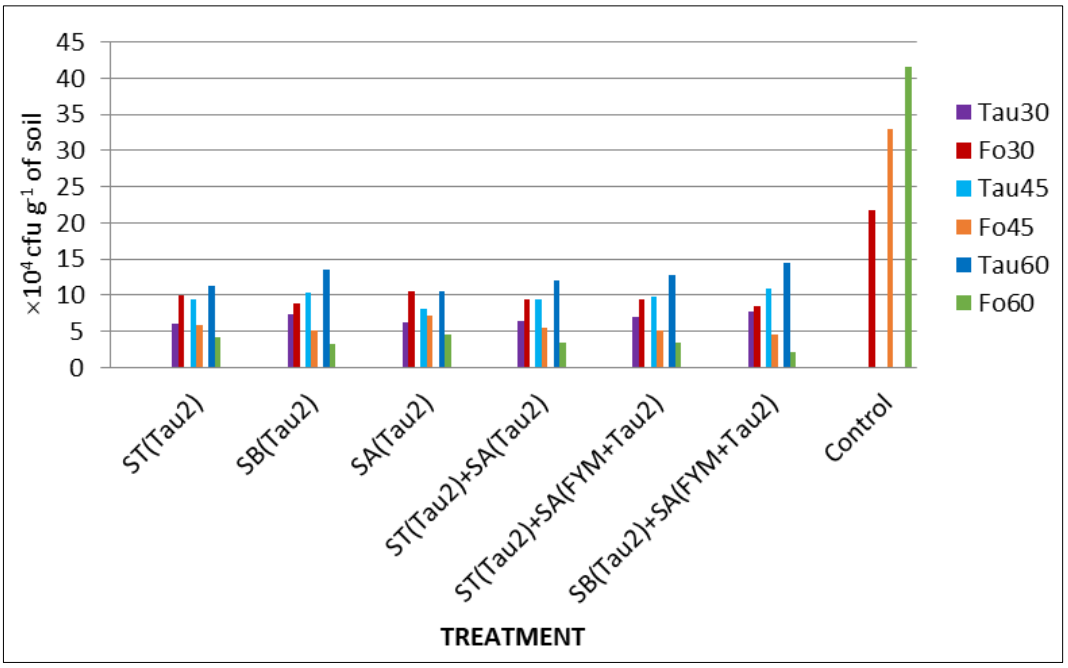

Fig 2: Population (×10^4 cfu g^{-1} of soil) of *T. aureoviride* (Tau_2) and *F. oxysporum* f. sp. *cucumerinum* in cucumber rhizosphere at 30, 45 and 60 days after sowing

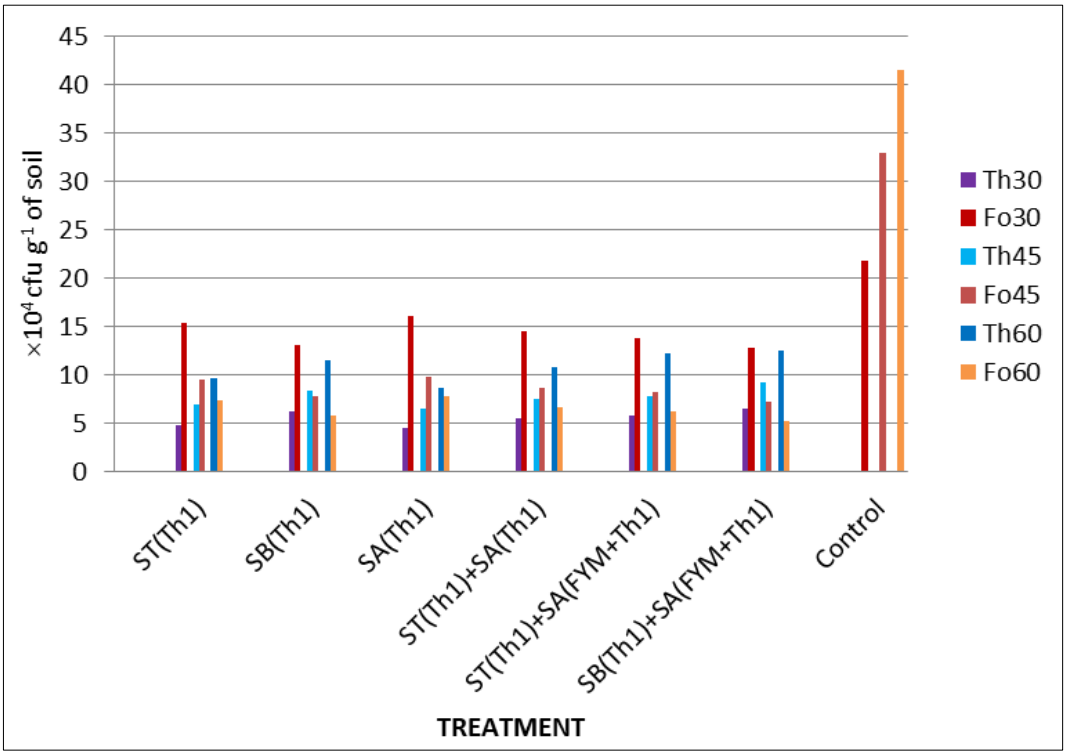

Fig 3: Population (×10^4 cfu g^{-1} of soil) of *T. harzianum* (Th_1) and *F. oxysporum* f. sp. *cucumerinum* in cucumber rhizosphere at 30, 45 and 60 days after sowing

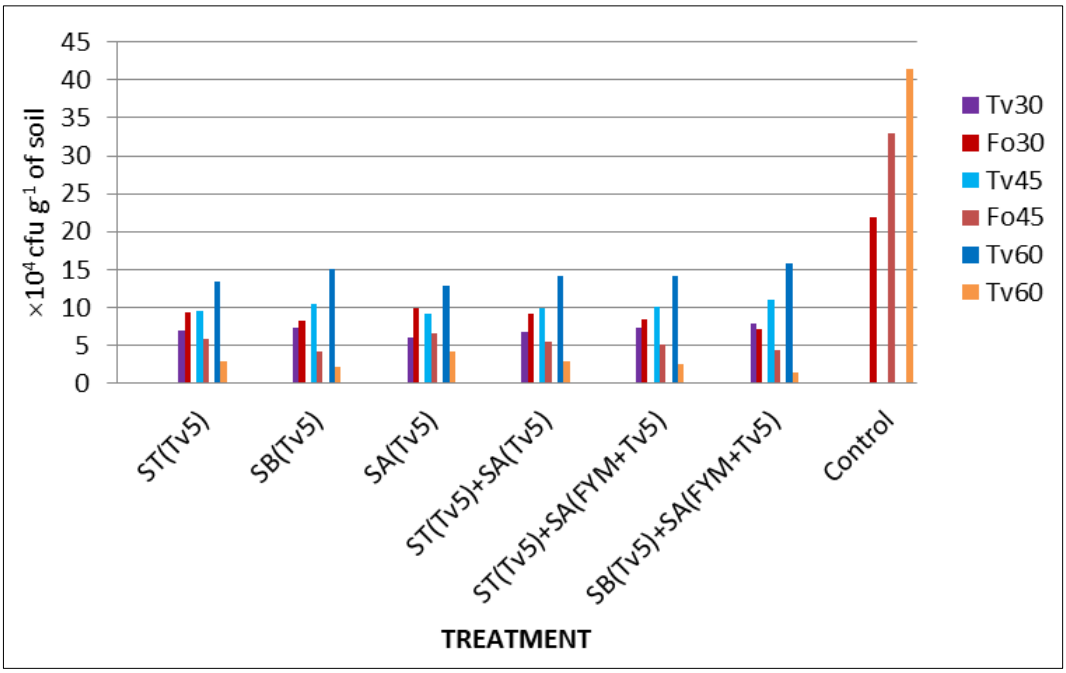

Fig 4: Population (×10^4 cfu g^{-1} of soil) of *T. viride* (Tv_5) and *F. oxysporum* f. sp. *cucumerinum* in cucumber rhizosphere at 30, 45 and 60 days after sowing

Effect of *Trichoderma* isolates on pre and post-emergence mortality and growth of cucumber seedlings

Pre-and post-emergence mortality

T. viride (Tv_2) treatments showed significant reduction on pre-emergence mortality as compared to control. Least pre-emergence mortality of 12.67 percent was observed with the treatment SB (Tv_2) + SA (FYM + Tv_2) as against 44.67 percent with control (Table 1). Whereas, treatment SB (Tv_2) + SA (FYM + Tv_2) is highly effective treatment in checking post-emergence mortality as the treatment recorded lowest post-emergence mortality of 11.34 percent as against 34.67 percent in control. In *T. aureoviride* (Tau_2) treatments, SB (Tau_2) + SA (FYM + Tau_2) is significantly superior treatment that reduced the pre-emergence mortality to 10.00 percent. Treatment ST (Tau_2) + SA (Tau_2) was followed by ST (Tau_2) and SA (Tau_2) which resulted in respective pre-emergence mortality of 27.34 and 32.00 percent as against 41.88 percent with control (Table 1). The treatment SB (Tau_2) + SA (FYM + Tau_2) noticed least post-emergence mortality of 8.67 percent. In *T. harzianum* (Th_1) treatments, SB (Th_1) + SA (FYM + Th_1) is highly effective in reducing pre-emergence mortality to 16.00 percent as against 44.67 percent with control. Highest pre-emergence mortality (44.67%) was observed in control (Table 1). Least post-emergence mortality of 14.00% was observed with treatment SB (Th_1) + SA (FYM + Th_1). In *T. viride* (Tv_5, local isolate) treatments, least pre-emergence mortality of 7.34 percent was obtained with treatment SB (Tv_5) + SA (FYM + Tv_5) as against 44.67 percent with control. Least post-emergence mortality (6.67%) was observed with the treatment SB (Tv_5) + SA (FYM + Tv_5) as against 34.67 percent with control (Table 1).

The treatments, Seed biopriming (*Trichoderma*) + Soil application (FYM + *Trichoderma*), Seed treatment (*Trichoderma*) + (FYM + *Trichoderma*) and Seed treatment (*Trichoderma*) + Soil application (*Trichoderma*) were most effective treatments and reduced pre and post-emergence mortality and increased root and shoot length of cucumber seedlings. Application of *Trichoderma* species through seed treatment and soil application individually proved inferior to other treatments. Among the four [*T. viride* (Tv_2), *T. aureoviride* (Tau_2), *T. harzianum* (Th_1) and *T. viride* (T v_5)] *Trichoderma* species evaluated, *T. viride* (Tv_5), a local isolate was superior in reducing pre- and post-emergence mortality and increasing root and shoot length of cucumber seedlings as compared to other three *Trichoderma* isolates tried under pot culture study. Decrease in pre-and post-emergence mortality and increased root and shoot length obtained through different treatments with *Trichoderma* species in the present study owe support from Akter *et al.* (2007) [2] who also obtained improved germination rate, higher shoot and root biomass, root and leaf area of cucumber on application of *Trichoderma*-based fertilizers.

Shoot and root length

T. viride (Tv_2) treatment, SB (Tv_2) + SA (FYM + Tv_2) enhanced shoot length upto 23.96 cm which was superior to other treatments. This was followed by SB (Tv_2), ST (Tv_2) + SA (FYM + Tv_2), ST (Tv_2) + SA (Tv_2), ST (Tv_2) and SA (Tv_2) which recorded 21.70, 20.40, 19.70, 18.76 and 17.09 cm shoot length, respectively (Table 2). The data marked significant increase in root length with treatment SB (Tv_2) + SA (FYM + Tv_2) and SB (Tv_2) which recorded 11.51 and 10.89 cm root length, respectively. These were followed by treatments ST (Tv_2) + SA (FYM + Tv_2), ST (Tv_2) + SA (Tv_2), ST (Tv_2) and SA (Tv_2) which enhanced root length by 10.09, 9.88, 9.78 and 8.01 cm, respectively as against only 6.32 cm in control. In *T. aureoviride* (Tau_2) treatments, SB + (FYM + Tau_2) and SB recorded enhanced shoot length of 27.56 and 26.91 cm, respectively. The next best treatments were ST+ (FYM + Tau_2), (ST + SA), ST and SA which gave shoot length of 23.47, 22.17, 19.15 and 18.31 cm, respectively, as against 12.36 cm with control and 16.04 cm in uninoculated control (Table 2). The treatment SB (Tau_2) + SA (FYM + Tau_2) induced maximum root length of 12.33 cm followed by SB (Tau_2) which recorded 11.36 cm root length. The treatments ST (Tau_2) + SA (FYM + Tau_2), ST (Tau_2) + SA

(Tau_2), ST (Tau_2) and SA (Tau_2) enhanced root length by 11.02, 10.59, 9.66 and 8.56 cm, respectively. Control and uninoculated control observed root lengths of only 6.32 and 6.57 cm, respectively (Table 2). In *T. harzianum* (Th_1) treatments, SB (Th_1) + SA (FYM + Th_1) and SB (Th_1) enhanced maximum shoot length by 20.90 and 19.89 cm, respectively. These were followed by treatment ST (Th_1) + SA (FYM + Th_1), ST (Th_1) + SA (Th_1), ST (Th_1) and SA (Th_1) which induced shoot length of 19.13, 18.25, 17.82 and 16.65 cm, respectively as against 12.36 and 16.04 cm with control and uninoculated control, respectively. Maximum root length of 10.68 cm was observed with treatments SB (Th_1) + SA (FYM + Th_1) followed by SB (Th_1) and ST (Th_1) + SA (FYM + Th_1) which recorded root length of 10.02 and 9.25 cm, respectively. The treatments ST (Th_1) + SA (Th_1), ST (Th_1) and SA (Th_1) enhanced root length by 9.05, 8.43 and 8.06 cm, respectively, as against control and uninoculated control that recorded root length of 6.32 and 6.57 cm, respectively (Table 2). In *T. viride* (Tv_5,local isolate) treatments, the highly effective treatment SB (Tv_5) + SA (FYM + Tv_5) enhanced the shoot length to the maximum of 32.09 cm followed by SB (Tv_5), ST (Tv_5) + SA (FYM + Tv_5), ST (Tv_5) + SA (Tv_5), ST (Tv_5) and SA (Tv_5) treatments which recorded 31.18, 29.67, 26.72, 23.64 and 20.83 cm shoot length, respectively, as against 12.36 and 16.04 cm in control and uninoculated control. The treatments SB (Tv_5) + SA (FYM + Tv_5) and SB (Tv_5) were superior by observing respective root lengths of 13.30 and 12.24 cm (Table 2). These were followed by treatments ST (Tv_5) + SA (FYM + Tv_5), ST (Tv_5) + SA (Tv_5), ST (Tv_5) and SA (Tv_5) that attained the root length of 11.85, 11.07, 10.41 and 9.99 cm, respectively, as against 6.57 and 6.32 in uninoculated control and control, respectively. Chet (1987) [6] also observed early germination and increased plant length, leaf area and dry weight of cucumber plants when conidia of *T. harzianum* were added to melon, tomato, cucumber, pepper, radish and bean seedlings. Later, Yedidia *et al.* (2001) [29] also reported that treatment of cucumber pants in soil with *T. harzianum* (T-203) resulted in large increase in root length, significant increase in dry weight, shoot length and leaf area over that of untreated control. Sultana and Ghaffar (2010) [26] also support our observations as they also reported reduced seedling mortality and root infection in cucumber by *T. harzianum, T. viride* and *G. virens.* In the present study, Seed biopriming (*Trichoderma*) + Soil application (FYM + *Trichoderma*) and Seed treatment (*Trichoderma*) + Soil application (FYM + *Trichoderma*) proved better treatments. It could be due to additive effect of FYM supplemented with *Trichoderma* species that might have resulted in better and faster multiplication and survival of *Trichoderma* species as biocontrol is primarily linked to sustained increase in active propagules of the antagonist. All the *Trichoderma* isolates which are amended with FYM must have proliferated quickly and got established in the soil. Earlier, Saju *et al.* (2002) [23] have also observed better growth and survival of antagonists in the soils amended with material like neem cake, farm yard manure and coir compost. Reese and Mandels (1959) [22] found *Trichoderma* to produce β-1, 3-glucanase when grown on such substrates as starch, cellulose, mannitol and laminarin. In our study, the seeds which were bioprimed with *Trichoderma* spore suspension were previously coated with starch that is why the treatments Seed biopriming (*Trichoderma*) + Soil application (FYM + *Trichoderma*) and Seed biopriming (*Trichoderma*) must have increased biological control activity of *Trichoderma* species over other treatments where seeds were not coated with starch prior to sowing. This suggest that compounds incorporated into seed treatment that are promotive to *Trichoderma* activity may have provided the substrate for increased production of lytic enzymes important for biological control activity. Nelson *et al.* (1988) [19] also reported that adding specific compounds to seed treatments provide conditions favourable for the growth and activity of seed-introduced *Trichoderma* spp. while at the same time creating a spermosphere environment unfavourable to the activity of seed-rotting *Pythium* spp. According to Mukhtar (2008) [16], seed germination was enhanced when okra seeds were coated with spore suspension of *T. viride, T. harzianum* and *T. koningii*, individually, supplemented with 2% starch as an adhesive. The improved efficacy of all the tested *Trichoderma* isolates with Seed biopriming may also be attributed to the good establishment and adherence of *Trichoderma* on the seeds in the presence of starch and can show positive effect on growth related parameters and prevent pathogen attack.

In the present study the treatments with *T. viride* (Tv_5), a local isolate proved much superior over other *Trichoderma* species in increasing root and shoot length and decreasing pre- and post-emergence mortality under pot culture study. It could be attributed to its better adaptability to the native soil where the experiment was laid. This view point is ably supported by Sen (2000) who also advocated use of indigenous isolates of bioagents to tackle endemic soil borne diseases through biocontrol programme due to its higher adaptability and acclimatization to the native soil environment and subsequently better proliferation and colonization within a short period of time as compared to exogenous isolates. Native strains of different *Trichoderma* species are better mycoparasites than the commercial available formulations or antagonists (Dubey and Patel, 2001) [8]. The superiority of seed treatment with biocontrol agent over soil application in suppressing the test pathogen *F. oxysporum* f. sp. *cucumerinum* in the present study was observed. Because in soil application of *Trichoderma* species, the bioagent first gets multiplied in the introduced environment and then move near to the root region to prevent the entry of the pathogen but in seed treatment, bioagent readily multiplies on the seed surface which, in turn, prevents the entry of the pathogen. This view point is also supported by Uma Maheshwari (1991) [27] who reported that seed treatment with *T. longibrachiatum* recorded less incidence of root rot as compared to furrow application in ground nut. The treatments in which FYM was used for multiplication of *Trichoderma* isolates and thereafter evaluated together with seed treatment and seed biopriming were superior over other treatments in increasing their own population and suppressing the population of the test pathogen under the pot culture experiments. Bhasker *et al.* (2007) [5] also reported effectivity of *T. harzianum* in combination with either FYM or neem cake in reducing root rot complex.

Palate 1: Mortality of cucumber seedlings

Table 1: Effect of different treatments of *Trichoderma* species on the mortality of cucumber seedlings under pot culture study

Treatment	Mortality (%)							
	Pre-emergence				Post-emergence			
	Tv_2	Tau_2	Th_1	Tv_5	Tv_2	Tau_2	Th_1	Tv_5
ST (Tau_2)	30.00 (33.17)^{bc}	27.34 (31.45)^{bc}	35.33 (36.45)^{b}	26.00 (30.60)^{b}	26.67 (31.03)^{bc}	25.34 (30.18)^{bc}	26.67 (31.06)^{b}	22.00 (27.95)^{c}
SB (Tau_2)	18.00 (24.98)^{e}	15.34 (22.98)^{e}	20.67 (26.93)^{d}	12.67 (20.73)^{d}	15.34 (22.83)^{de}	12.67 (20.73)^{e}	18.00 (25.02)^{d}	9.34 (17.71)^{e}
SA (Tau_2)	35.34(36.46)^{b}	32.00 (34.44)^{b}	39.34 (38.84)^{b}	30.67 (33.60)^{b}	32.00 (34.39)^{ab}	29.34 (32.75)^{b}	31.34 (34.02)^{a}	27.33 (31.44)^{b}
ST (Tau_2) + SA (Tau_2)	25.34 (30.10)^{cd}	23.00 (28.61)^{cd}	30.00 (33.20)^{c}	20.67 (26.93)^{c}	24.67 (29.72)^{c}	22.67 (28.35)^{c}	24.67 (29.75)^{bc}	18.00 (25.07)^{c}
ST (Tau_2) + SA (FYM + Tau_2)	22.00 (27.95)^{de}	18.67 (25.29)^{de}	26.00 (30.60)^{c}	15.34 (22.98)^{d}	19.34 (26.03)^{d}	17.34 (24.52)^{d}	21.33 (27.44)^{cd}	14.00 (21.93)^{d}
SB (Tau_2) + SA (FYM + Tau_2)	12.67 (20.73)^{f}	10.00 (18.35)^{f}	16.00 (23.53)^{e}	7.34 (15.62)^{e}	11.34 (19.54)^{e}	8.67 (17.07)^{f}	14.00 (21.93)^{e}	6.67 (14.63)^{f}
Control	44.67 (41.88)^{a}	44.67 (41.88)^{a}	44.67 (41.88)^{a}	44.67 (41.88)^{a}	34.67 (36.06)^{a}	34.67 (36.06)^{a}	34.67 (36.06)^{a}	34.67 (36.06)^{a}
Uninoculated Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SEm(±)	1.22	1.33	1.02	1.18	1.19	0.92	0.92	0.99
CD (P=0.05)	3.54	3.86	2.96	3.41	3.44	2.65	2.67	2.88

Figures in parenthesis are arcsine transformed values
*Figures followed by same letter are statistically at par.
ST= Seed Treatment, SB= Seed Biopriming, SA= Soil Application, FYM= Farm Yard Manure, Tv_2= *T. viride*, Tau_2= *T. aureoviride*, Th_1= *T. harzianum*, Tv_5= *T. viride* (local isolate)

Table 2: Effect of different treatments of *Trichoderma* species on the growth of cucumber seedlings under pot culture study

Treatment	Shoot length (cm)				Root length (cm)			
	Tv_2	Tau_2	Th_1	Tv_5	Tv_2	Tau_2	Th_1	Tv_5
ST (Tau_2)	18.76^{cd}	19.15^{c}	17.82^{cd}	23.64^{d}	9.78^{b}	9.66^{cd}	8.43^{c}	10.41^{c}
SB (Tau_2)	21.70^{b}	26.91^{a}	19.89^{ab}	31.18^{ab}	10.89^{ab}	11.36^{ab}	10.02^{ab}	12.24^{ab}
SA (Tau_2)	17.09^{de}	18.31^{cd}	16.65^{de}	20.83^{e}	8.01^{c}	8.56^{d}	8.06^{c}	9.99^{c}
ST (Tau_2) + SA (Tau_2)	19.70^{bc}	22.17^{b}	18.25b^{cd}	26.72^{c}	9.88^{b}	10.59^{bc}	9.05^{bc}	11.07^{bc}
ST (Tau_2) + SA (FYM + Tau_2)	20.40^{bc}	23.47^{b}	19.13^{bc}	29.67^{b}	10.09^{b}	11.02^{b}	9.25^{abc}	11.85^{b}
SB (Tau_2) + SA (FYM + Tau_2)	23.96^{a}	27.56^{a}	20.90^{a}	32.09^{a}	11.51^{a}	12.33^{a}	10.68^{a}	13.30^{a}
Control	12.36^{f}	12.36^{e}	12.36^{f}	12.36^{g}	6.32^{d}	6.32^{e}	6.32^{d}	6.32^{d}
Uninoculated Control	16.04^{e}	16.04^{d}	16.04^{e}	16.04^{f}	6.57^{d}	6.57^{e}	6.57^{d}	6.57^{d}
SEm(±)	0.75	0.79	0.58	0.66	0.45	0.40	0.51	0.41
CD (P=0.05)	2.17	2.29	1.69	1.92	1.30	1.15	1.47	1.19

Figures in parenthesis are arcsine transformed values
*Figures followed by same letter are statistically at par.
ST= Seed Treatment, SB= Seed Biopriming, SA= Soil Application, FYM= Farm Yard Manure, Tv_2= *T. viride*, Tau_2= *T. aureoviride*, Th_1= *T. harzianum*, Tv_5= *T. viride* (local isolate)

Conclusion

Research involving combinations of seed biopriming or seed treatment and soil application or FYM treated with *Trichoderma* against *Fusarium oxysporum* f. sp. *cucumerinum* revealed that among the selected treatments, combined treatments of seed biopriming along with FYM treated with *Trichoderma*, seed treatment along with FYM treated with *Trichoderma* and seed treatment along with soil application resulted in significantly higher growth parameters and less disease incidence as compared to seed treatment and soil application alone. Seed biopriming was also at par with the combined treatments. Highest population count of *Trichoderma* spp. and subsequently least population count of *F. oxysporum* f. sp. *cucumerinum* were recovered with seed biopriming along with FYM treated with *Trichoderma* when the tested *Trichoderma* isolates, *T. viride* (Tv_2), *T. aureoviride* (Tau_2), *T. harzianum* (Th_1) and *T. viride* (Tv_5), were evaluated individually after 30, 45 and 60 days of sowing. Seed biopriming along with FYM treated with *Trichoderma* was the best treatment in achieving least pre and post-emergence mortality. Maximum increase in root and shoot length was observed with the treatment seed biopriming along with FYM treated with *Trichoderma*. Also, amongst the various *Trichoderma* isolates evaluated, *T. viride* (Tv_5), a local isolate was proved superior while as *T. harzianum* was comparatively less effective.

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