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Evaluation of Trichoderma Harzianum's Biocontrol Abilities against Rhizosphere Pathogens on Crop Plants

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Abstract: The purpose of study it to be *T.harizianum* as a biocontrol agent in agricultural field instead of chemical pesticides for sustainable farming practices. The importance of biological microscopic organisms like bacteria, fungi, protozoans and nematodes are one of the key entities in the process of nutrient cycle, and energy transfer in soil ecosystem. The use of chemical pesticides or fertilizer alters the biological diversity of soils. Biocontrol agents are prime important in soil ecosystems as well as reduce hazardous effects of chemical fertilizers. The pretreatment with *T.harzianum* increased seed germination and plant growth parameters, including shoot height, root length, the number of leaves total fresh weight and dry weight. The combined *T.harzianum* with pathogens *Fusarium oxysporium, A.niger* and *A.flavus* also decreased the disease index and reduced root rot and seedlings' death. The fungal pathogen *Fusarium oxysporium, A.niger* and *A.flavus* treatments significantly affected the growth and development of Okra, Fenugreek, Dolichos and those impact was significantly reduced with the administration of T.harzianum. Chlorophyll quantification study on Okra, Fenugreek and Dolichos plants was influenced by biotic stress in particularly fungal pathogens and treated plants, the reduction in chlorophyll concentration. *T.harzianum* pretreatment increased the chlorophyll pigmentation with or without *F.oxysporum, A.niger* and *A.flavus*.

***Keywords*:** *T.harzianum*, Biocontrol agent, Pathogen, Chlorophyll.

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1. Introduction

Persoon published the original report of Trichoderma genus in 1794, also Tulasne and Tulasne in 1865, hypothesized about the sexual status of a

species of Hypocrea. According to (Cook and Baker, 1983), Trichoderma is a typical soil fungus whose conidiophores end in phialides and (Doi and Doi, 1980) designated 63 species under this genus in addition to assigning some anamorphs of the genus Hypocrea to Verticillium and Gliocladium due to their shared characteristics of retaining conidiophores on irregular branches and elongated phialides (Bissett, 1984), Trichoderma species can be divided into five groups, including Longibrachiatum, Trichoderma, Pachybasium, Saturnisporum, and a brand-new category called Hypocreanum.

Preserve the environment and advance sustainable agriculture, there is a persistent call for a reduction in the use of chemical-based fertilizers and fungicides in the agricultural sector. The use of beneficial microorganisms as inoculants for bio fertilization and biocontrol is now increasing. It has been utilized to boost plant crop development while reducing environmental impact by treating seeds or seedlings with both biofertilizers and biocontrol systems (Kumar and Sindhu *et al*., 2022). A filamentous fungus with various uses in agronomy and the environs, Trichoderma is among the most investigated genera (Mukherjee *et al*., 2013; Joo and Hussein 2022). In agronomic soils and forest soils all over the world, there are several species of Trichoderma (Bitas *et al*., 2013). According to (Contreras-Cornejo *et al*., 2016), these species actively engage in interactions with plant roots and rhizosphere microbes.

Trichoderma may mitigate agrochemical pollution, prevent disease, encourage plant development, increase plant resilience, and improve nutrient utilization efficiency (Tilocca *et al*., 2020; Fontana *et al*., 2021; Sánchez-Montesinos *et al*., 2021; Al-Surhanee, 2022; Tyśkiewicz *et al*., 2022). Many investigations have demonstrated that the majority of Trichoderma spp. are capable of producing compounds that are bioactive and have antagonistic effects on nematodes and fungi that lead to plant diseases (Druzhinina *et al*., 2018). These biologically active substances, which include cell wall-degrading enzymes and secondary metabolites, can effectively increase crop resistance, minimize plant diseases, and stimulate development of plants.

The main biological control mechanisms employed by Trichoderma spp. to deal with pathogens involve (a) recognition and invasion of fungal-like plant pathogens through cell wall disruption and absorption of released nutrients known as mycoparasitism (Bhat, 2017), (b) achieving plant disease resistance through modifying root architecture during interactions with pathogens (Pandey and Senthil-Kumar, 2019), and (c) attacking root-knot and cyst nematodes by destroying nematode eggs and second phase juvenile, also some segment of adult nematodes (Heidari and Olia, 2016) .

The aim of this study is to evaluate the biocontrol efficiency of *T.harzianum* against F.oxysporum, *A.niger* and *A.flavus* soil born pathogenic fungi. Different combination of *T.harizianum* with pathogens and also with three selected plant species like Okra (*Abelmoschus esculentus* L.), Fenugreek (*Trigonella foenum-graceum* L) and Dolichos Bean (*Lablab purpureus* L.) their antagonistic effects, disease manifestations on plants; seed germinations, growth and developmental activities on plants of these fungal entities.

2. Materials and Methods

The present study evaluates the biocontrol and bio fertility activity of fungal isolate *T.harzianum*, and it's effect against the different fungal pathogens like *F.oxysporum, A. niger* and *A. flavus* in crop plants like Okra (*Abelmoschus esculentus L.*), Fenugreek *(Trigonella foenum-graceum L.)* and Dolichus bean*(Lablab purpureus L.)* Experiments were designed in order to know the different growth performance of the selected plant species.

2.1. Fungal Isolates

The pure culture of *T.harzianum* and pathogens strains *F. oxysporum*, *A.niger, A. flavus* collected from Department of Plant pathology Horticulture College, University of Horticultural Sciences, Bagalkote, Karnataka ,India.

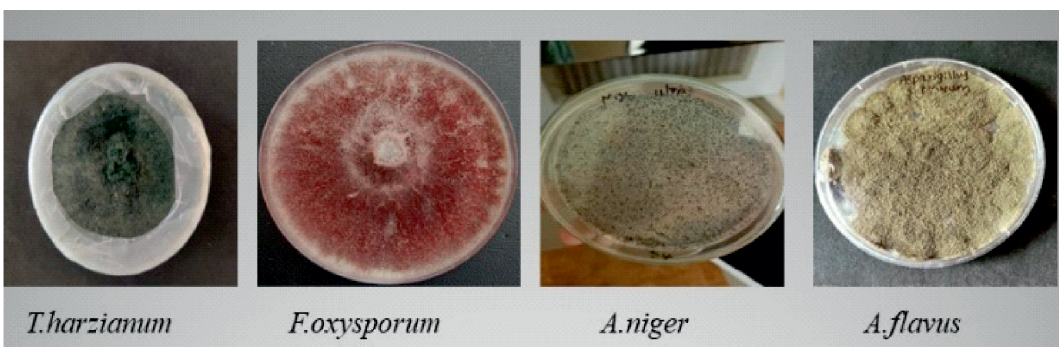

Figure 1: Pure cultured fungal strains-*T.harzianum; F. oxysporum; A.niger; A. flavus*

2.2. Medium and Culture

To culture fungal strains Potato Dextrose Agar (PDA) is more suitable and easily prepared. The components of PDA media are; (potato 200 g, Dextrose 20g, and agar 15 g).Take 200 gm of potato for 1L of PDA media preparation. Wash the potato to remove dirt, peel off the skin and dice them. Add the pieces to 1 L of distilled water, boil for 20-25 min on a hot plate. Collect the broth

through the muslin cloth in a conical flask. Add 20 gm of Dextrose and 15 gm of Agar to the potato broth and autoclave for 15 min at 15 PSI on liquid cycle. The sterilized media is ready for microbial work.

2.3. Fungal Inoculation Preparation

T.harzianum, as well as pathogenic fungi *F.oxysporum, A.niger, A.flavus*, were further grown on PDA for 7 and 14 days at 25°C, respectively. For spore suspension culture preparation, add 1 ml of sterilized distilled water to the culture plates and the spores were dispersed gently scraped using sterile spatula. Transfer the mixture to the conical flask containing 100 ml sterilized distilled water (Stock solution). This solution is used for further serial dilution preparation.

2.4. Dual-Culture Assay of Antagonistic Fungi and Pathogens

An alternate culturing approach was developed for the antagonistic assay. The inhibitory effects of the biocontrol fungus isolate against(Rahman *et al*., 2009) examined *F.oxysporum*, *A.niger*, and *A.flavus* in vitro utilizing a dual-culture plate method under modified circumstances. The research was carried out with a colony-diameter growth approach on PDA media. Using the dual-culture approach, mycelial pads (diameter in 5 mm) were parallel transferred to a PDA plate in an opposite sides of 1.5 cm from the plate's edge, from the cultures of *F.oxysporum* (14 days old), *A.niger*, *A.flavus* (7 days old), and *T.harzianum* (7 days old). The only organisms utilized as controls in the entirely randomized experiment were F. oxysporum, *A. niger,* and *A. flavus*. Each set consisted of two duplicates. After the inoculation, the plates were incubated for seven days at 25ºC. After seven days, the pathogens (F.oxysporum, A.niger, and A. flavus) in the treatment and control groups were compared in terms of growth distance. After that, the antifungal effect was calculated with a minor change to the formula presented by (Lu ZhiXiang *et al*., 2020). The following formula was used to get the inhibition rate:

$$\text{Inhibition rate (\%)} = \frac{(\textit{Average diameter of the control} - \textit{Average diameter of Treatments})}{(\textit{Average diameter of the control} - 0.5)} \times 100$$

Where the growth distance of the pathogen colony is denoted by the control, and the growing distance of the pathogens in the course of action dual cultures is denoted by the treatment. In the meantime, the mycelium plug's diameter can be seen by 0.5.

3. Effects of T.harzianum on Dolichos bean, Fenugreek and okra seeds under A.niger, A.flavus and F.oxysporum infections

Dolichos bean, Fenugreek and Okra seeds of relatively uniform size were thoroughly sterilized with 5% Formaldehyde for 5 min and washed with sterile distilled water 4-5 times. Afterwards the seeds were soaked in a fungal spore suspension (10^6 spores per ml) and the control was soaked in distilled water. According to the method of (Zhang XiangXiang *et al*., 2015), seeds were air-dried overnight under aseptic conditions before being sown. The soil used in the experiment was obtained from a field. The experiment was set up in a completely randomized manner with two controls: plants inoculated with *A.niger*, *A.flavus* and *F.oxysporum* (positive control) and plants inoculated with sterile water only (negative control). The treatments are detailed in Table1.

Table 1: The selected Bio-control and pathogens and their combinations in this study

Group	*Name*	*Treatment*
T0	Negative control	Distilled water
T1	Positive control	*Fusarium oxysporum*
T2	Positive control	*Aspergillus niger*
T3	Positive control	*Aspergillus flavus*
T4	T. *harzianum*	*Trichoderma harzianum*
T5	T. *harzianum* + *F.oxysporum*	Inoculation of strain T. *harzianum* +F.*oxysporum*
T6	T. *harzianum* +*A.niger*	Inoculation of strain T. *harzianum* +*A.niger*
T7	T. *harzianum* +*A.flavus*	Inoculation of strain T. *harzianum* +*A.flavus*

For each treatment, two plastic glasses, each containing 100 g of sterile soil was used. Seeds of equal size were planted 1 cm deep in the soil and gently covered. The plants were watered every 24 h, kept at a constant temperature of 25º C with supplemental day/night lighting of 16/8 h, and a relative humidity of 65%. Seedling germination was determined using the method described by Oluwaranti *et al*. (2015), and the percentage of seed germination potential (GP %) was determined as follows:

G.P% = {(seedlings germinated after 3 days)/seeds planted} × 100

The calculated formula of germination rate [GR (%) = (NGS/TNS) X100], where NGS stands for the number of seeds that germinated five days after planting and TNS for the total number of seeds placed in each glass. The calculation of germination index is [GI (%) = NGSi/Ti× 100], where the number of seeds that germinated at a given time (NGSi) and the incubation period (Ti), based on the computation of (Niu *et al*., 2013).

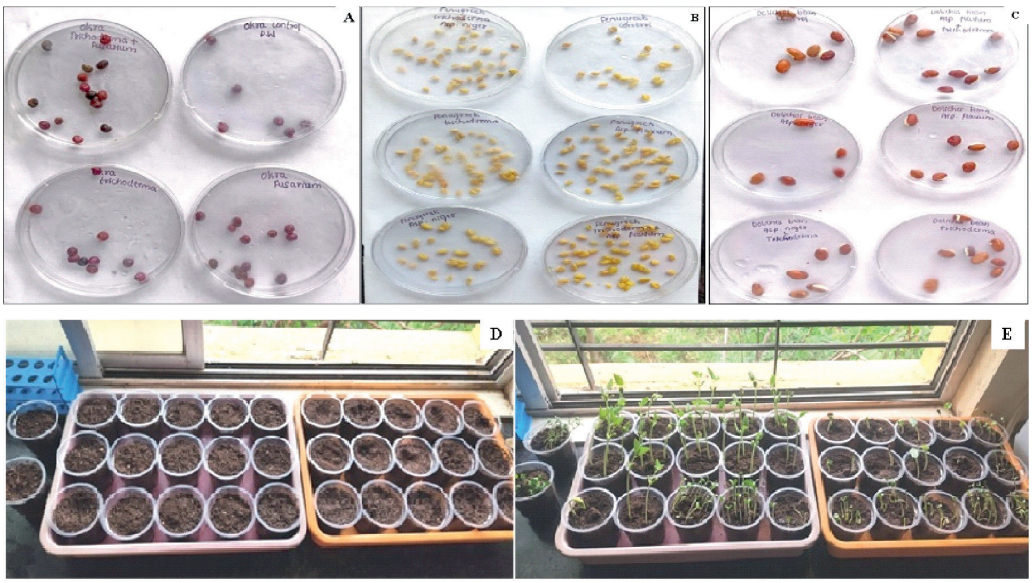

Figure 2: Seeds are pretreated with different fungal strains; A- Okra seeds; B- Fenugreek seeds; C- Dolichos seeds- Pretreated seeds are sown- Germinated seeds after 4 days

3.1. Growth Parameters

The Dolichos, Fenugreek and Okra seedlings were harvested 21 days after treatment. The shoots and roots of the seedlings were removed, washed three times with distilled water, dried, and weighed. The length and weight of the shoots and roots were determined by the use of measuring tape and weighing balance. The shoots and roots were oven-dried at their dried weight at 105ºC for 30 min, and then kept at 80ºC to ensure a steady weight before being weighed. Each preservation and control procedure was carried out three times.

3.2. Disease Assessment

The five-point rating system is used to measure disease. Twenty-one days following treatment, the root rot disease index was calculated. A disease index based on root rot, yellowing, and chlorosis of leaves and cotyledons at 21 days was used to describe the disease symptoms. (Zhang *et al*., 2015) described a 5-point rating system (0–5) for disease severity, where 0 represents no disease, 1 represents a trace to 10% of the roots were rotted, 2 represents 11–25% of the roots were rotted, 3 represents 26–50% of the roots were rotted, 4 represents 51–75% of the roots were rotted, and 5 represents 76–100% of the roots were rotted. The formula below was used to calculate the DI:

DI (%) = [Σ (number of diseased plants × disease index) / (total number of plants investigated ×highest disease index)] × 100

3.3. Determination of Chlorophyll Content

Chlorophyll was extracted with Acetone using the method described by (Miazek and Ledakowicz, 2013). The fresh leaves of Dolichos bean, Fenugreek and okra seedlings weighing 0.2 g were ground to a powder using acetone and it is homogenized to 10 ml. Centrifuge at 5000 rpm for 5 minutes. Take the supernatant to read the absorbance of the solution. The chlorophyll content was measured using a dual-wavelength spectrophotometer at 663 and 645 nm absorbance.

3.4. Colony morphology of Fungal isolates

Colony morphology refers to the observable characteristics of a fungal colony grown on a solid agar medium. These characteristics can provide valuable information about the type of fungus and its growth conditions. The colony morphological characteristics were noted and documented (Table 2).

Table 2: Colony characteristics of different fungal stains

SL. No.	*Fungal Strains*	*Media Nature*	*Colony Morphology*	*Colony Colour*	*Colony Diameter(Cm)*
1.	T. harzianum	Solid	Oval	Green	3.5
2	F.oxysporum	Solid	Round	Red	2.6
3	A.nigar	Solid	Round	Black	1.8
4	A.flavus	Solid	Oval	Pale green	3.3

4. In Vitro Colony Growth Inhibition of *T.harzianum on F.oxysporum, A.niger* and *A.flavus*

A dual culture method was adopted in order to know the degree of inhibition of Trichoderma with Pathogens. The colony growth of *F.oxysporum, A.nige*r and *A.flavus* was significantly inhibited by *T.harzianum* on different days succeeding incubation. The average inhibitory rates of *T.harzianum* on *F.oxysporum* were 58.13% and the inhibitory rates of *T.harzianum* on *A.niger* and *A.flavus* were 25.5% and 58.4% respectively on 7th day compared to 1st day and 3rd day incubation at 25^{o}C (Table 3).

Table 3: In vitro colony growth inhibition of *T.harzianum* against selected pathogens

1st day observation					
Sl. No	*Fungal Strains*	*Nature of media*	*Diameter of the colony (cm)*		*Inhibition rate %*
			T.harzianum	*Pathogens*	
1	T.harzianum+F.oxysporum	Solid	1.3	0.7	75.00
2	T.harzianum+A. niger	Solid	1.5	0.6	90.00
3	T.harzianum+ A. flavus	Solid	1.2	0.9	42.85
3rd day observation					
1	T.harzianum+F.oxysporum	Solid	2.3	0.7	88.88
2	T.harzianum+ A.niger	Solid	2.6	1.8	38.09
3	T.harzianum+ A. flavus	Solid	2.1	2.0	6.25
7th day observation					
1	T.harzianum+F.oxysporum	Solid	4.8	2.3	58.13
2	T.harzianum+A.niger	Solid	4.5	3.7	25.58
3	T.harzianum+ A. flavus	Solid	5.8	2.7	58.49

5. Effects of *T.harzianum* on Okra, Fenugreek and Dolichous bean Seeds Germination under *F.oxysporum, A.niger* and *A.flavus* Infections

The effect of *T.harzianum on* selected plants such as Okra, Fenugreek, and Dolichos were studied in laboratory conditions using sterilized soil. The characters were observed periodically and documented: results were illustrated**.** Seeds were pretreated with *T.harzianum* stimulated seed germination. The germination rate (GR), germination potential (Lu *et al*., 2020), and germination index (GI) increased respectively in comparison with pathogens and sterile water treatment. However, compared to the control, *T.harzianum* increased the GP, GI, and GR by 82%, 17.04% and 82% in okra and in Fenugreek it is increased by 93.3%(Lu ZhiXiang *et al*., 2020), 58.3%(GI), 93.3% (GR) and in Dolichos bean it is increased by 88%(Lu ZhiXiang *et al*., 2020), 17.04%(GI), 88% (GR).

The *F.oxysporum* treatment of the seeds reduced the GP, GI, and GR in Okra by 40%, 8.3%, and 40%, respectively, in comparison to the control. The

GP, GI, and GR were reduced in fenugreek by 54.6%, 34.1%, and 54.6% in the *A. niger* treatment, and in Dolichos by 61.3%, 38.3%, and 61.3% in the *A. flavus* treatment, respectively. *A.niger* treatment also decreased the GP, GI, and GR by 44% (Lu ZhiXiang *et al*., 2020), 9.13% (GI), and 44% (GR) in comparison to the control **(Fig.3.).**

5.1. *Effect of T.harzianum and Fusarium on Okra*

T.harzianum increased the length of shoot and root by 18.75cm and 8.43cm respectively, total fresh weight of shoot and root by 2.49gm and 0.36gm respectively as well as dry weight of shoot and root by 1.24gm and 0.18 gm respectively compared to the control. The pathogen (*F.oxysporum)* treated plant decreased the length of shoot and root by 13.47cm and 2.12 cm respectively, total fresh weight of shoot and root by 1.4gm and 0.07 gm respectively, similarly dry weight of shoot and root by 0.7gm and 0.03gm respectively as compared to *T.harzianum* treated plants. Again, there was an increase in the plant height, total fresh weight, and dry weight in the combined *T. harzianum+F.* oxysporium treatment compared to the control (Figure 4-5)**.**

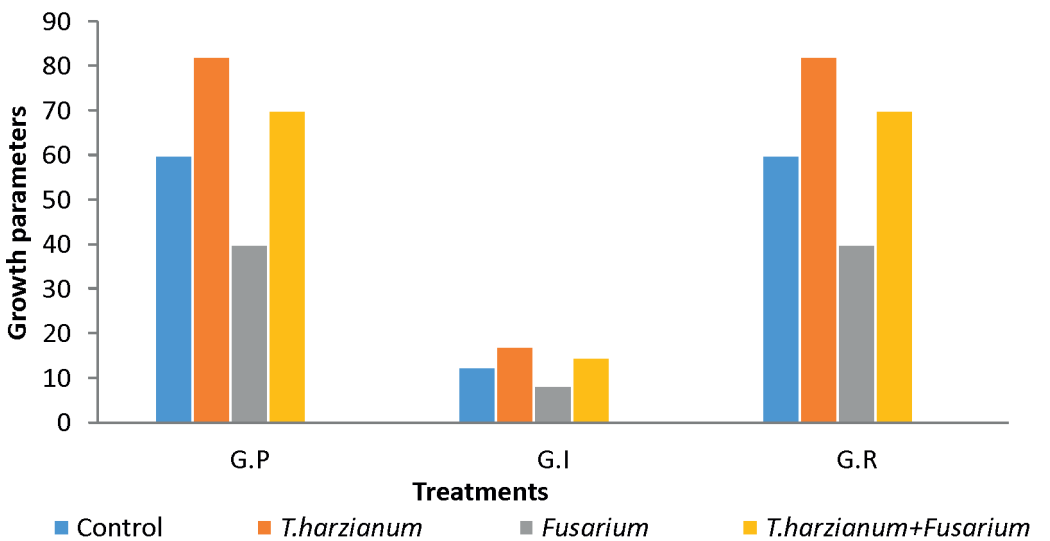

Figure 3: Effect of T.harzianum, F.oxysporum on seed germination of Okra plant

5.2. *Effect of T.harzianum, A.niger and A.flavus on Fenugreek*

T.harzianum increased the shoot and root length by 8.36cm and 5.21cm respectively, total fresh weight of shoot and root by 0.29gm and 0.15gm respectively as well as dry weight of shoot and root by 0.14gm and 0.07 gm

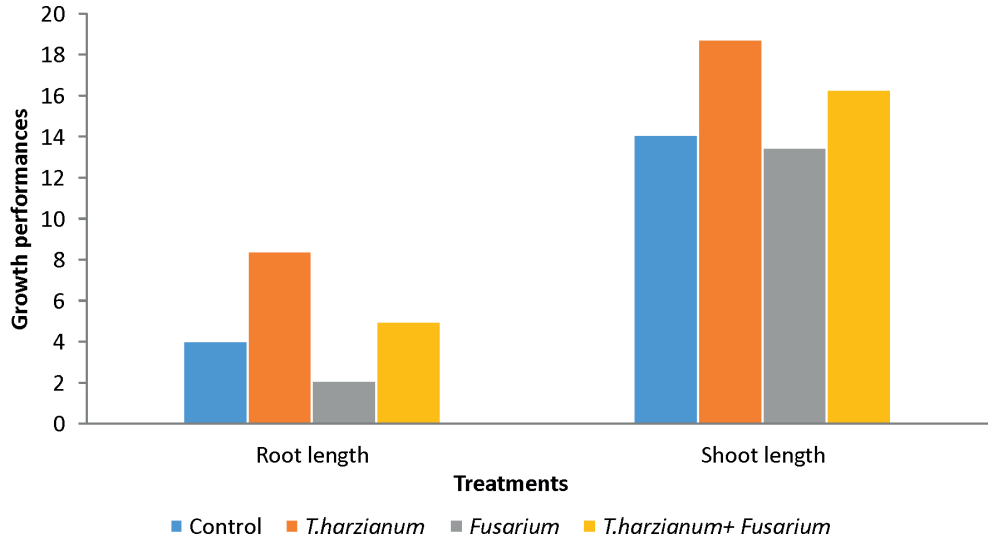

Figure 4: Performance of Root and Shoot length of treatments on Okra plant

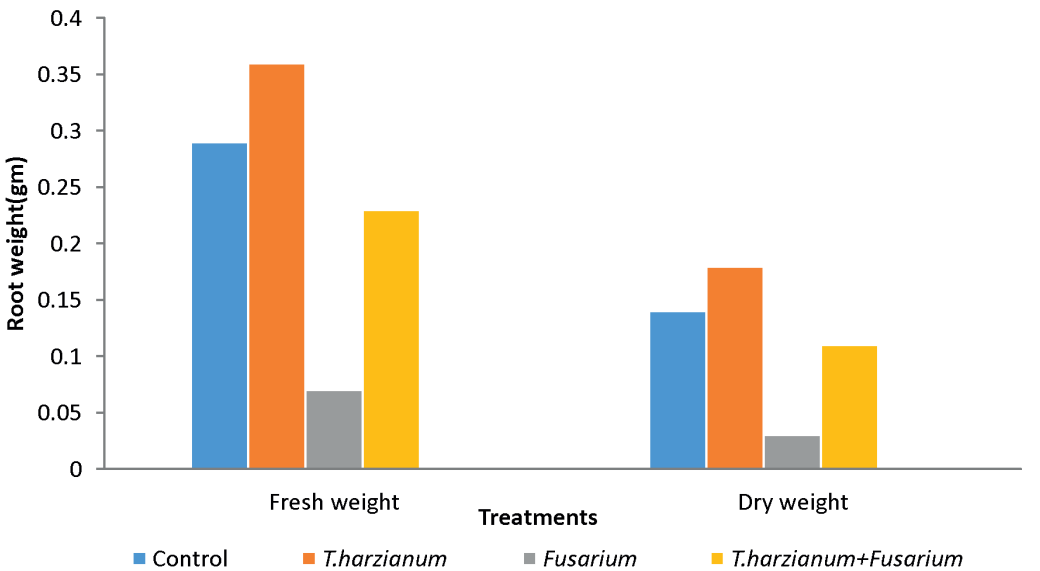

Figure 5: Performance of Root weight of Treatments on Okra plant

respectively compared to the control. The pathogen (*A.niger*) treated plant decreased the shoot and root length by 4.17cm and 1.28cm respectively, total fresh weight of shoot and root by 0.01gm and 0.013gm respectively, similarly dry weight of shoot and root by 0.06gm and 0.006gm respectively and The pathogen (*A.flavus*) treated plant decreased the shoot and root length by 4.26cm and 2.05cm respectively, total fresh weight of shoot and root by 0.15gm

and 0.016gm respectively, similarly dry weight of shoot and root by 0.07gm and 0.008gm respectively as compared to *T.harzianum* treated plants. Again, there was an increase in the plant height, total fresh weight, and dry weight in the combined *T. harzianum+F. oxysporum* treatment compared to the control (Figure 6-8).

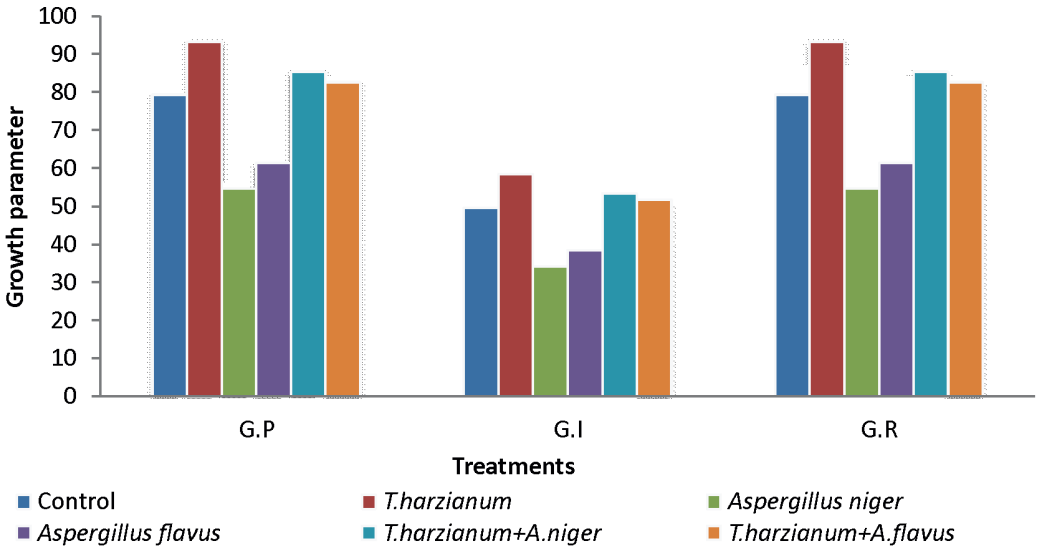

Figure 6: Effect of *T.harzianum, A.niger A.flavus* on seed germination of Fenugreek plant

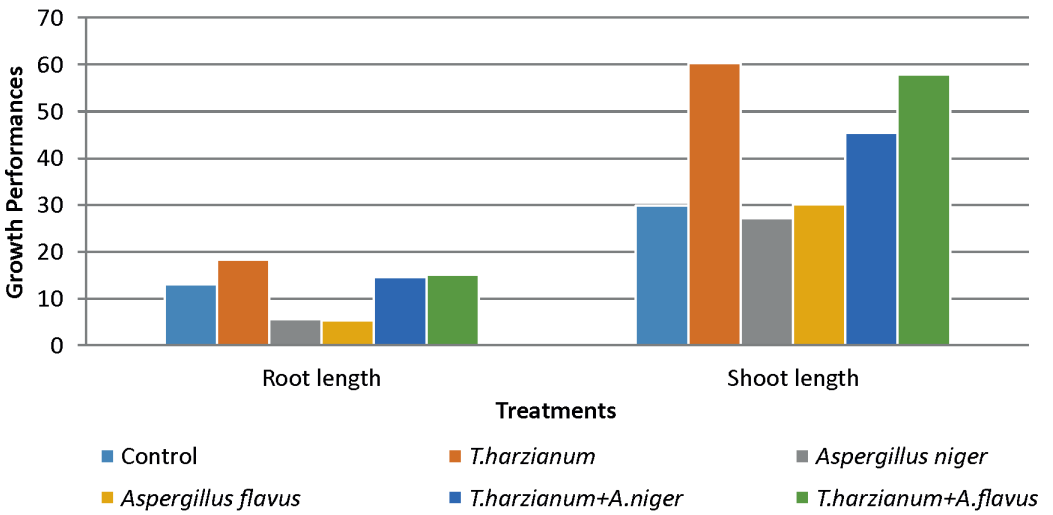

Figure 7: Performance of Root and Shoot length of treatments on Fenugreek plant

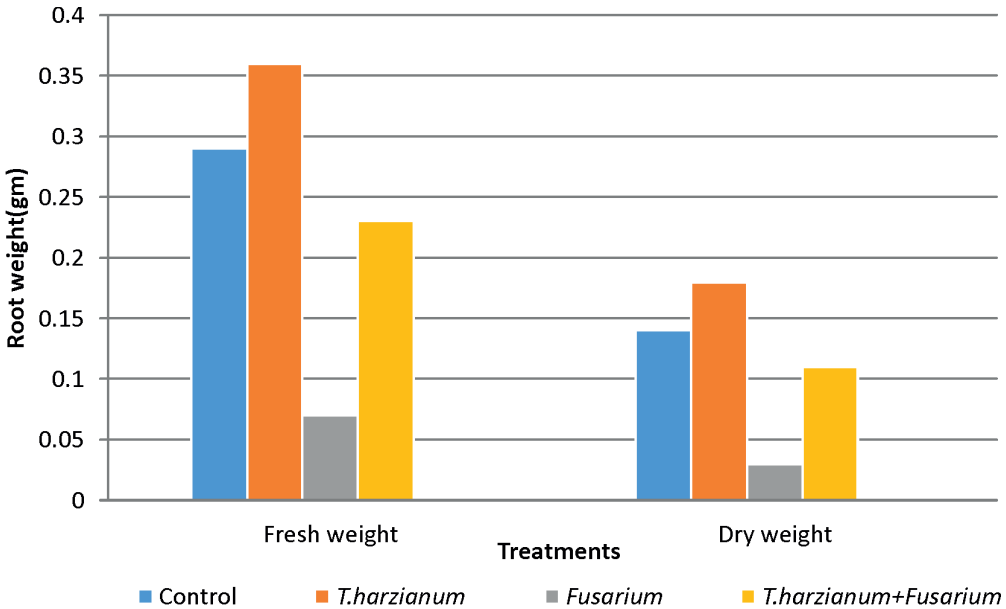

Figure 8: Performance of Shoot weight of Treatments on Fenugreek plant

5.3. *Effect of T.harzianum, A.niger and A.flavus on Dolichos*

T.harzianum increased the shoot and root length by 60.29cm and 18.33cm respectively, total fresh weight of shoot and root by 6.71gm and 0.15gm respectively as well as dry weight of shoot and root by 0.14gm and 0.6gm respectively compared to the control. The pathogen (*A.niger*) treated plant decreased the shoot and root length by 4.17cm and 1.28cm respectively, total fresh weight of shoot and root by 1.82gm and 0.17gm respectively, similarly dry weight of shoot and root by 0.91gm and 0.09gm respectively and The pathogen (*A.flavus*) treated plant decreased the shoot and root length by 30.16cm and 5.24cm respectively, total fresh weight of shoot and root by 2.13gm and 0.22gm respectively, similarly dry weight of shoot and root by 1.06gm and 0.12gm respectively as compared to *T.harzianum* treated plants. Again, there was an increase in the plant height, total fresh weight, and dry weight in the combined *T. harzianum+F.oxysporum* treatment compared to the control (Figure 9-11).

5.4. *Disease Assessment*

The disease index of Okra, Fenugreek and Dolichos bean were determined 15 days after treatment. The disease symptoms were characterized using disease index based on wilting, root rot, yellowing of leaves and leaf curlings were recorded. The findings demonstrated that disease occurrence was extremely

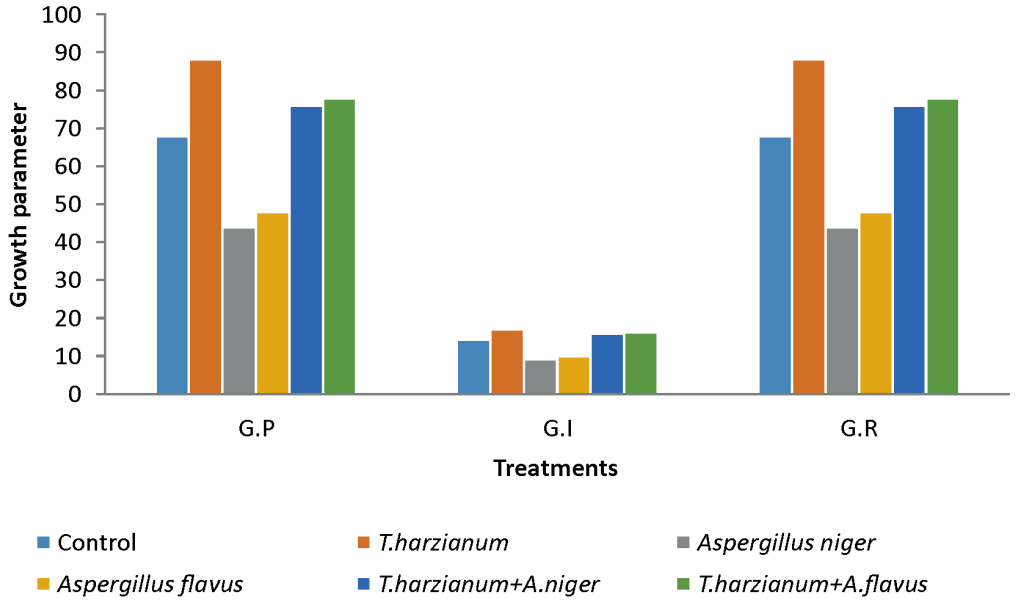

Figure 9: Effect of *T.harzianum, A.niger A.flavus* on seed germination of Dolichos plant

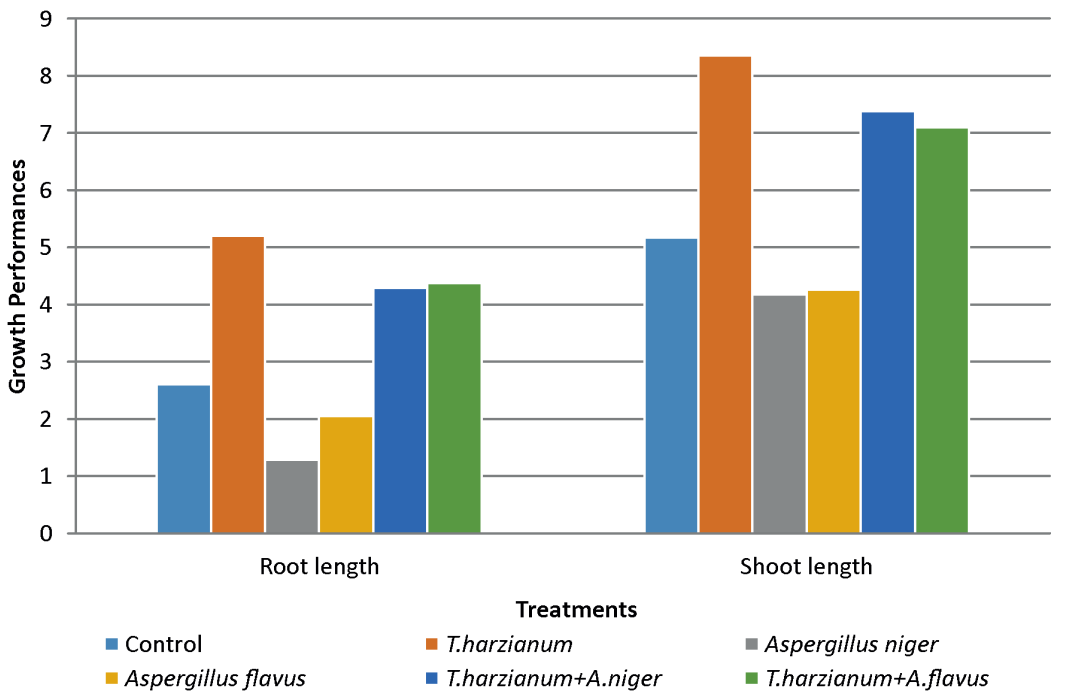

Figure 10: Performance of Root and Shoot length of treatments on Dolichos bean plant

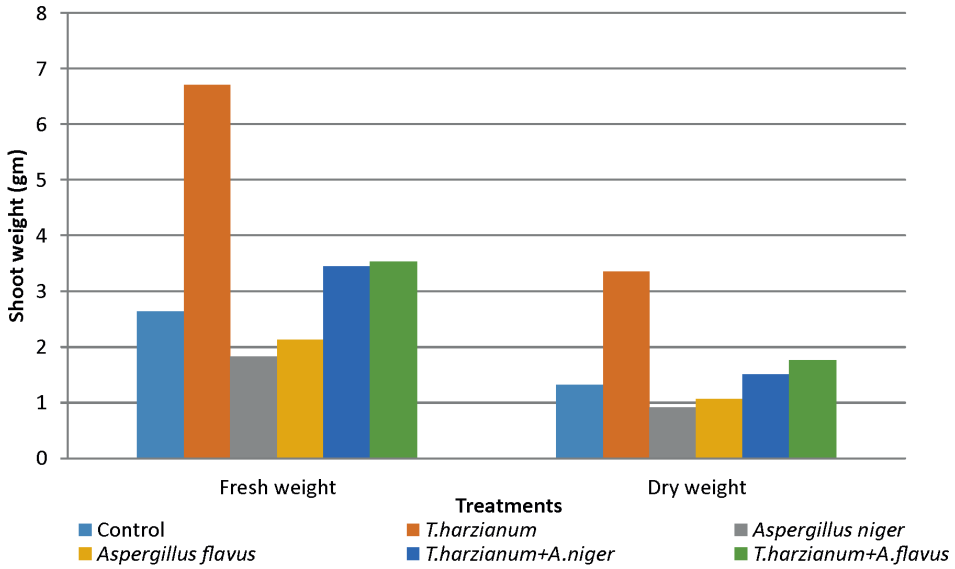

Figure 11: Performance of Shoot weight of Treatments on Dolichos bean plant

severe, as high as 50% by *Fusarium* infection in the Okra plant compared to *T.harzianum* (3%). (Fig 12.) In case of Dolichos bean the highest infection shown by A.flavus is 38.33% compared to *T.harzianum* (5.49%). The highest percentage of disease index in Fenugreek shown by *A.niger* is 53.8% compared to *T.harzianum* (1.96%).This method of disease scale is valuable information of fungal pathogens with Trichoderma. This method was described by Zhang *et al.* (2014). It is one of the simplest and commonly used methods for disease index preparations in plant's fungal diseases.

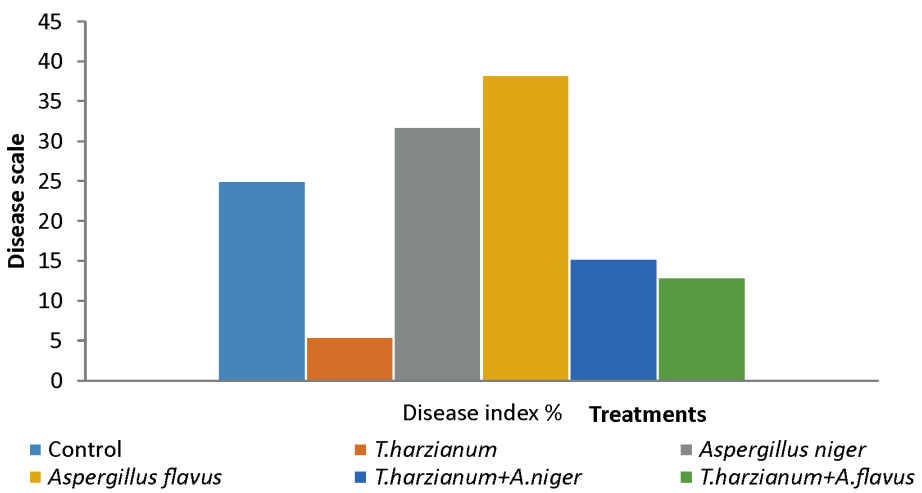

Figure 12: Disease assessment in Okra plant

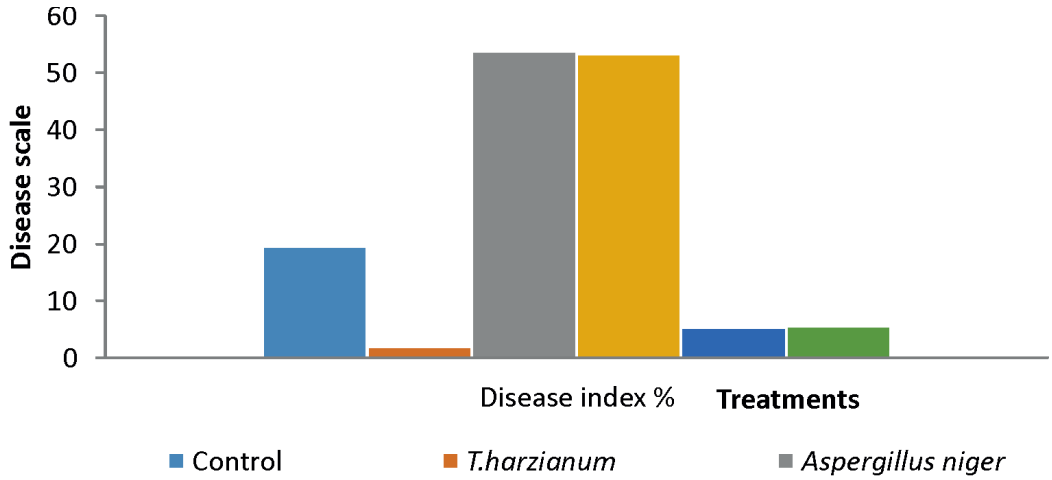

Figure 13: Disease assessment in Fenugreek plant

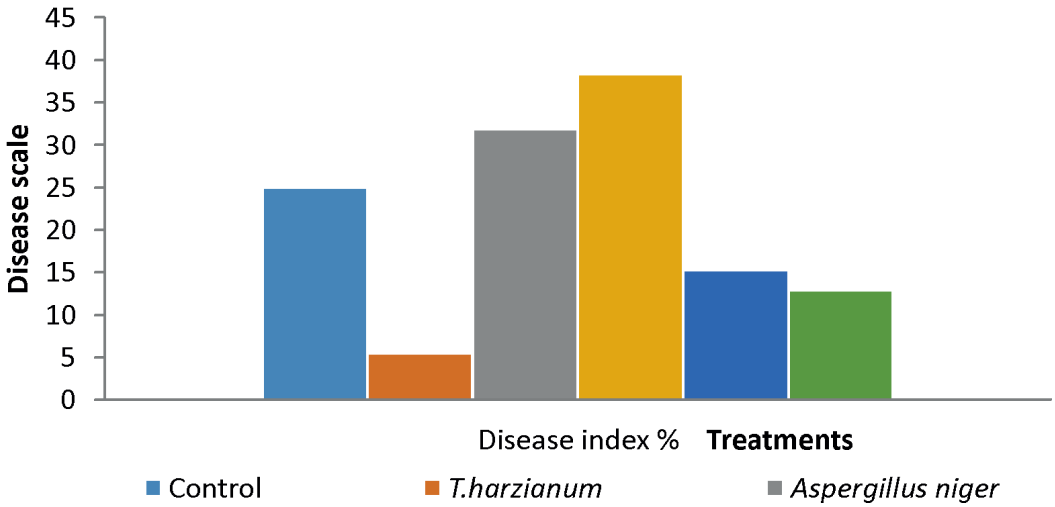

Figure 14: Disease assessment in Dolichos plant

The amount of chlorophyll pigment in a plant indicates its health. Any variation in its intensity distributions suggested physiologic issues or general plant health. In order to estimate various combinations of treatments for both Trichoderma and pathogens, researchers performed as follows. The *F.oxysporum* treatment alone reduced the levels of Chl-a, Chl-b, and Total chlorophyll in Okra by 0.35, 0.19, and 0.54 in relation to the control (Figure 13). Similarly, compared to *T.harzianum*, the *A.niger* treatment in dolichos bean reduced Chl-a, Chl-b and Total chlorophyll 0.68, 0.37, and 1.14, and *A.flavus* by 0.69,

0.35, and 1.12, respectively. In contrast to *T.harzianum*, the *A.niger* treatment in fenugreek reduced Chl-a, Chl -b, and the total chlorophyll by 0.43, 0.27, and 0.79, and *A.flavus* by 0.45, 0.28, and 0.82, respectively (Figure 14).The Chl-a, Chl -b, and the total chlorophyll content in the okra, fenugreek, and dolichos bean plants—caused by *F.oxysporum* , *A. niger* and *A.flavus*, respectively—were therefore restored to a level similar to the control after the *T.harzianum* treatments were given.

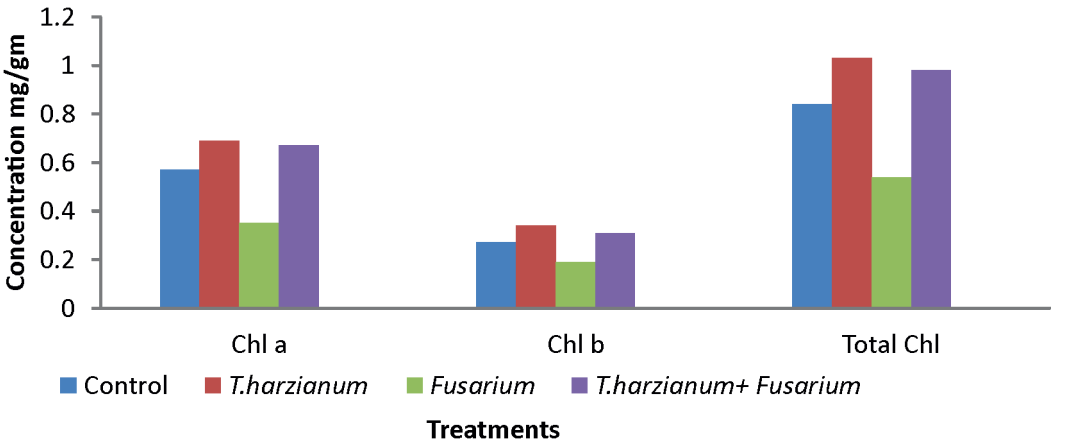

Figure 15: Estimated Chlorophyll pigments in Okra with different Treatments

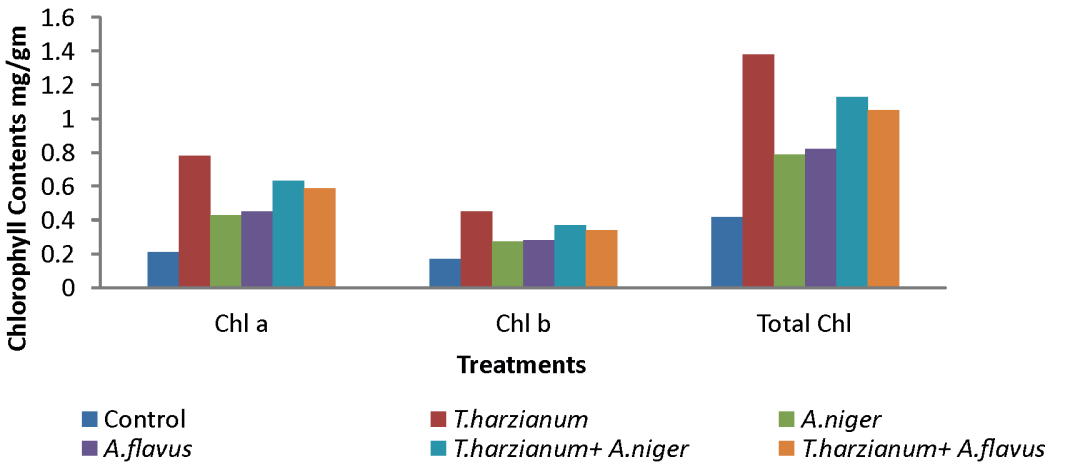

Figure 16: Estimated Chlorophyll pigments in Fenugreek with different Treatments

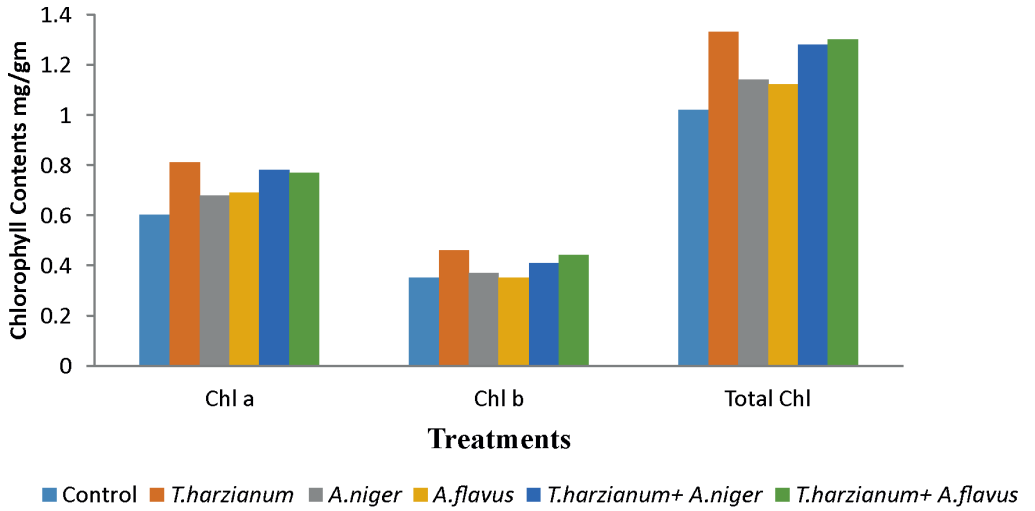

Figure 17: Estimated Chlorophyll pigments in Dolichos bean with different Treatments

6. Discussion

Plant disease needs to be controlled to keep the quality and enhance the productivity of the yields. One of the main constraints that contribute to the low quality and productivity of food products in India is failure due to various plant diseases including fungal, bacterial, viral, nematode, mycoplasma etc. Among them, fungal diseases are a more severe concern to other disease causing entities. In this study three fungal strains with Trichoderma strains were carried out by dual culture method and comparable observations were noticed. (Ibarra-Medina *et al*., 2010), were conducted to study a biocontrol efficiency of Trichoderma, where antagonists with over 70% inhibition of pathogen mycelial growth are considered effective biocontrol. Similarly in this experiment, one Trichoderma, three pathogenic fungi were considered effective antagonists. In contrast, (Kannangara, Dharmarathna, and Jayarathna 2017) described the Trichoderma antagonists with inhibition efficiency higher than 40% as a better biological control agent. According to this description, all the isolates indicate higher inhibition (>40%) efficiency; the selected isolates can be considered as better biocontrol control agents.

Various review literatures in biocontrol agents of Trichoderma indicated the mode of mechanisms as like; the biocontrol agent *T.harzianum* used against different rhizosphere pathogens. *Trichoderma* isolates can suppress fungal phytopathogens directly through mycoparasitism, or indirectly through nutrition and space competition, the promotion of plant development, and by

enhancing defense mechanisms. We observed that *T.harzianum* grows parallel and tightly wraps around the hyphae of *F.oxysporum, A.niger* and *A.flavus* showing mycoparasitism in dual culture techniques. Furthermore, *T.harzianum* develop appressoria and hook like structures that enable solid adhesion to the *F.oxysporum, A.niger* and *A.flavus* hosts. The attachment and germination of *T.harzianum* spores were detected on *F.oxysporum, A.niger* and *A.flavus* host surfaces, indicating nutrient absorption from the fungi host. This shows that the host identification and lysis mechanisms used by *Trichoderma* may vary depending on the Trichoderma–host interactions. Rhizosphere competence has also been reported to be the mechanism by which Trichoderma isolates exclude phytopathogens and endophytic organisms from the rhizosphere of host plants (Druzhinina *et al*., 2010). The capability of Trichoderma isolates to mobilize and utilize nutrients has enabled them to be more efficient and competitive than many other soil microbes (Mokhtar and Aid, 2013; Vinale *et al*., 2008).

The pretreatment with *T.harzianum* increased seed germination and plant growth. The effects of the *T.harzianum* treatments resulted in an increase in plant growth parameters, including shoot height, root length, and the number of leaves. The combined *T.harzianum* with pathogens *F.oxysporum, A.niger* and *A.flavus* also decreased the disease index and reduced root rot and seedlings' death. Although *Trichoderma spp*. has been shown to promote plant development, there is limited evidence of the systemic responses of plants to *T.harzianum* under *F.oxysporum, A.niger* and *A.flavus* stress conditions. Our results showed that *F.oxysporum, A.niger* and *A.flavus* treatments significantly affected the growth and development of okra, fenugreek and dolichos bean plants respectively. However, this impact was significantly reduced with the administration of Trichoderma in particular *T.harzianum*. The increase in root density, height, and dry matter of these plants, as well as other agronomic traits like the number of buds per plant and their fresh weight, may be attributed to Trichoderma harzianum's capacity to solubilize minerals like Fe, Mn, Zn, and P,(Smith and Read, 2010), provide extra N to the plant (Gorelick and Bernstein, 2014), and reduce the distance that nutrients must diffuse towards the roots (Powell and Bagyaraj, 1984).

In this study, *T.harzianum* enhanced the plant height, total fresh weight, and dry weight. On the other hand, the infections with *F.oxysporum*, *A.niger*, and *A.flavus* reduced the amount of leaves, plant height, total fresh weight, dry weight, and relative water content. *Fusarium spp*., specifically *A.niger* and *A.flavus*, frequently caused plants to become stunted; their leaves changed

color from pale green to golden yellow before wilting, withering, dying, and eventually falling off the stem base. The xylem vascular tissue of the roots and the lower stem were dark streaks, and the roots decayed. The Fusarium, *A.niger* and *A.flavus* increased ROS (Reactive Oxygen Species) production in the seedling, leading to low water and nutrient absorption. The above mentioned reasons decreased the photosynthetic activity and led to reduced biomass production in the seedlings.

Here, okra, fenugreek and dolichos bean plants chlorophyll was influenced by biotic stress in particularly fungal pathogens. A crucial component of photosynthesis is chlorophyll, and by regulating cellular osmotic processes, plant bio-regulators help enhance chlorophyll quality. Under pathogen stress, the reduction in chlorophyll concentration in selected plants could be attributed to the breakdown or reduced activities of chlorophyll production enzymes. The decrease in chlorophyll content could be linked to an increase in stress symptoms. However, the *T.harzianum* pretreatment increased the chlorophyll pigmentation with or without Fusarium, *A.niger* and *A.flavus* stress.

7. Conclusion

Biocontrol evaluation of *Trichoderma harzianum* against rhizosphere pathogens on crop plants involves assessing the efficacy of this fungus in controlling harmful pathogens present in the soil around the roots of crops. Selected target crop plants are economically important crops that are susceptible to soil-borne pathogens. These selected rhizosphere pathogens are specific pathogens known to be problematic in the rhizosphere of these crop plants. These could include fungal pathogens such as *Fusarium spp*, *Aspergillus spp*. or bacterial pathogens. Obtained the *T. harzianum* strain and characterize this strain to ensure they are indeed *T.harzianum* and assess their potential for biocontrol. Conducted in vitro assays to evaluate the antagonistic activity of *T. harzianum* against the rhizosphere pathogens of *Fusarium spp*, *Aspergillus spp*. This involved dual-culture assays, where *T.harzianum* is grown alongside the pathogen on agar plates, and the inhibition zones are measured. Conducted field study assessed the biocontrol efficacy of *T. harzianum* under controlled conditions. This was involved inoculating the crop plants with both the pathogen and *T.harzianum* and monitoring disease development, plant growth, and chlorophyll content estimation compared to controls. Conducted field trials evaluated the effectiveness of *T.harzianum* under more natural conditions. Treatments were involved applying *T. harzianum* as a seed treatment, monitoring disease incidence, plant health. *T. harzianum* is a good candidate for Integration with

Integrated diseases Management strategy, including its compatibility with other control methods and it's long

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