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Biocontrol potentials of *Trichoderma harzianum, Glomus hoi*, and Neem extract on Fusarium wilt of Okra incited by *Fusarium oxysporum*

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ABSTRACT

Fusarium wilt, incited by Fusarium oxysporum, limits okra productivity. Eco-friendly control strategies involving botanicals and microbial agents offer reliable mitigation options. This study investigated the in vitro effect of Trichoderma harzianum and Neem extract on F. oxysporum and evaluated the effect of T. harzianum, Glomus hoi, and Neem extract on the growth and disease severity (DS) of Okra infected with Fusarium oxysporum. Aqueous extract of Neem leaf at varying concentrations was incorporated into molten PDA and allowed to set for in vitro experiments, while 5 ml of 10^5 spores/ml T. harzianum and F. oxysporum, 30 ml of neem extract (0.5%), and 30 g soil inoculum of G. hoi were applied to the soil surrounding the roots of okra plants in different treatment combinations in vivo. The least radial mycelia growth (RMG) of 1.6 cm was recorded when F. oxysporum was introduced 72 hours after T. harzianum in vitro. The highest RMG of F. oxysporum (3.2 cm) was observed in plates containing 0.1% Neem extract, while the least RMG was observed on plates containing 0.5% Neem extract. Application of T. harzianum at planting and at two- and four-weeks before F. oxysporum inoculation reduced the DS, while G. hoi application 4-weeks before F. oxysporum also reduced DS. Neem extract and T. harzianum effectively reduced DS of F. oxysporum in okra. Both treatments inhibited fungal growth in vitro, while G. hoi, neem extract, and T. harzianum lowered DS in vivo. Single and combined application of G. hoi and T. harzianum improved okra growth.

Keywords: *Botanicals, Biocontrol, Disease Assessments, Plant Disease Management.*

INTRODUCTION

Okra (*Abelmoschus esculentus (L.) Moench)* is a widely utilized species belonging to the family Malvaceae [1]. The vegetable is extensively consumed in India, but the crop is thought to have originated in Ethiopia, North-Eastern Africa. It is recognised as one of the earliest cultivated crops and is currently grown in numerous countries, with a wide distribution across Africa, Asia, southern Europe, and the Americas [2,3]. Over the years, the total area under cultivation has increased, and as such production has also increased, with India being the world's largest producer, followed by Nigeria and Sudan [3].

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In Nigeria, production was estimated to be around 1,807,284.29 tonnes [4]. Despite being an important vegetable, the production and productivity of the okra plant are constrained, resulting in a reduction of growth and yield potential of okra. These constraints include damages caused by insect pest infestation and infection by pathogens, resulting in diseases [5]. Okra plants are susceptible to a number of fungal diseases. Notable among the fungal diseases of okra in Africa are damping-off disease caused by *Rhizoctonia solani*, *Macrophomina phaseolina*, and *Pythium aphanidermatum*. Others important diseases include; vascular wilt incited by *Fusarium oxysporum*, Cercospora blight caused by *Cercospora abelmoschus*, and powdery mildew incited by *Erysiphe cichoracearum* and *Oidium abelmoschi* [2].

Fusarium wilt caused by *Fusarium oxysporum* is a devastating disease, of okra and commonly associated with regions where the crop is intensively cultivated. The pathogen was reported to cause damping-off and wilt in okra [6,7] with chlorosis and necrosis of the leaf margins as initial symptoms [7]. Severely diseased plants may die as seedlings, or if they survive, may often remain stunted throughout the season. Additionally, the vascular system of infected plants exhibits discoloration due to systemic infection of the fungus [8]. Okra is reported to be highly susceptible to *Fusarium oxysporum* in Nigeria, resulting in damping-off and wilt [6,7]. Upon infection, the pathogen invades the plant through the roots and progressively colonizes and blocks the vascular tissues. It obstructs the xylem vessels in the stem, thereby restricting the translocation of water to the aerial parts, including branches and leaves. This disruption leads to physiological starvation of the plant, ultimately resulting in reduced yield and deterioration in fruit quality [9,10].

The term "Biological control" has been used in plant pathology to describe the 'use of microbial antagonists in suppressing diseases, as well as the use of host-specific pathogens in controlling weed population' [11]. Biological control of Fusarium wilt disease has gained increasing attention as a sustainable and environmentally friendly disease management option because it offers a viable alternative to the use of chemicals and has led to the discovery of diverse mechanisms of plant protection mediated by selected beneficial microorganisms [12,13]. Any organism that possesses the ability to suppress pests or pathogens is known as a Biological Control Agent (BCA) [14]. In broad terms, this also includes the use of natural products extracted or fermented from various sources, and these natural products are termed "Botanicals" [14]. Examples of biological control agents are *Trichoderma* spp, *Glomus* spp, *Bacillus* spp, and *Pseudomonas* spp. [15], while botanicals include; *Azardirachta indica*, *Datura stramonium*, *Juglans regia* L. extracts, etc. The search for microbial antagonists has led to the discovery of highly effective fungal and bacterial agents with strong inhibitory activity [16]. For example, *Trichoderma* species have been reported in the management of soil-borne plant pathogens such as *Rhizoctonia solani* Khun., *Sclerotium rolfsii* (Sacc.) Curzi, *Pythium* spp and *Fusarium* spp. [17].

Trichoderma spp. are free-living fungi commonly found in soil environments and plant root ecosystems [11]. Over the past decades, considerable research efforts have been devoted to the mycoparasitic capacities of *Trichoderma* and their roles in enhancing plant health [18]. These investigations have revealed the disease suppressing abilities and plant growth enhancing potentials of *Trichoderma* spp. in the greenhouse and under field conditions, [19]. Additionally, *Glomus,* a genus of arbuscular mycorrhiza (AM) fungi, which forms symbiotic relationships (mycorrhizas) with plant roots have been associated with plant disease management. *Glomus*, being the largest genus of AM fungi, with 85 species described, is currently defined as non-monophyletic [20]. Species such as *G. facultative, G. hoi, G. mosseae, G. chappys,* etc. are found in this genus. The association of AM fungi with most agricultural crops and their roles in improving plant growth and yield is well documented [21]. Many disease management practices have been employed over the years in the control of *Fusarium* wilt. The use of fungicides has been found to be very effective. However, its toxic effects on not only target organisms but also non-target organisms, plants and their products, humans, and all other forms of life have been a major source of concern [4]. Alternative methods that are reliable and eco-friendly are now being explored with particular interest in microbial control agents and botanicals. However, limited information is available on the comparative and combined effects of *Trichoderma harzianum*, *Glomus hoi*, and neem extract on the suppression of *Fusarium oxysporum* wilt and growth response of okra under both *in vitro* and screenhouse conditions. This creates a need to evaluate these biocontrol agents individually and in combination as sustainable alternatives for managing Fusarium wilt in okra. It was hypothesized that early application of *T. harzianum*, *G. hoi*, and neem extract would reduce disease severity caused by *F. oxysporum* and improve the growth of okra plants. The present study investigated the *in vitro* effect of *T. harzianum* and Neem extract on *F. oxysporum*, evaluated the *in vivo* effect of *T. harzianum*, *Glomus hoi,* and Neem extract on the growth and disease severity of Okra plant infected with *Fusarium oxysporum.*

MATERIALS AND METHODS

Experimental Location

The *in vitro* experiment was carried out in the Mycology Laboratory, Department of Crop Production and Protection, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, whereas the *in vivo* study was conducted in the screenhouse of the same Faculty at Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

Source of Planting Material

The seeds of *Abelmoschus esculentus* were obtained from the Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Osun state, Nigeria.

Source of Inoculum

Pure cultures of *Fusarium oxysporum* and *Trichoderma harzianum* maintained in slant bottles were obtained from the Mycology Laboratory, Department of Crop Production and Protection, Obafemi Awolowo University, Ile-Ife. The isolates were sub-cultured onto Potato Dextrose Agar (PDA; HiMedia), prepared according to the manufacturer's instructions, and dispensed into sterile Petridish. Soil inoculum of *Glomus hoi* was also sourced from the same laboratory.

Preparation of Neem Extract

Neem leaf extract was prepared using a hot-water extraction procedure for both *in vitro* and *in vivo* experiments [11]. Briefly, 10, 20 and 50g of powdered neem leaf was soaked in 100 ml of hot water (80°C), overnight. The mixture was sieved through a muslin cloth to remove the residue while the filtrate was subsequently passed to filter paper (Whatman No 1). The filtrate (extract) was concentrated into different percentages viz-a-viz; 0.1%, 0.2% and 0.5% v/v kept at refrigerated condition until use.

In vitro effect of *Trichoderma harzianum* on *Fusarium oxysporum*

A 5 mm diameter mycelial disc, excised from the advancing margin of seven-day-old culture of both *T. harzianum* and *F. oxysporum* using a sterile cork borer, was inoculated on opposite sides of a calibrated petridish at equidistant from the periphery (4.5 cm apart). Radial mycelia growth of the organisms was measured and percentage inhibition was calculated accordingly using the following formula [11];

$$P_i = \frac{C-T}{C} X\ 100$$

P_i= Percentage inhibition
C= Radial mycelia growth of *T. harzianum* (mm)
T= Radial mycelia growth of test pathogen, *F. oxysporum* (mm).

In vitro effect of neem extract on *Fusarium oxysporum*

The plant extract was evaluated *in vitro* using the Poison Food Technique [22]. Two millilitres each of neem extract prepared at concentration of 0.1%, 0.3%, and 0.5% (v/v) was dispensed into 9 cm Petri dishes. Afterwards, 18 ml of molten Potato Dextrose Agar (PDA) was added to each plate to make up a final volume of 20 ml per plate. After solidification, inoculations were done with 5 mm mycelial plugs from a 7-day-old culture of *F. oxysporum* and were placed centrally in each of the petridish. The inoculated plates were incubated at room temperature, while the radial mycelia growth was recorded at 24 hours interval. The percentage growth inhibition was calculated using the following formula [23].

$$\text{Mycelial inhibition} = \frac{\text{Radial growth in control} - \text{Radial growth in treatment}}{\text{Radial growth in control}} X\ 100$$

Screenhouse Experiment

The potted experiment tested the effect of *A. indica, G. hoi* and *T. harzianum* in the management of Fusarium wilt disease of Okra. Twenty-three treatments (Supplementary Table 1) were applied and replicated three times. The experiment was arranged in a completely randomized design, with uninoculated and pathogen-inoculated controls included as control treatments.

Disease Severity (DS) Assessment

Assessment for DS was done following symptom development. Observed symptoms include vein clearing and dropping of petioles, chlorosis of lower leaves and subsequent wilt. Wilt severity was determined using a modified scale of [24].

Data Collection and **Statistical Analysis**

Following the application of treatments, data were collected on a weekly basis on plant height (cm), stem girth (cm), number of leaves (counts), and disease severity. The data collected were subjected to analysis of variance (ANOVA) using Statistical Analysis Software (SAS) version 9.1. Data in percentages and counts were square root transformed. Means were separated using Least Significant Difference (LSD). Microsoft Excel 2016 was used to plot charts and graphs.

Ethical Consideration

This study involved only plants and fungal cultures; therefore, human participant consent and animal ethics approval were not applicable.

Limitation of the Study

In vivo evaluation was conducted under screenhouse conditions, which may not perfectly fit growing conditions in the field.

RESULTS AND DISCUSSION

Glomus hoi, Trichoderma harzianum, and Neem extract influenced the growth of *Abelmoschus esculentus* and reduced the disease severity of *Fusarium oxysporum* in the *in vitro* and *in vivo* experiments.

In vitro* evaluation of *Trichoderma harzianum* against *Fusarium oxysporum

The *in vitro* evaluation of *Trichoderma harzianum* against *Fusarium oxysporum* showed that the radial mycelial growth of *F. oxysporum* decreased with time for the treatments where *T. harzianum* was inoculated on solidified PDA before *F. oxysporum* (T-F). The radial mycelia growth of *F. oxysporum* also increased with time in treatments where *F. oxysporum* was inoculated on PDA before *T. harzianum* (F-T). The highest radial mycelia growth (RMG) of *F. oxysporum* 5.0 cm was observed when *T. harzianum* was introduced 72 hours after *F. oxysporum* (F-T72), followed by a RMG of 4.8 cm when *T. harzianum* was introduced 48 hours after *F. oxysporum* (F-T48), then RMG of 3.6 cm when *T. harzianum* was introduced 24 hours after *F. oxysporum* (F-T24) (Figure 1). RMG of 3.6 cm was recorded for *F. oxysporum* when *T. harzianum* and *F oxysporum* were inoculated on the growth media at the same time (T-F0), followed by 2.4 cm when *F. oxysporum* was introduced 24 hours after *T. harzianum* (T-F24). The RMG for *F. oxysporum* of 1.6 cm was recorded when *F. oxysporum* was introduced 72 hours after *T. harzianum* (T-F72) in the petri dish (Figure 1).

T. harzianum showed an antagonistic effect against *F. oxysporum* when plated together in the same petridish. This was similar to a report by [15], where *T. harzianum* was able to overgrow a test pathogen when inoculated in the same plate. The highest percentage inhibition of 82.2% was observed when *T. harzianum* was present 72 hours before *F. oxysporum* (T-F72), followed by 81.6% when *T. harzianum* was present 48 hours before *F. oxysporum* (T-F48), followed by 53.85% when *T. harzianum* was present 24 hours before *F. oxysporum* (T-F24), and the least percentage inhibition of 35.7% was observed when *T. harzianum* and *F. oxysporum* mycelia disc were placed in the petri dish at the same time (T-F0) (Figure 1). This inhibition is associated with the early presence of *T. harzianum,* which facilitated the establishment of *T. harzianum* on the growth media before the introduction of *F. oxysporum.* This supported the report of [18], who described the mechanism of action of *T. harzianum* to include direct parasitism and competition.

In vitro* evaluation of Neem extract against *Fusarium oxysporum

The highest RMG of *F. oxysporum* (3.2 cm) was observed in a plate containing 0.1% Neem extract, followed by 3.0 cm in a plate containing 0.2%, while the least RMG (2.9 cm) was observed on plates containing 0.5% Neem extract. Although there are no significant differences between the three concentrations used, there was a significant difference between the treatments with Neem extract and the control plate with *F. oxysporum* only (F), which had a RMG of 5.0 cm (Figure 2). Consequently, 0.5% Neem extract was selected for use in subsequent trials involving Neem extract.

Greenhouse Experiments

Effect of treatments on the disease severity of infected okra

The mean disease severity used was square root-transformed percentage data. Treatments containing *Glomus hoi, Trichoderma harzianum,* and *Fusarium oxysporum* at planting (GlTF) had the least disease severity value of 1.58 compared with the control (*F. oxysporum* only, (F)), which had a disease severity value of 3.00. Among the treatments containing *T. harzianum,* the least disease severity value was recorded in treatments with *T. harzianum* and *F. oxysporum* at planting (TF0) and was followed by treatments with *T. harzianum* two weeks before inoculating the plant with *F. oxysporum* (TF2) and four weeks before inoculating the plant with *F. oxysporum* (TF4) (Figure 3). Although these treatments were not significantly different from each other, the treatments had reduced disease severity compared to the control F. This implied that the early presence of *T. harzianum* would reduce the disease severity of *F. oxysporum.* Among the treatments containing *Glomus hoi,* the least disease severity value was observed in the treatment containing *G. hoi* four weeks before *F. oxysporum* (GF4) compared to the control. This may be alluded to the symbiotic association that *G. hoi* forms with plant root [11].

Mycorrhiza is an association that benefits the plants because the fungus aids nutrient uptake, enhances water transport in the plant, and provides protection for the plant [11]. The early establishment of the arbuscular mycorrhizal fungus before the introduction of the pathogen would increase the chances of the plant to oppose the ingress of the pathogen. While treatment containing *G. hoi* and *T. harzianum* at planting (GlTF) had a reduced disease severity compared to *G hoi* four weeks before *Fusarium oxysporum* (GlF4), both treatments were significantly different from the treatment with *F. oxysporum* (Figure 4).

This is because *Glomus hoi* establishes a relationship with plant roots, not seeds; therefore, the presence of *T. harzianum* conferred the ability to ward off the ingress of *F oxysporum* while *Glomus hoi* is yet to establish a relationship with the emerging roots of the plant. Thus, offering the plant a form of protection while the mycorrhizal fungus builds up its structures.

Among the treatments containing 0.5% Neem extract, the least disease severity value was observed in the treatment containing 0.5% Neem extract, two weeks before inoculation (NF2), followed by NF4, where neem extract was introduced to the soil four weeks before inoculation with *F. oxysporum* (Figure 5). It was observed that the treatments FN2 and FN4, where *F. oxysporum* was introduced into the soil two weeks and four weeks before the introduction of neem extracts, respectively, were not significantly different from each other but were significantly different from the control. This showed that neem extract contains anti-fungal properties that fight the ingress of *F. oxysporum*. This is similar to a report by [6], who reported that neem leaves contain vital phytochemicals that are responsible for their antifungal and antimicrobial ability.

Effect of treatments on selected Okra growth parameters

Effect of treatments on the number of leaves and stem girth

There was no significant difference between the treatments the different treatments applied in this study for the number of leaves. This indicated that the number of leaves was not affected by the applied treatments. However, for stem girth at 3 weeks after planting (WAP), there were observed significant differences between the F and treatments with *Trichoderma harzianum* (T, TF2, TF4, and FT4 except for TF0 and FT2). There were also significant differences between F and treatments with *Glomus hoi* (GlF0, GlF2, GlF4, FGl2, and GlF4). Significant differences were also observed in treatments containing Neem extract (NF0, FN4, except NF2, NF4, and FN2). The treatment containing *G. hoi* two weeks before the introduction of *F. oxysporum* (GlF2) had the largest stem girth, and the treatment containing Neem extract and *F. oxysporum* at planting (NF0) had the least stem girth, although not significantly different from NGF and NTF (Figure 6a,b,c).

At 10 WAP, there was a significant difference between the F and treatments with *Glomus hoi* (GlF2, GlF4, FGl2, and FGl4 except GlF0) (Figure 6a,b,c). At the end of the experiment, the treatment with *G. hoi* had the largest stem girth, and this was significantly different from other treatments except GLF4 (Figure 6).

Other treatments with *G. hoi* (GLF2, FGL4, FGL2, and NGT) had larger stem girth compared to other treatments. This indicated that *G. hoi* played a significant role in improving the growth, especially the stem girth of the okra plant. This is in support of the report of [21] that Arbuscular mycorrhizal (AM) fungi form a symbiotic association that enhances the growth and yield of crops.

Effect of treatments on plant height

At 3 WAP, there were significant differences between F and other treatments with *Trichoderma harzianum*. In addition, there were significant differences between F and treatments with *G. hoi.* Significant differences were also observed in treatments containing Neem extract (NF0, NF4, FN2, and FN4 except NF2) (Figure 7a,b,c).

At the end of the experiment, treatment NGT had the highest height, although not significantly different from *Glomus hoi,* only GLF2, and GLF4 (Figure 7c). This displayed the ability of *G. hoi* to improve the growth of the okra plant. [25] reported phosphorus to be the most established nutrient uptake associated with mycorrhizal symbioses. Hence, okra plants inoculated with *G. hoi,* which is able to extract phosphorus and other nutrients, had increased growth with regard to height compared to other treatments.

CONCLUSIONS

This study showed that *Trichoderma harzianum* possessed an antagonistic effect against *Fusarium oxysporum* and that the early presence of *T. harzianum* inhibited the establishment of *F. oxysporum* in the *in vivo* and *in vitro* experiments. Also, the early presence of *Glomus hoi* helped the host (plant) in warding off the ingress of *F. oxysporum*. Neem extract inhibited *Fusarium oxysporum* in the *in vivo* and *in vitro* experiments. Hence, the penetration, colonization, and establishment of *F oxysporum* in the okra plant were suppressed by the presence of *G. hoi*, Neem extract, and *T. harzianum*. This study also showed that the application of *G. hoi* and *T. harzianum* enhanced the growth of the okra plant. Therefore, this study recommends the dual inoculation of okra seedlings with *T. harzianum* and *G. hoi* as it significantly reduced the disease severity of *F. oxysporum*. *Trichoderma harzianum* could be used as a seed treatment prior to sowing.

Declarations: Authors declare no conflict of interest.

Table 1: Mean square values from the analysis of variance of treatments applied on the number of leaves of Okra plants

Source of Variation	Degree of Freedom	Number of Leaves							
		3WAP	4WAP	5WAP	6WAP	7WAP	8WAP	9WAP	10WAP
Replication	2	0.001^{ns}	0.091^{ns}	0.034^{ns}	0.043^{ns}	0.022^{ns}	0.008^{ns}	0.017^{ns}	0.170^{ns}
Treatments	22	0.110^{**}	0.040^{ns}	0.048^{ns}	0.039^{ns}	0.065^{ns}	0.092^{ns}	0.038^{ns}	0.070^{ns}
Error	44	0.040	0.040	0.034	0.037	0.042	0.068	0.046	0.060
CV		10.06	9.560	8.310	8.760	9.170	11.920	9.710	11.910
R^2		56.22	36.44	42.56	36.23	44.26	40.63	29.87	39.34

WAP: Weeks after planting

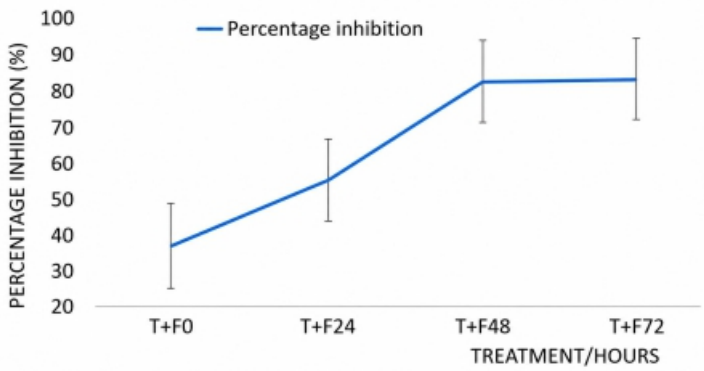

Figure 1: Radial mycelia growth inhibition of *Fusarium oxysporum* by *Trichoderma harzianum.* **F-T:** *Fusarium oxysporum* before *Trichoderma harzianum,* **T-F:** *Trichoderma harzianum* before *Fusarium oxysporum*

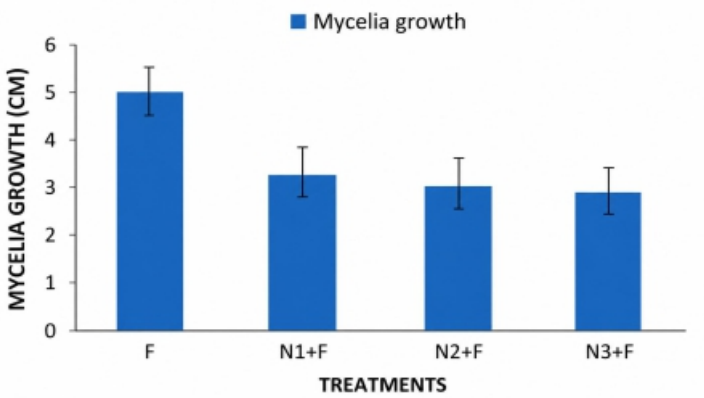

Figure 2: Percentage inhibition of *Fusarium oxysporum* by Neem extract at different concentrations. F: *Fusarium oxysporum,* N1; 0.1% Neem extract, N2; 0.2% Neem extract, N3: 0.5% Neem extract.

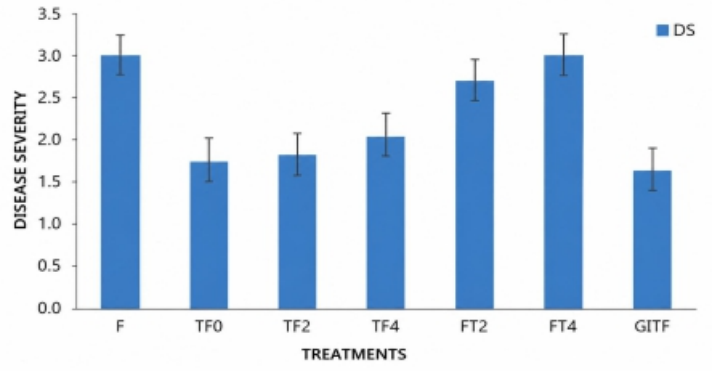

Figure 3: Average disease severity scores of okra plants inoculated with *Fusarium oxysporum* when treated *Trichoderma harzianum*. F: *Fusarium oxysporum*: *Trichoderma harzianum*, Gl: *Glomus hoi*

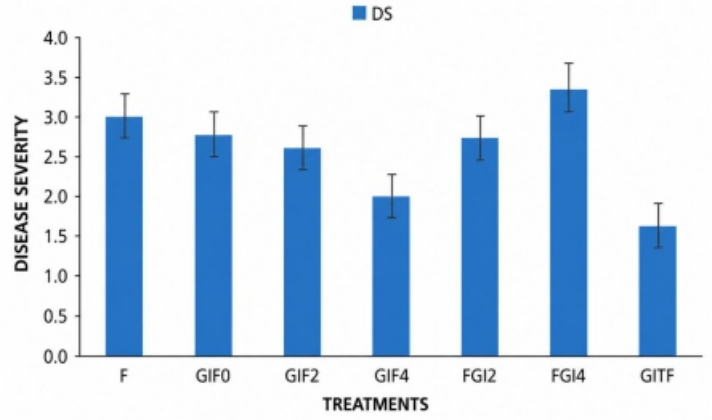

Figure 4: Biocontrol potential of *Glomus hoi* against *Fusarium oxysporum*. F: *Fusarium oxysporum*: Gl: *Glomus hoi*

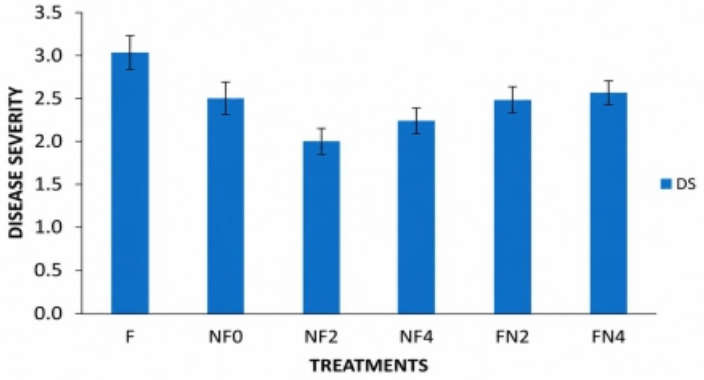

Figure 5: Biocontrol potential of Neem extract against *Fusarium oxysporum* at different time intervals. F: *Fusarium oxysporum,* N: neem extract.

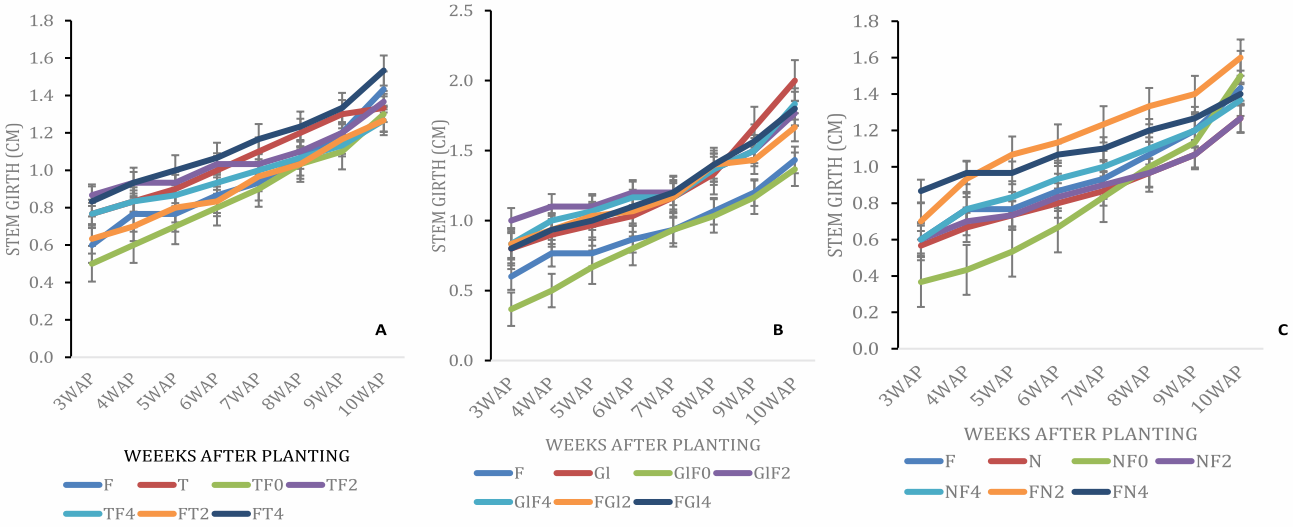

Figure 6: Response of *Fusarium oxysporum*- infected Okra stem girth to tested biocontrol agents (WAP: Weeks after planting)

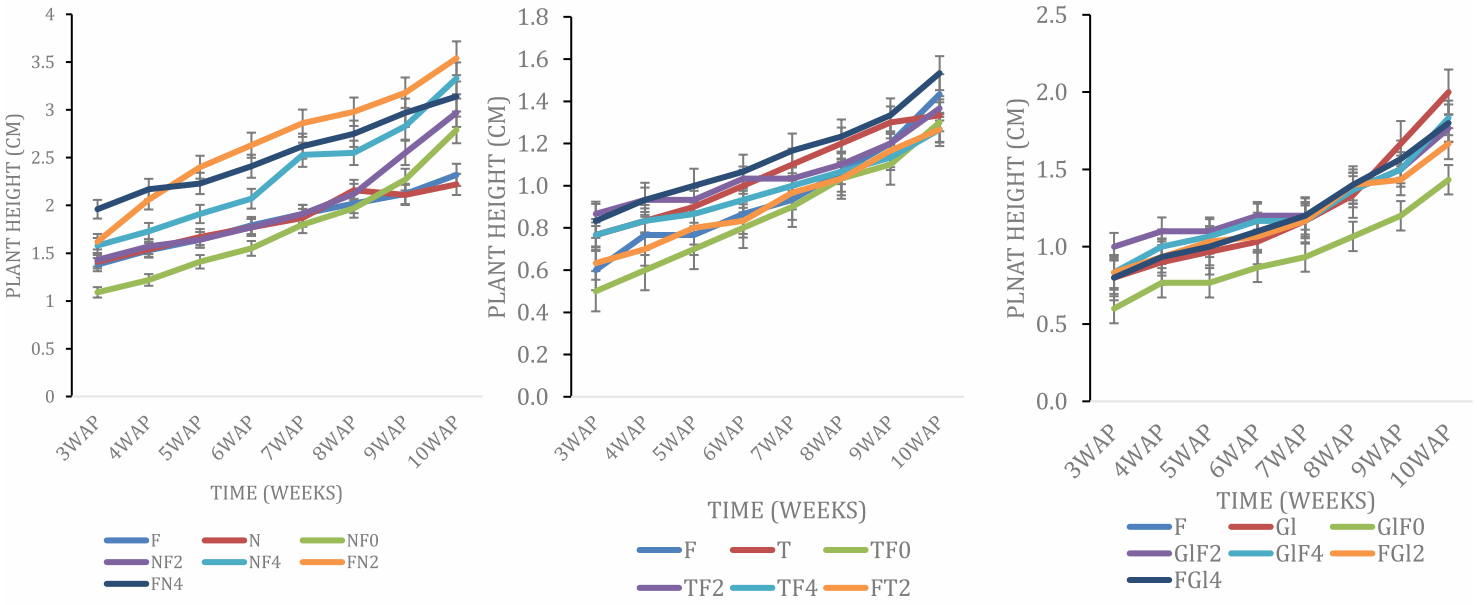

Figure 7: Response of *Fusarium oxysporum* infected Okra plant height to tested biocontrol agents (WAP: Weeks after planting)

Supplementary Table 1: Treatment combination for in vivo experiment

Treatments	Combination
T_1	Okra + *Trichoderma harzianum* at planting
T_2	Okra + *Glomus hoi* at planting
T_3	Okra + Neem extract at planting
T_4	Okra + *Fusarium oxysporum* at planting
T_5	Okra + *Trichoderma harzianum* + *Fusarium oxysporum* at planting
T_6	Okra + *Glomus hoi* + *Fusarium oxysporum* at planting
T_7	Okra + Neem extract + *Fusarium oxysporum* at planting
T_8	Okra + *Trichoderma harzianum* + *Fusarium oxysporum* at 2 WAP
T_9	Okra + *Trichoderma harzianum* + *Fusarium oxysporum* at 4 WAP
T_{10}	Okra + *Glomus hoi* + *Fusarium oxysporum* at 2WAP
T_{11}	Okra + *Glomus hoi* + *Fusarium oxysporum* at 4WAP
T_{12}	Okra + Neem extract + *Fusarium oxysporum* at 2WAP
T_{13}	Okra + Neem extract + *Fusarium oxysporum* at 4WAP
T_{14}	Okra + *Fusarium oxysporum* + *Trichoderma harzianum* at 2WAP
T_{15}	Okra + *Fusarium oxysporum* + *Trichoderma harzianum* at 4WAP
T_{16}	Okra + *Fusarium oxysporum* + *Glomus hoi* at 2WAP
T_{17}	Okra + *Fusarium oxysporum* + *Glomus hoi* at 4WAP
T_{18}	Okra + *Fusarium oxysporum* + Neem extract at 2WAP
T_{19}	Okra + *Fusarium oxysporum* + Neem extract at 4WAP
T_{20}	Okra + *Glomus hoi* + *Trichoderma harzianum* + *Fusarium oxysporum*
T_{21}	Okra + Neem extract + *Trichoderma harzianum* + *Fusarium oxysporum*
T_{22}	Okra + Neem extract + *Glomus hoi* + *Trichoderma harzianum*
T_{23}	Okra + Neem extract + *Glomus hoi* + *Fusarium oxysporum*

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