Enhanced disease resistance in Yard-long bean *(Vigna unguiculata)* against Brown Rust and Fusarium Wilt diseases using *Trichoderma* and antioxidants

A THESIS

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ANTARIN ZULFIKER

Student No. 1701185

Session: 2023-2024

Thesis Semester: January-June, 2024

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IN

PLANT PATHOLOGY

DEPARTMENT OF PLANT PATHOLOGY

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JUNE, 2024

DADICATED

TO MY BELOVED
PARENTS

AND

SISTERS

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The Author

ABSTRACT

Yard-long bean (*Vigna unguiculata)* is a significant leguminous crop in tropical countries. The vegetable is becoming more and more popular, because of its excellent flavour and high nutritional content. Brown rust and Fusarium wilt disease of yard-long beans caused by *Uromyces phaseoli typica* and *Fusarium oxysporum* f. sp. *phaseoli* are two devastating diseases responsible for maximum yield loss in Bangladesh. The present study was aimed at the bio-control of Brown rust and Fusarium wilt disease as an alternative means of chemical control. For enhancing systemic resistance in yard-long bean plants against *Uromyces phaseoli typica* and *Fusarium oxysporum* f. sp. *phaseoli* as well as for the sustainable and eco-friendly management of Brown rust and Fusarium wilt disease, two previously isolated *Trichoderma* (*T. asperellum* and *T. harzianum*) were used to control these diseases. This study was also concerned with the application of antioxidants; salicylic acid and tartaric acid. In the pot experiment, the triple combination of charcoal-based formulated *T. asperellum*, salicylic acid (4 mM), and tartaric acid (10 mM) exhibited the highest reduction of Rust incidence (75.02 %) and Rust severity (75.38 %) over control as well as Wilt disease incidence (69.86 %) and Wilt disease severity (80.56 %) over control at 60 days after sowing (DAS). The triple combination of charcoal-based formulated *T. asperellum*, salicylic acid (4 mM), and tartaric acid (10 mM) resulted in the highest yield in yard long bean (5.3 Kg/plant) compared to other treatments and control. This combination also exhibited the best results in all tested parameters in plant height (199.43 cm), leaf number (60.33), number of pod/plant (84.67) at 90 DAS, and pod length (65.63 cm). Furthermore, this combined application showed maximum total chlorophyll content (151.33 mg/g FW) as well. The study explored the use of charcoal-based formulated *T. asperellum*, salicylic acid (4 mM), and tartaric acid (10 mM) for the preparation of bio-fertilizer and/or bio-pesticides for the field application to control Brown rust and Fusarium wilt disease of yard-long bean.

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CHAPTER I

INTRODUCTION

Yard-long bean *(Vigna unguiculata)* is one of the most widely consumed vegetables in many Southeast Asian nations. It is a typical crop of vegetables in Bangladesh, India, Indonesia, the Philippines, and Sri Lanka (Ullah *et al*., 2011). Bangladesh produces 41895.45 metric tons yard-long bean in 17937.32 acres of land (BBS, 2023).

It belongs to the family Fabaceae and is a crucial summer vegetable in Bangladesh (Haque *et al*., 2021). The yard-long bean is a highly nutritious vegetable that is rich in vitamins, minerals, and a fair quantity of crude protein (Ano and Ubochi, 2008). It improves the soil by fixing nitrogen through a symbiotic relationship with nodule bacteria (*Bradyrhizobium* spp.) and serving as a ground cover (Singh *et al*., 1997; Haque *et al*., 2021). Additionally, it improves the soil's structure and raises the amount of organic matter in the soil following soil incorporation (Valenzuela and Smith, 2002). Yard-long bean fibre is used to construct fishing lines and has also been thought to be a viable source of pulp for high-grade paper (Summerfield and Roberts, 1985). The hulls of yard-long beans are highly digestible and can be as feed for livestock and therefore significant for farmers (Savadogo *et al*., 2000; Ajeigbe *et al*., 2003).

Every year yard-long bean production in Bangladesh faces numerous obstacles, such as inadequate high-producing varieties, poor seed quality, biotic and abiotic stressors, etc. Diseases are one of the main biotic stressors limiting Bangladesh's reduced yard-long bean yield. However, the major diseases contributing to yield losses are Brown rust and Fusarium wilt disease.

Rust disease is caused by *Uromyces phaseoli typica* that directly enter epidermal cells (Mellersh *et al*., 2002). On any portion of the plant above ground, the rust pustules can be seen. The infection first appears as tiny, nearly white, slightly elevated pustules that subsequently develop into distinctive, Rust-coloured, circular sori (Ramangoud B. Honnur *et al*, 2016). By the middle of the flowering period, very vulnerable lines can be nearly totally defoliated, resulting in a likely devastating yield loss.

Wilt disease caused by *Fusarium oxysporum* f. sp. *phaseoli* is also a devastating disease that is responsible for about 60% yield reduction in cultivated yard-long beans (Pirayesh *et al*., 2018; Metsena *et al*., 2021). It is a systemic disease that attacks the vascular bundles of yard-long beans where the pathogen secretes extracellular polysaccharides that interfere with water absorption and translocation processes in the xylem and phloem vessels, respectively (Porttorf *et al*., 2014; Gordon, 2017). This impairs plant growth and ultimately reduces yield among susceptible yard-long bean varieties. The initial symptoms are expressed in older leaves which show mild discoloration of leaves that later change to greenish yellow (Sabo *et al*., 2014). The advanced stage of infection shows drooping of leaves, wilting, and ultimate death of infected plants. Wilting often begins on one side, then the whole part, and later the death of the plant (Wamalwa, 2018).

These diseases are extremely challenging to control because the intricate features of the target pathosystems are layered on top of the inherent complexities of the management strategy. However, management of fungal diseases requires an integrated approach where using a chemical like sulphur compounds would be the last option. Farmers are using chemicals anonymously without being economically benefitted to control such kind of devastating disease. In addition, using of chemicals is also causing a polluted environment and human health hazards (Bonjar *et al*. 2004).

Biological control methods have been widely used to address soil-borne vegetable diseases since they are generally safe and beneficial to the environment (Naranjo *et al*., 2015). Increasing the use of systemic acquired resistance (SAR) is a novel approach to managing several crop diseases (Hoitink and Boehm, 1999; Vallad *et al*., 2000; Nelson and Boehm, 2002).

Currently, one of the most promising approaches to managing plant diseases is induced systemic resistance (ISR). It has been suggested that a few fungi may be able to cause systemic resistance in plants (Van Loon *et al*., 1998).

T. harzianum and *T. asperellum* can be considered an ideal biocontrol agents for their good characteristics. *Trichoderma* not only prevents Rust and Wilt disease but also promotes yard-long bean growth and yield. This antagonist is highly isolable and grows quickly on organic materials. *T. harzianum* and *T. asperellum* have several modes of action, including mycoparasitism (Lumsden *et al*., 1995; Abada, 2002 and Ahmed, 2013), the synthesis of antifungal compounds (Robinson *et al*., 2009), and the possession of an enzymatic system that breaks down pathogens (Elad and Kapat, 1999 and Ziedan *et al*., 2005). Apart from these methods of operation, *Trichoderma* spp. additionally stimulate resistance in treated plants to protect them from specific diseases (Homer, 1993; Harman, 2006; Ahmed, 2005) develop under a broad range of environmental circumstances, including temperature (Bailey *et al*., 2008 and Singh and others, 2010). *Trichoderma* spp. have a beneficial impact on plant protection if colonized on roots, as they trigger the plant defence mechanism including the production of phytoalexins, Pathogenesis Related (PR) proteins, jasmonic acid, peroxidases, and chitinases (Djonovic *et al*., 2006).

Salicylic acid (SA) is a phytohormone that is essential to defensive signalling (Vlot *et al*., 2009). It is necessary for SAR as well as basal defence. Whereas the application of exogenous SA or SA analogues induces resistance to pathogens (White, 1979; Ward ER, 1991; Görlach *et al*., 1996), degradation of SA by transforming plants with the bacterial salicylate hydroxylase gene NahG blocks SA accumulation and SAR (Gaffney T., *et al*. 1993). So far, no limited studies carried out in Bangladesh regarding the application of single use of *Trichoderma* or in combinations with some antioxidants to enhance the systemic resistance in long-yard bean. Therefore, the present research aimed to study the role of several selected antioxidants including salicylic Acid (SA), and tartaric acid (TA), for possible enhancement of systemic acquired resistance in yard long bean to control Rust and Wilt disease.

Objectives:

The following objectives were selected for the current study:

1. To enhance the disease resistance in yard long beans against Fusarium wilt disease and Brown rust disease.
2. To observe the efficacy of *Trichoderma* spp. and antioxidants in enhancing disease resistance and improving agronomic attributes of yard-long bean.

CHAPTER II

REVIEW OF LITERATURES

Brown rust and Fusarium wilt disease caused by *Uromyces phaseoli typica* and *Fusarium oxysporum* f. sp. *phaseoli*, respectively have attracted the attention of plant pathologists and professional researchers throughout the world. An attempt has been made to review the available literature about the management of Brown rust and Fusarium wilt disease with *Trichoderma* and antioxidants.

2.1 Brown rust disease of yard-long beans caused by *Uromyces phaseoli typica*

Ndalira *et al.*, (2020) reported that one of the most harmful diseases that negatively affects yard long bean output and foliar health is the Rust disease caused by *Uromyces phaseoli typica.*

López *et al.*, (2015) observed that there is a great physiological variation among the races of *Uromyces appendiculatus var. appendiculatus* (Pers.) in Mexico; this pathogen causes bean Rust, which is one of the main fungal diseases of bean (*Phaseolus vulgaris L.*) crops.

De Souza *et al.*, (2008) and Kolmer *et al.* (2009) reported that Common bean Rust, caused by the autoecious and macrocyclic pathogen *Uromyces appendiculatus (Pers) Unger*, is one of the major common bean diseases worldwide.

Uma and Salimath (2003) observed that among the various diseases that threatening the production of cowpea, cowpea Rust caused by *Uromyces phaseoli var. vignae* causes the huge loss to the producers.

Khare MN *et al.*, (1993) described that Rust caused by *Uromyces viciae-fabae* is another widespread foliar disease of lentil in countries like- Ethiopia, Morocco, Chile, Ecuador, Bangladesh, India, and Pakistan, which can result in crop damage of up to 100% i.e. complete failure of the crop.

Heath (1989) reported that *Uromyces vignae Barclay* causing cowpea (Vigna unguiculata L. Walp.) Rust is a macrocyclic, autoecious fungus which is the major disease contributing to yield losses.

2.2 Fusarium wilt disease of yard-long beans caused by *Fusarium oxysporum* f. sp. *Phaseoli*

Hasan (2023) observed that Fusarium wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici* is a harmful disease responsible for huge yield loss in Bangladesh.

Roy (2023) revealed that Fusarium wilt of cucumber caused by *Fusarium oxysporum* f. sp. *cucumerinum* is a deadly disease responsible for maximum yield loss in Bangladesh.

Siyin, Yang *et al.,* (2023) reported that faba bean fusarium Wilt disease is a devastating soil-borne disease, which is caused by *Fusarium oxysporum* f. sp. *fabae*.

Piyaresh *et al.,* (2018) and Metsena *et al.* (2021) described that Wilt disease caused by *Fusarium oxysporum* f. sp. *tracheiphilum* (Fot) (E.F. Sm.) Snyd. and Hans is a devastating disease that is responsible for about 60% yield reduction in cultivated cowpea. It is a systemic disease which attacks the vascular bundles of cowpea where the pathogen secretes extracellular polysaccharides that interfere with water absorption and translocation processes in the xylem and phloem vessels.

Chittarath *et al.,* (2018) observed that Fusarium Wilt disease caused by *Fusarium oxysporum* f. sp *ciceris*) is one of the deadly diseases that cause catastrophic damage to numerous legume crops worldwide. Chickpea is one of the most susceptible crops for Fusarium Wilt disease attack, which causes significant production losses.

Bertoldo *et al*., (2015) described that more than 150 agricultural crop species are affected by the severe vascular Wilt disease caused by the pathogenic strains of Fusarium, including cotton, banana, tomato, melon, legumes, and watermelon.

Martyn (2014) observed that Fusarium wilt disease (FW) is among the world's most dangerous soil-borne and seed-transmitted plant diseases. Tomato (*Solanum lycopersicum L.*), banana (*Musa* spp. *L.*), melon (*Cucumis melo L.*), yard-long bean *(Vigna unguiculata)*, and cotton (*Gossypium* spp. L.) are among the numerous plant species that have been infected by Fusarium Wilt disease.

Pottorff *et al.*, (2014) revealed that Fusarium Wilt disease caused by fungal pathogen, *Fusarium oxysporum f.* sp. *tracheiphilum* (fot), is one of the soil-borne diseases that cause major threat in many of the cowpea growing area; substantially reduces the yield of 30 to 100% with high plant mortality and severe problem in cowpea production.

2.3 *Trichoderma* spp. against *Uromyces phaseoli typica* and *F. oxysporum* f. sp. *phaseoli*

Sharma *et al.*, (2019) shows that the species of *Trichoderma*, a genus of hyphomycetes, are ubiquitous in the environment, but especially in soils. They have been used or encountered in many human activities, including commercial applications in production of enzymes and biological control of plant disease.

Lieckfeldt *et al*. (1999) reported that *Trichoderma* (Ascomycetes, Hypocreales) strains that have warted conidia are traditionally identified as *T. viride*, the type species of *Trichoderma*.

Bissett *et al*., (1991) reported that phialides are ampulliform to lageniform, usually constricted at the base, more or less swollen near the middle, and abruptly near the apex into short sub cylindric neck. They are disposed in verticals terminally on branches of the conidiophore, or less frequently singly or in whorls directly beneath septa along the conidiophore and its branches.

Bissett *et al.,* (1984) described that the genus *Trichoderma* is characterized by rapidly growing colonies bearing tufted or repeatedly branched conidiophores with lageniform phialides and hyaline or green conidia borne in slimy heads.

Domsch *et al.* (1980) evaluated that conidiophore may end up in sterile appendages with the phialides borne on lateral branches in some species. Conidia are hyaline or, more usually, green, smooth-walled or roughened. Hyaline chlamydospores are usually present in the mycelia of older cultures.

Casas-Flores *et al*., (2014) studied that *Trichoderma* spp. antagonize phytopathogens by competing for nutrients, space, by producing antibiotics as well as by inducing systemic resistance of plants. In addition, *Trichoderma* spp. stimulate plant growth and development by means of the production of plant growth promoting molecules. Activation of the processes that regulate biocontrol involve components of diverse signal transduction pathways, such as mitogen-activated protein kinase, cyclic adenosine monophosphate-dependent protein kinase, and heterotrimeric G- proteins.

Sharma *et al*., (2012) revealed that *Trichoderma* is soil borne eukaryotic, multicellular fungus. It is most commonly used for biocontrol agent against plant pathogen that contain chitin and glucans in their cell wall. The mechanism of *Trichoderma* includes direct competition with the target organism, antibiosis, predation or parasitism of the target organism and induced resistance of the host plant.

Verma *et al.,* (2007) concluded that *Trichoderma* spp. have been widely used as antagonistic fungal agents against several pests as well as plant growth enhancers. Faster metabolic rates, anti-microbial metabolites, and physiological conformation are key factors which chiefly contribute to antagonism of these fungi, Mycoparasitism, spatial and nutrient competition, antibiosis by enzymes and secondary metabolites, and induction of plant defence system are typical biocontrol actions of these fungi. On the other hand, *Trichoderma* spp. have also been used in a wide range of commercial enzyme productions, namely, cellulases, hemi-cellulases, proteases, and B-1,3-glucanase.

Harman *et al*., (2004) reported that *Trichoderma* spp. is opportunistic, avirulent plant symbionts, as well as being parasites of other fungi. At least some strains establish robust and long-lasting colonization of root surfaces and penetrate into the epidermis and a few cells below this level. They produce or release a variety of compounds that induce localized or systemic resistance responses, and this explains their lack of pathogenicity to plants. These root-microorganism associations cause substantial changes to the plant proteome and metabolism. Plants are protected from numerous classes of plant pathogen by responses that are similar to systemic acquired resistance and rhizobacteria-induced systemic resistance. Root colonization by *Trichoderma* spp. also frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients.

2.4 Beneficial microorganism against Brown rust and Fusarium wilt disease

Chen *et al.* (2020) showed that bioorganic fertilizer developed by using *Bacillus licheniformis* (X-1) and *Bacillus methylotrophicus* (Z-1) exhibited a strong inhibition ability against the pathogen Fusarium sp. in comparison with the chemical and organic fertilizers allowing 80% disease free strawberry production together with improved physical and biochemical indexes in the pot 7 experiments. The enzyme activity analysis of SOD, PPO, POD, and CAT in the bioorganic fertilizer (BOF) group showed significant increase with values; 48.8 %, 68.7 %, 85.9 %, and 41.1% than that of the control group, respectively.

Xie *et al.* (2021) found that inactivated *F. oxysporum* f. sp. *niveum* (FON) mycelia significantly decreased rhizosphere FON number and Fusarium wilt disease severity. In addition, inactivated FON mycelia inoculation significantly enhanced soil enzyme activity levels and plant systemic resistance, which promoted resistance to Fusarium Wilt disease and thus watermelon *(Citrullus lanatus L.)* growth.

Bubici *et al.* (2019) found that Fusarium wilt disease of banana (FWB) had been controlled up to 79 % by using Pseudomonas spp. strains, and up to 70% by several endophytes and *Trichoderma* spp. strains. Lower biocontrol efficacy (42-55 %) had been obtained with arbuscular mycorrhizal fungi, *Bacillus* spp., and non-pathogenic Fusarium strains.

Sani *et al.* (2020) showed that the combined application of *Trichoderma* and biochar increased the growth attributes positively and produced 101.45 % and 11.33 % higher yield compared to half dose and standard dose of N-P-K, respectively with eliciting an increase in mineral contents, total soluble solids as well as bioactive molecules such as lycopene and ascorbic acid, thereby increased the nutritional and functional quality of the tomato fruits. Collectively, *Trichoderma* and biochar improved soil fertility, nutrient uptake and promoted the growth of rhizosphere fungal and bacterial populations, which combinedly resulted in higher tomato yields, antioxidants, and minerals.

Siddiqui *et al.* (2008) showed that there was a reduction of 85.04 % in Choanephora wet rot severity on okra treated with *Trichoderma*-fortified rice straw (RST) extracts during 12 weeks of assessment in the field, which was comparable to the conventional fungicide Dithane M-45®.

Harman *et al.* (2004) opined that some strains of *Trichoderma* spp. establish robust and long-lasting colonization of root surfaces and penetrate 8 into the epidermis and a few cells below this level. They produce or release a variety of compounds that induce localized or systemic resistance responses. These root-microorganism associations cause substantial changes to the plant proteome and metabolism and protect plants from numerous classes of plant pathogen by responses that are similar to systemic acquired resistance and rhizobacteria-induced systemic resistance. Root colonization by *Trichoderma* spp. also frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients.

Harman (2021) found that combination of *T. harzianum* and salicylic acid led to a significant decrease in powdery mildew disease severity in sunflower in comparison to control. The integration of antioxidants with bioagents showed a better response to control powdery mildew disease than single treatments.

Rajput *et al.* (2021) found that the combined application of *T. pseudokoningii* BHUR2 and vermiwash increased fresh weight of root (4.8-fold) and shoot (5.8-fold), dry weight of root (6.9-fold) and shoot (6.4-fold) and number of fruits per plant (4.2-fold) in tomato as compared to control under *Sclerotium rolfsii* inoculated condition. Plants treated with *T. pseudokoningii* BHUR2 and vermiwash exhibited higher defence response against *S. rolfsii*, mediated by higher activity of superoxide dismutase (3.57-fold), peroxidase (2.05-fold) and phenylalanine ammonia lyase (2.98-fold) enzymes and accumulation of total phenol content (5.35-fold) as compared to control plants.

El-Mougy and Abdel-Kader (2018) observed that the use of *T. harzianum* and antioxidants in combination had superior effect for reducing disease incidence and severity on faba bean plants compared to fungicide and the control.

Sun *et al.* (2021) performed a pot experiment and revealed that the addition of the Pb-resistant microorganism *T. asperellum* SD-5 promoted growth and increased biomass in ryegrass under Pb stress, in addition to significantly enhancing photosynthesis by increasing the leaf chlorophyll 9 content and improving the total protein content and expression of the PAPX, POD, SOD, and GPX genes, evidence of an improved antioxidant system and the alleviation of Pb stress.

Doley *et al.* (2014) found that the inoculation of *Glomus fasciculatum* and *T. asperelloides* reduced the severity of disease but their combinations showed the best result against *Sclerotium rolfsii* causing root-rot of groundnut. The defence related physiological, biochemical and anti-oxidant activities observed in roots of groundnut plant significantly increased by single inoculation of AM fungi or *Trichoderma*. But, the combined inoculations of AM fungi and *Trichoderma* species showed the highest defence related activities.

Sudantha *et al.* (2020) showed that *Trichoderma* spp. can increase the induced resistance of onion to Fusarium wilt disease diseases and increase the growth and yield of onion.

Khruengsai *et al.* (2021) concluded that the volatile compounds produced by each strain of *T. afroharzianum* strain MFLUCC19-0090 and *T. afroharzianum* strain MFLUCC19-0091 inhibited pathogen growth and *Trichoderma*-derived volatile compounds significantly reduced Fusarium related disease severity and incidence percentages in the inoculated fresh chilies. Phenyl ethyl alcohol was found to possess the strongest antifungal activity against both pathogens.

Panwar (2014) found *T. harzianum* (Th-M) most effective in significantly reducing the radial growth of all *F. graminearum* (Fusarium head blight pathogen of wheat) isolates followed by *T. viride*. Foliar application of *T. harzianum* (M) and *T. viride* alone and in combination significantly reduced the disease severity as compared to control. Maximum reduction in disease severity was achieved with combined application of *T. harzianum* and *T. viride*.

Boruah and Dutta (2021) performed in vitro assay of *T. asperellum* and chitosan nanoparticle against *F. oxysporum, Rhizoctonia solani* and *S. rolfsii* and found the combination of *T. asperellum* and chitosan nanoparticle as 10 superiors in suppression of mycelial growth of the pathogens as compared to *Trichoderma* alone and carbendazim @0.1%.

Ayyandurai *et al.* (2021) revealed that *T. asperellum* (TB3) isolate was found to effectively inhibit the radial mycelial growth of the pathogen *F. solani* f.sp. *melongenae* causing Fusarium Wilt disease of brinjal by (85.77 %) when compared to all other isolates where most of the *Trichoderma* species produced toxic volatile metabolites, having significant effects on growth and development of the plant.

Dubey *et al.* (2007) found the integration of *T. harzianum* (106 spores/ml/10 g seed) and carboxin (2 g/Kg seed) for seed treatment to be the best which enhanced seed germination against Fusarium Wilt disease by 12.0-14.0% and grain yields of chickpea (Cicer arietinum L.) by 42.6-72.9 % and reduced Wilt disease incidence (44.1-60.3%) during experimentations.

Khan *et al*. (2017) found that *T. harzianum* produced up to 75.5 % inhibition of colony growth of the pathogen *F. oxysporum* f. sp. *Cubense*, a soil loving pathogen of banana causing distortion of vascular system, followed by incubation for 72 h at 28±2°C in vitro. In pot culture *T. harzianum* considerably reduced disease severity.

Pavlovskaya *et al.* (2020) found that among three *Trichoderma* isolates, *T. atrobrunneum* VKPM F-1434, *T. harzianum* 5/14, T. lixii T4/14, the *T. atrobrunneum* fungus strain VKPM F-1434 showed the highest degree of inhibition on 10 days of cultivation with phytopathogenic microorganisms *F. oxysporum* isolate B/14, *F. oxysporum* isolate MOS509, *F. oxysporum* isolate IMI58289 in vitro which was 100 %. The study revealed that all *Trichoderma* species are capable of producing lytic enzymes VI (2018) found in the result of in vitro interactions that the highest percentage growth inhibition of mycelia of the pathogen *F. oxysporum* causal agent of White Yam (*Dioscorearotundata poir*). Tuber Rot (77.99 %) was recorded when *T. harzianum* was introduced two days before the inoculation of 11 *F. oxysporum*, followed by introduction of *T. harzianum* same time with *F. oxysporum* (45.69 %) and the least percentage growth inhibition (13.72 %) was recorded when the antagonist was introduced two days after inoculation of the pathogen.

Ramírez-Olier and Botero-Botero (2019) showed that both the isolates of *Trichoderma* (*T. asperellum* (GRB-HA1 and GRB-HA2) resulted 100% in percentage inhibition of radial growth (PIRG) and grade 4 mycoparasitism in dual cultures against *Colletotrichum gloeosporioides* and *Curvularia lunata* although GRB-HA1 led to 70% PIRG and grade 3 mycoparasitism and GRBHA2 led to 84% PIRG and grade four mycoparasitism against *F. oxysporum.*

Yassin *et al.* (2021) found that *T. viride* showed antagonistic activity against *F. proliferatum* and *F. verticillioides* with mycelial inhibition rates of 80.17% and 70.46%, while *T. harzianum* exhibited rates of 68.38% and 60.64%, respectively. The culture filtrates of *T. viride* and *T. harzianum* strains exhibited antifungal activity against F. verticillioides strain with suppressive rates of 56.7% and 32.2%, while the mycelial inhibition rates against *F. proliferatum* strain were 44.09% and 23.50%, respectively. Moreover, the acetonic extracts of *T. viride* and *T. harzianum* strains exerted the highest antifungal potency against *F. proliferatum* strain.

Sinha *et al.* (2018) concluded that 1% talc-based formulation of *T. harzianum* significantly reduced the Wilt disease caused by *F. oxysporum* f. sp. *capsici* causing Wilt disease in chilli as 87.5 percent followed by 1% talc-based formulation *T. viride* as 83.93 percent. A 1% talc-based formulation of *T. harzianum* has also recorded maximum germination along with enhanced plant height, root length and yield.

Andoji (2021) used *T. virens,* T. *atroviride, T. viride, T. harzianum, T. koningiopsis, T. stilbohypoxyli,* and *T. pseudokoningii* species for the antagonistic study against *F. solani* inciting root rot of chickpea (*Cicer arietinum L.*) and found that all *Trichoderma* species showed great antagonistic 12 activity. But among them*, T. virens, T. atroviride, T. viride* showed 90% and 80 % antagonistic activity than others in case of a sensitive isolate of test fungus. The resistant isolate of the pathogen was restricting the antagonism to some extent.

Moosa *et al.* (2017) evaluated isolates of *Trichoderma* and cow manure in vitro and in vivo individually and in combination and concluded that in in vitro the T. *viride* and cow manure mix produced the best pathogen inhibition. In vivo the *T. viride, T. harzianum* and cow manure mix produced the best inhibition.

Hussein *et al.* (2022) found the antagonistic activities of *T. harzianum* against the growth of *F. solani* AJA2 (62.3%), followed by *F. oxysporum* AJA (55.2%), *F. incarnatum* AJA (53.2%), and *F. solani* AJA1 (50.8%). The highest inhibition rate (85 %) was shown by *T. harzianum* against *F. incarnatum*. The volatile compounds of *T. harzianum* led to a high percentage of inhibition of *Fusarium* species.

El-Sharkawy and Abdelrazik (2022) observed that *T. harzianum* caused the greatest reduction in mycelial growth of *F. solani* (75.17%), the causal agent of root rot in squash under in vitro conditions, followed by *T. album* and *T. koningii*.

You *et al.* (2022) reported that the volatile organic compounds (VOCs) produced by *T. koningiopsis* T-51 showed high inhibitory activity against plant pathogenic fungi *Botrytis cinerea* and *F. oxysporum*. The percentage of *B. cinerea* and *F. oxysporum* mycelial growth inhibition by T-51 VOCs was 73.78% and 43.68%, respectively. In both B. cinerea and *F. oxysporum*, conidial germination was delayed, and germ tube elongation was suppressed when exposed to T-51 VOCs, and the final conidial germination rate of *B. cinerea* decreased significantly after T-51 treatment. The VOCs from T-51 reduced the Botrytis fruit rot of tomato compared with that noted when using the control.

2.5 Salicylic acid and disease resistance

Roy1 (2023) described that an essential part of defensive signalling is played by the phytohormone salicylic acid (SA). Both basal defence and SAR depend on it. According to early research, pathogen infections raise SA levels in three areas of plants—both local and distant.

Saengchan (2022) found that salicylic acid and B. subtilis strain JN2-007 reduced mycelial growth of *F. solani* (11.83%–57.73% at day 7), as well as disease severity in the cassava plants at 14 days after the inoculation compared to that of the negative control (28.12%–39.58% compared to 68.75%). Furthermore, salicylic acid at a concentration of 500 µl. L−1 could induce H2O2, level of peroxidase, polyphenol oxidase, and catalase activities that were highest at 24 h after pathogen inoculation.

Abo-Elyousr *et al.* (2009) revealed that foliage sprays of 2mM SA significantly suppressed disease development in onion plants against *Stemphylium vesicarium* up to 40.39 % by day 15 after inoculation under greenhouse conditions.

CHAPTER III

MATERIALS AND METHODS

The study was aimed for the eco-friendly management of Brown rust and Fusarium wilt disease caused by *Uromyces phaseoli typica* and *Fusarium oxysporum* f. sp. *phaseoli* by the using of formulated *Trichoderma* and antioxidants. The detailed methodology used to carry the experiments is as follows:

3.1 Collection of seeds of yard-long bean

Seeds of long-yard bean (Hong Kong Excel) were collected from Laltir Seed Company, Bangladesh.

3.2 Collection and preparation of *Trichoderma* spp.

Trichoderma (*T. asperellum* and *T. harzianum*) were previously isolated and identified in our laboratory which again sub-cultured on PDA (200 g of potato, 20 g of dextrose, 10 g of agar, 1000 mL of distilled water, and neutral pH for 7 d in the dark at 28 °C (Fig. 1).

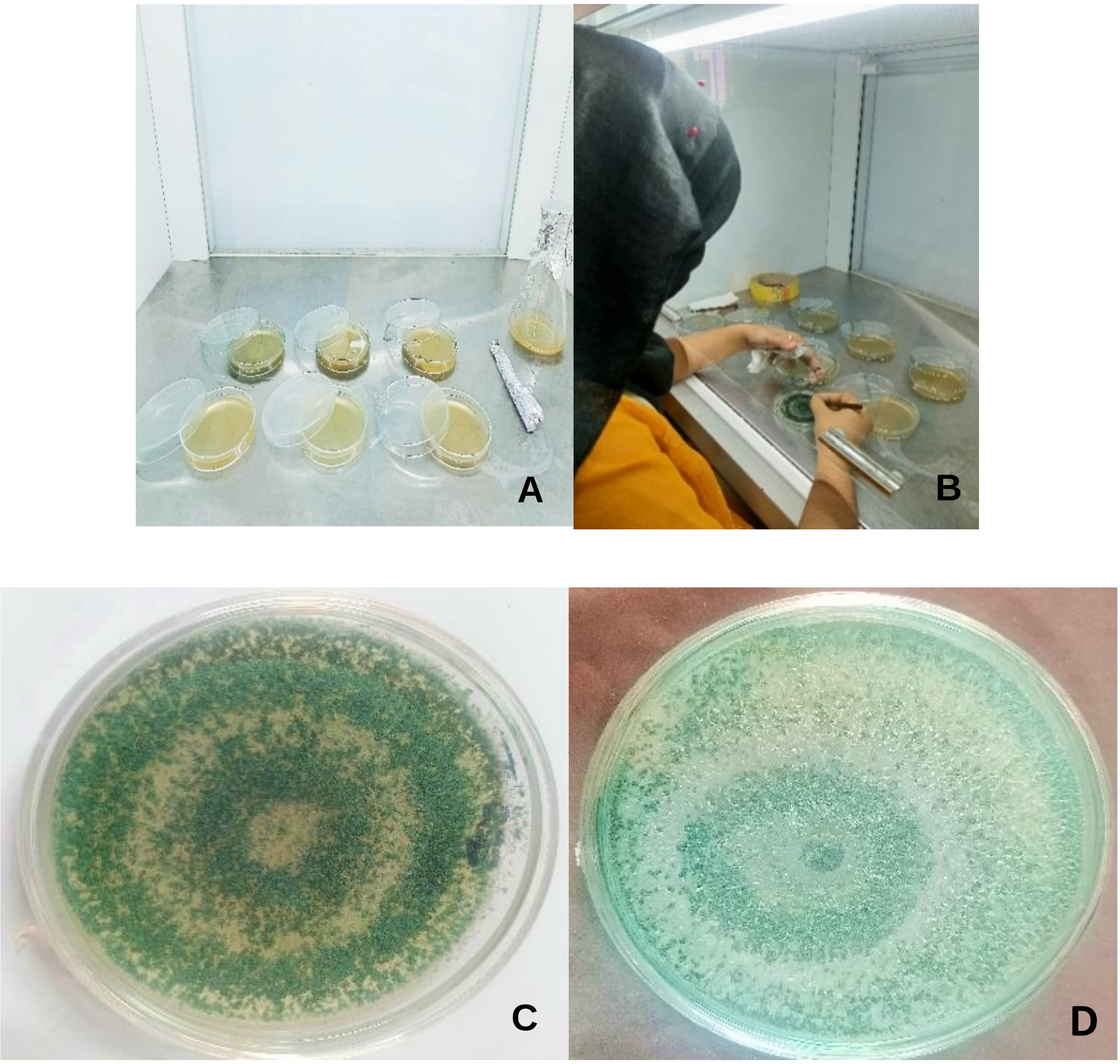


Figure 1. Preparation of *Trichoderma* A. PDA media for pure culture, B. Prepared pure culture in laminar air flow, C. Obtained pure culture of *Trichoderma harzianum* after 7 days, and D. Obtained pure culture of *Trichoderma asperellum* after 7 days

3.2.1 Preparation of formulated *Trichoderma*

Charcoal was prepared by soaking tap water for four hours. After soaking, 200g of substrates were poured into 500 mL conical flasks and autoclaved at 121ºC with 15 PSI for 15 minutes. Each conical flask was inoculated with five mycelia discs of 7 days old culture of *T. asperellum* and *T. harzianum*. After incubation at room temperature, conical flasks were opened and colonized materials were air dried and packaged for soil application (Islam *et al.*, 2007) (Fig. 2).

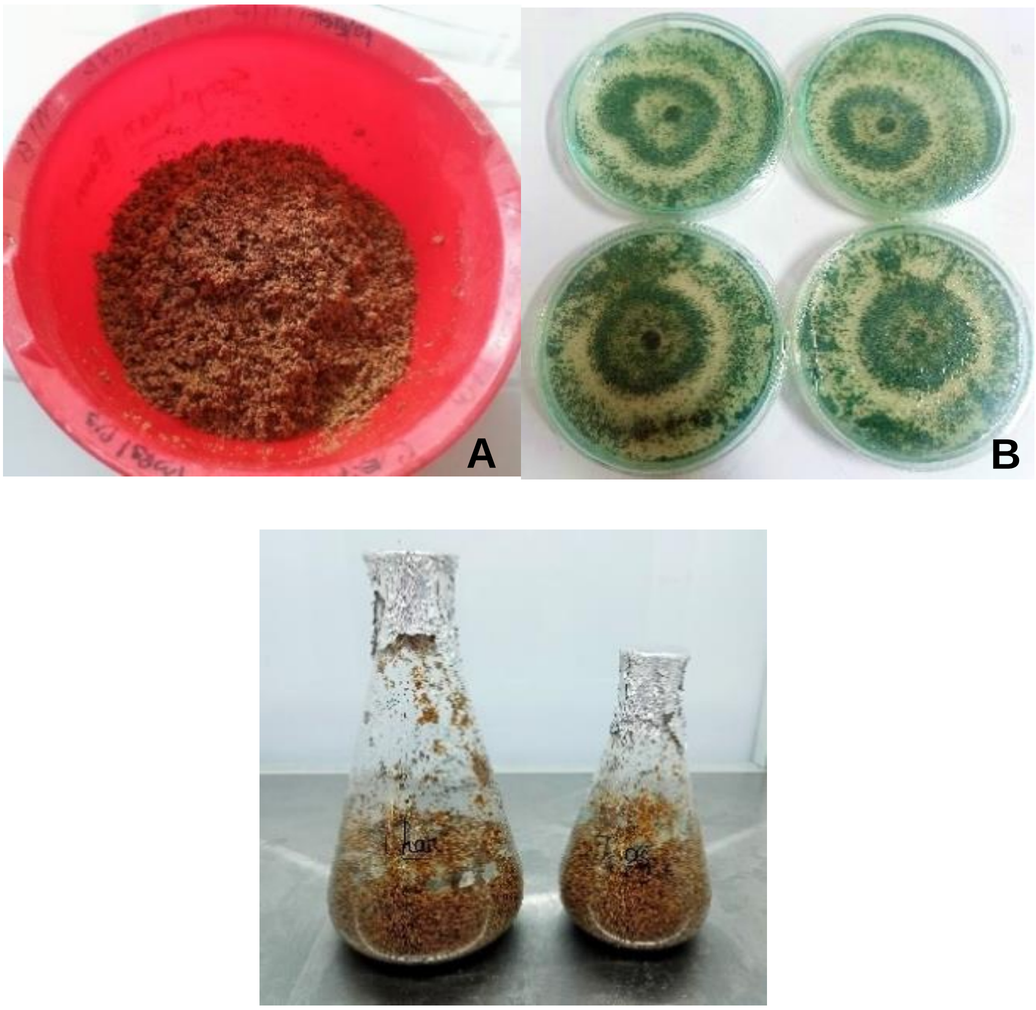


Figure 2. Preparation of charcoal-based formulated *Trichoderma* (*T. asperellum* and *T. harzianum*) (A. Water-soaked charcoal, B. 7 days old pure culture of *Trichoderma harzianum* and *Trichoderma asperellum*, and C. Prepared charcoal-based *Trichoderma*.

3.2.2 Preparation of antioxidant solution

The antioxidants salicylic acid and tartaric acid were dissolved in distilled water to obtain the desired concentrations of 4 mM and 10 mM, respectively.

3.3 Application of *Trichoderma* spp. and antioxidants to enhance the systemic resistance against brown Rust and fusarium Wilt disease of yard-long bean

3.3.1 Experimental site

Geographically the experimental site was located at 25038/ N latitude and 88041/ E longitude, with a height of 38 m above the sea level belonging to Agroecological Zone-I (AEZ-I) named old Himalayan piedmont plain. Yard-long bean performs best on well-drained sandy loams or sandy soils where soil pH ranges from 5.5 to 6.5 (Davis *et al*., 1991) and can grow well in temperature from 20 to 35 °C (Valenzuela and Smith, 2002).

3.3.2 Experimental period

The experiments were carried out during the period of March,2023 to June, 2023.

3.3.3 Preparation of pot soil and potting

Sandy loam soil, sand and well decomposed cow-dung were collected and mixed properly at the ratio of 2:2:1 and then the mixed soil was sterilized with formalin @ 3% per cubic feet soil. The treated soil was covered with brown paper for 72 hours without disturbance. After 72 hours, the brown paper was removed and the sterilized soil was exposed to air drying for 48 hours in order to remove excess vapour of formalin. Total 36 plastic pots (20 cm diameter) were washed with water and sterilized with formalin. After the sterilization, each pot was filled with 3 Kg dried sterilized mixed soil.

3.3.4 Sowing of seeds

Before sowing the seeds, surface sterilization with low concentration of mercuric chloride solution (0.001%) for 1 minute and subsequently washing was done with water for thrice. Five (5) seeds were sown in each plastic pot containing sterilized soil.

3.3.5 Treatments Combinations

T. asperellum, *T. harzianum,* salicylic acid and tartaric acid were applied in order to enhance the systemic acquired resistance in yard-long bean against Rust and Wilt disease. However, the experiments comprised of twelve (12) treatments were as follows:

T0: Control
T_1: Charcoal-based *T. asperellum*
T_2: Salicylic Acid (SA) (4.0 mM)
T_3: Tartaric Acid (TA) (10.0 mM)
T_4: Charcoal-based *T. asperellum* with SA
T_5: Charcoal-based *T. asperellum* with TA
T_6: Charcoal-based *T. asperellum* with SA and TA
T_7: Charcoal-based *T. harzianum*
T_8: Charcoal-based *T. harzianum* with SA
T_9: Charcoal-based *T. harzianum* with TA
T_{10}: Charcoal-based *T. harzianum* with SA and TA
T_{11}: Carbendazim

3.3.6 Experimental design and layout

The experiment was laid out following Completely Randomized Design (CRD) with three replications. There were 36 pots altogether in the experiment where pot to pot distance was 10 cm.

3.3.7 Application of formulated *Trichoderma*

Trichoderma formulations (@5g/pot) were applied into the soil near the root zone of long yard-bean seedling of 7 days old when true leaves unfolded. *Trichoderma* formulations were again applied after 14 and 21 days after transplantation (DAT) in the soil near the root zone of yard-long bean seedling (Fig. 3).

3.3.8 Application of antioxidants

When the true leaves started to unfolded 7 days after sowing, long-yard bean seedlings of relatively consistent size were supplemented with antioxidants solutions of salicylic acid (4 mM) and tartaric acid (10 mM) through root (Fig. 3).

Figure 3. Application of formulated *Trichoderma* (*T. asperellum* and *T. harzianum*) and antioxidants in yard-long bean plants (A. *Trichoderma* formulations, antioxidants, and carbendazim treatment samples. B. Application of *Trichoderma* formulations, antioxidants, and carbendazim treatment in yard-long bean plants).

3.3.9 Stacking

Stacking was done with bamboo stick for each long yard bean plant.

3.3.10 Application of fertilizers

Well decomposed cow dung was applied at the time of final soil preparation. The sources of N, P and K fertilizers were urea (180mg/pot), TSP (90mg/pot), MP (75 mg/pot), respectively (Chowdhury and Hassan, 2013). The entire amounts of TSP, MP were applied during soil preparation where urea was applied in two equal instalments at 20 and 30 days after sowing of seeds.

3.3.11 Application of pesticides

In order to prevent insect infestation, pesticides were applied when needed.

3.3.12 Interculture operations

Weeding, thinning and watering were done as and when necessary.

3.4 Data Collection

3.4.1 Recording of disease

The disease resistance indexes included plant survival rate, disease incidence rate, disease severity, and control effect. Disease incidence and disease severity of Brown Rust and Fusarium Wilt disease was evaluated at 15, 30, and 45 DAT. Disease incidence was recorded as the number of plants infected by Brown rust and Fusarium wilt disease out of thirty-six plants. Disease assessment was done using a 6-point Godoy *et al.* (1997) and Arafa *et al.* (2016) rating scale in terms of abundance of the Rust disease on the plants.

Brown Rust disease grading scale:

Rating Scale	Foliage affected	Description
0	Less than 1%	No pustules on the leaves
1	1-10%	Pustules are small, round, powdery brown uredospores covering 1 per cent or less than the leaf area
2	11-25%	Rust pustules covering 11 to 25 per cent of the leaf area
3	26--50%	26 to 50 per cent of the leaf area
4	51-75%	51 to 75 per cent of the leaf area
5	More than 75%	75 per cent or more of the leaf area

Disease severity was determined on a 6-point rating scale in terms of abundance of the Fusarium Wilt disease on the plants using the modified method of Lebeda and Buczkowski, (1986).

Fusarium Wilt disease grading scale:

Rating Scale	Percent Infection	Description
0	Less than 1%	Whole plants with no visible symptoms
1	1-5%	Very mild Wilting, 1-5% Wilt diseasing of one or two leaves
2	6-10%	Mild Wilting, 6 – 10% plant tissue Wilt diseased, few leaves affected
3	11-20%	Moderate Wilting, 11 – 20% yellowing of lower leaves and necrosis
4	21-50%	Severe Wilting, 21 – 50% plant tissue Wilt diseased, drooping of leaves
5	More than 50%	Very severe Wilting, >50% plant tissue Wilt diseased, drooping and death

Disease incidence and severity were calculated as follows:

$$\text{Disease incidence (DI\%)} = \frac{\text{No. of infected plant parts}}{\text{Total number of plants parts}} \times 100$$

$$\text{Disease severity (DS)} = \frac{\text{Sum of individual ratings}}{\text{Number of plants assessed} \times \text{highest rating in the scale}} \times 100$$

3.4.2 Plant height (cm)

The plant height (cm) was measured by scale from ground level to tip of the plant expressed in centimetres and mean was computed at 30, 45, and 60 days after the sowing of seeds.

3.4.3 Number of fruits per plant

Total number of marketable fruits was harvested. Total number of fruits was counted and the average number of fruits per plant was calculated. Fruits were picked on the basis of horticultural maturity, size, colour and age being determined for the purpose of consumption as the fruit grew rapidly and soon get beyond the marketable stage. Picking was done throughout the harvesting period.

3.4.4 Weight (Kg/plant) of fruits

Weight (Kg) of pods/plant was recorded from one plant selected randomly for each replication and total yield was calculated through average plant yield by multiplying plant population.

3.4.5 Estimation of chlorophyll content of leaves

Chlorophyll content of 2^{nd} leaf from the top of each treatment was estimated according to Witham *et al.* (1971). In brief, 0.25g leaf tissue from middle of the leaf was taken in brown bottles containing 25 mL of 80% aqueous acetone. The bottles were kept in dark for 48 hours. The optical density of the coloured solutions was determined against 80% acetone as blank using spectrophotometer (SPECTRO UV-VIS RS Spectrophotometer, Labo Med, Inc.) at 645 nm and 663 nm. Total chlorophyll was measured using the following formula:

Total chlorophyll = [20.2*D (645nm) + 8.02*D (663nm)] *[V/W*1000]

Where,

V=Volume of 80% aqueous acetone (mL)

W=Weight of fresh leaf (g)

D645=Absorbance at 645 nm wave length

D663= Absorbance at wave 663 nm length

3.4.6 Statistical analysis

Data obtained from different parameters were statistically analysed to find out the level of effectiveness of different treatments to control Rust and Fusarium Wilt disease. The analysis of variance was performed by using Statistix 10 software. The mean difference among the treatment were estimated by DMRT (Duncan`s Multiple Range Test) at 5% level of probability (Gomez and Gomez, 1984)

CHAPTER IV

RESULT

The present study aimed to evaluate the efficacy of different formulated *Trichoderma* alone or in combination with different antioxidants against Brown rust and Fusarium wilt disease of yard-long bean. The efficacy of different treatment combinations to control the Brown rust and Fusarium wilt disease are as follows:

4.1 Efficacy of *Trichoderma* spp. and antioxidants against Rust disease incidence (%) at 30 DAS

Triple combination of *T. asperellum*, salicylic acid and tartaric acid showed the statistically significant lowest Rust disease incidence (16.57 %) followed by the triple combination of *T. harzianum*, salicylic acid and tartaric acid (25.84 %). These results were followed by the application of the combination of *T. asperellum* with salicylic acid and *T. harzianum* with salicylic acid (33.13 % and 36.31 %); the combination of *T. asperellum* with tartaric acid and *T. harzianum* with tartaric acid (39.21 % and 44.66 %); single use of salicylic acid and tartaric acid (54.76 % and 58.48 %); single application of *T. asperellum* and *T. harzianum* (67.78 % and 69.91 %). The control showed the highest result of disease incidence (76.66 %) (Table 1).

Table 1. Efficacy of *Trichoderma* spp. and antioxidants against Rust disease incidence (%) at 30 DAS**.**

Treatments	**Rust disease incidence (%)**	
	At 30 DAS	**Reduction over control (%)**
T_0	76.66a ± 2.36	00
T_1	67.78b ± 1.57	11.58
T_2	54.76c ± 3.37	28.57
T_3	58.48c ± 2.44	23.72
T_4	33.13f ± 1.85	56.78
T_5	39.21e ± 1.78	48.85
T_6	16.57h ± 1.82	78.38
T_7	69.91b ± 2.29	8.81
T_8	36.31ef ± 0.84	52.63
T_9	44.66d ± 2.11	41.74
T_{10}	25.84g ± 3.31	66.29
T_{11}	9.81i ± 1.95	87.20
LSD (0.05)	4.63	

*Means ± Standard deviation followed by the same letter did not differ at 5% level of probability (T_0= Control, T_1= Charcoal-based *T. asperellum*, T_2= Salicylic Acid (SA) (4.0 mM), T_3= Tartaric Acid (TA) (10.0 mM), T_4= Charcoal-based *T. asperellum* with SA, T_5= Charcoal-based *T. asperellum* with TA, T_6= Charcoal-based *T. asperellum* with SA and TA, T_7= Charcoal-based *T. harzianum*, T_8= Charcoal-based *T. harzianum with* SA, T_9= Charcoal-based *T. harzianum* with TA, T_{10}= Charcoal-based *T. harzianum with* SA and TA and T_{11}= Carbendazim).

4.1.1 Efficacy of *Trichoderma* spp. and antioxidants against Rust disease incidence (%) at 45 DAS

Triple combination of *T. asperellum*, salicylic acid and tartaric acid showed the statistically significant lowest Rust disease incidence (21.23 %). These results were followed by the application of the triple combination of *T. harzianum*, salicylic acid and tartaric acid (30.82 %); the combination of *T. asperellum* with salicylic acid and *T. harzianum* with salicylic acid (34.78 % and 46.61 %); the combination of *T. asperellum* with tartaric acid and *T. harzianum* with tartaric acid (58.75 % and 68.07 %); single use of salicylic acid and tartaric acid (72.82 % and 73.69 %); single application of *T. asperellum* and *T. harzianum* (81.18 % and 83.91 %). The control showed the highest result of disease incidence (87.88 %) (Table 2).

Table 2. Efficacy of *Trichoderma* spp. and antioxidants against Rust disease incidence (%) at 45 DAS.

Treatments	Rust disease incidence (%)	
	At 45 DAS	Reduction over control (%)
T_0	87.877a ± 1.18	00
T_1	81.187b ± 1.08	7.6
T_2	72.827c ± 0.89	17.1
T_3	73.690c ± 1.69	16.15
T_4	34.787g ± 1.07	60.41
T_5	58.750e ± 1.33	33.18
T_6	21.233i ± 1.78	75.85
T_7	83.913b ± 1.60	4.52
T_8	46.610f ± 2.48	46.96
T_9	68.077d ± 2.79	22.54
T_{10}	30.817h ± 2.11	64.94
T_{11}	13.617j ± 1.29	84.51
LSD (0.05)	3.52	

*Means ± Standard deviation followed by the same letter did not differ at 5% level of probability (T_0= Control, T_1= Charcoal-based *T. asperellum*, T_2= Salicylic Acid (SA) (4.0 mM), T_3= Tartaric Acid (TA) (10.0 mM), T_4= Charcoal-based *T. asperellum* with SA, T_5= Charcoal-based *T. asperellum* with TA, T_6= Charcoal-based *T. asperellum* with SA and TA, T_7= Charcoal-based *T. harzianum*, T_8= Charcoal-based *T. harzianum with* SA, T_9= Charcoal-based *T. harzianum* with TA, T_{10}= Charcoal-based *T. harzianum with* SA and TA and T_{11}= Carbendazim).

4.1.2 Efficacy of *Trichoderma* spp. and antioxidants against Rust disease incidence (%) at 60 DAS.

Triple combination of *T. asperellum*, salicylic acid and tartaric acid showed the statistically significant lowest Rust disease incidence (23.24 %). These results were followed by the application of the triple combination of *T. harzianum*, salicylic acid and tartaric acid (32.38 %); the combination of *T. asperellum* with salicylic acid and *T. harzianum* with salicylic acid (44.96 % and 50.63 %); the combination of *T. asperellum* with tartaric acid and *T. harzianum* with tartaric acid (60.41 % and 64.06 %); single use of salicylic acid and tartaric acid (74.43 % and 77.79 %); single application of *T. asperellum* and *T. harzianum* (81 % and 84.26 %). The control showed the highest result of disease incidence (93.02 %) (Table 3).

Table 3. Efficacy of *Trichoderma* spp. and antioxidants against Rust disease incidence (%) at 60 DAS.

Treatments	Rust disease incidence (%)	
	At 60 DAS	Reduction over control (%)
T_0	93.02a ± 2.07	00
T_1	81.00bc±0.99	12.92
T_2	74.43d ± 1.72	19.98
T_3	77.79c ± 0.85	16.37
T_4	44.96h ± 2.48	51.67
T_5	60.41f ± 1.29	35.06
T_6	23.24j ± 1.81	75.02
T_7	84.26b ± 2.25	9.42
T_8	50.63g ± 1.15	45.57
T_9	64.06e ± 0.94	31.13
T_{10}	32.38i ± 1.07	65.19
T_{11}	12.78k ± 1.38	86.26
LSD (0.05)	3.29	

*Means ± Standard deviation followed by the same letter did not differ at 5% level of probability (T_0= Control, T_1= Charcoal-based *T. asperellum*, T_2= Salicylic Acid (SA) (4.0 mM), T_3= Tartaric Acid (TA) (10.0 mM), T_4= Charcoal-based *T. asperellum* with SA, T_5= Charcoal-based *T. asperellum* with TA, T_6= Charcoal-based *T. asperellum* with SA and TA, T_7= Charcoal-based *T. harzianum,* T_8= Charcoal-based *T. harzianum with* SA, T_9= Charcoal-based *T. harzianum* with TA, T_{10}= Charcoal-based *T. harzianum with* SA and TA and T_{11}= Carbendazim).

4.2 Efficacy of *Trichoderma* spp. and antioxidants against Rust disease severity (%) at 60 DAS

Triple combination of *T. asperellum*, salicylic acid and tartaric acid showed the statistically significant lowest Rust disease severity (21.33 %) with the triple combination of *T. harzianum*, salicylic acid and tartaric acid (24.33 %). These results were followed by the application of the combination of *T. asperellum* with salicylic acid and *T. harzianum* with salicylic acid (31.33 % and 32 %); the combination of *T. asperellum* with tartaric acid and *T. harzianum* with tartaric acid (35.33 % and 37.33 %); single use of salicylic acid and tartaric acid (43.33 % and 47.33 %); single application of *T. asperellum* and *T. harzianum* (68 % and 72.67 %). The control showed the highest result of disease severity (86.67 %) (Table 4).

Table 4. Efficacy of *Trichoderma* spp. and antioxidants against Rust disease severity (%) at 60 DAS.

Treatments	Rust disease severity (%)	
	At 60 DAS	Reduction over control (%)
T_0	86.67a ± 2.49	00
T_1	68b ± 2.94	21.54
T_2	43.33c ± 3.39	50.00
T_3	47.33c ± 2.05	45.39
T_4	31.33e ± 1.88	63.85
T_5	35.33de ± 2.49	59.24
T_6	21.33f ± 1.89	75.38
T_7	72.67b ± 0.94	16.15
T_8	32e ± 2.83	63.07
T_9	37.33d ± 2.49	56.92
T_{10}	24.33f ± 3.68	71.92
T_{11}	13.33g ± 2.49	84.62
LSD (0.05)	5.29	

*Means ± Standard deviation followed by the same letter did not differ at 5% level of probability (T_0= Control, T_1= Charcoal-based *T. asperellum*, T_2= Salicylic Acid (SA) (4.0 mM), T_3= Tartaric Acid (TA) (10.0 mM), T_4= Charcoal-based *T. asperellum* with SA, T_5= Charcoal-based *T. asperellum* with TA, T_6= Charcoal-based *T. asperellum* with SA and TA, T_7= Charcoal-based *T. harzianum*, T_8= Charcoal-based *T. harzianum with* SA, T_9= Charcoal-based *T. harzianum* with TA, T_{10}= Charcoal-based *T. harzianum with* SA and TA and T_{11}= Carbendazim).

4.3 Efficacy of *Trichoderma* spp. and antioxidants against Fusarium Wilt disease incidence (%) at 30 DAS

Triple combination of *T. asperellum*, salicylic acid and tartaric acid showed the statistically significant lowest Wilt disease incidence (3.32 %) with the triple combination of *T. harzianum*, salicylic acid and tartaric acid (3.73 %). These results were followed by the application of the combination of *T. asperellum* with salicylic acid and *T. harzianum* with salicylic acid (5.54 % and 5.96 %); the combination of *T. asperellum* with tartaric acid and *T. harzianum* with tartaric acid (6.61 % and 7.34 %); single use of salicylic acid and tartaric acid (8.83 % and 9.80 %); single application of *T. asperellum* and *T. harzianum* (12.56 % and 13.30 %). The control showed the highest result of disease incidence (16.67 %) (Table 5).

Table 5. Efficacy of *Trichoderma* spp. and antioxidants against Fusarium wilt disease incidence (%) at 30 DAS.

Treatments	Wilt disease incidence (%)	
	At 30 DAS	Reduction over control (%)
T_0	16.67a ± 1.07	00
T_1	12.56b ± 0.75	24.66
T_2	8.83c ± 0.65	47.03
T_3	9.80c ± 0.87	41.21
T_4	5.54f ± 0.29	66.76
T_5	6.61e ± 1.29	60.34
T_6	3.32g ± 0.29	80.08
T_7	13.30b ± 1.6	20.21
T_8	5.96e ± 1.26	64.25
T_9	7.34d ± 0.87	55.97
T_{10}	3.73g ± 0.72	77.63
T_{11}	1.65h ± 0.72	90.10
LSD (0.05)	2.85	

*Means ± Standard deviation followed by the same letter did not differ at 5% level of probability (T_0= Control, T_1= Charcoal-based *T. asperellum*, T_2= Salicylic Acid (SA) (4.0 mM), T_3= Tartaric Acid (TA) (10.0 mM), T_4= Charcoal-based *T. asperellum* with SA, T_5= Charcoal-based *T. asperellum* with TA, T_6= Charcoal-based *T. asperellum* with SA and TA, T_7= Charcoal-based *T. harzianum*, T_8= Charcoal-based *T. harzianum with* SA, T_9= Charcoal-based *T. harzianum* with TA, T_{10}= Charcoal-based *T. harzianum with* SA and TA and T_{11}= Carbendazim).

4.3.1 Efficacy of *Trichoderma* spp. and antioxidants against Fusarium wilt disease incidence (%) at 45 DAS

Triple combination of *T. asperellum*, salicylic acid and tartaric acid showed the statistically significant lowest Wilt disease incidence (5.57 %) with the triple combination of *T. harzianum*, salicylic acid and tartaric acid (6.24 %). These results were followed by the application of the combination of *T. asperellum* with salicylic acid and *T. harzianum* with salicylic acid (7.72 % and 8.81 %); the combination of *T. asperellum* with tartaric acid and *T. harzianum* with tartaric acid (9.21 % and 11.50 %); single use of salicylic acid and tartaric acid (12.31 % and 13.67 %); single application of *T. asperellum* and *T. harzianum* (14.42 % and 16.10 %). The control showed the highest result of disease incidence (19.30 %) (Table 6).

Table 6. Efficacy of *Trichoderma* spp. and antioxidants against Fusarium wilt disease incidence (%) at 45 DAS.

Treatments	**Wilt disease incidence (%)**	
	At 45 DAS	**Reduction over control (%)**
T_0	19.30a ± 1.25	00
T_1	14.42bc ± 0.50	25.28
T_2	12.31cd ± 0.87	36.22
T_3	13.67cd ± 0.87	29.17
T_4	7.72f ± 0.50	60
T_5	9.21e ± 0.75	52.28
T_6	5.57g ± 0.75	71.14
T_7	16.10b ± 1.00	16.58
T_8	8.81f ± 0.50	54.35
T_9	11.50e ± 0.21	40.41
T_{10}	6.24g ± 0.87	67.67
T_{11}	3.87h ± 1.37	79.95
LSD (0.05)	2.25	

*Means ± Standard deviation followed by the same letter did not differ at 5% level of probability (T_0= Control, T_1= Charcoal-based *T. asperellum*, T_2= Salicylic Acid (SA) (4.0 mM), T_3= Tartaric Acid (TA) (10.0 mM), T_4= Charcoal-based *T. asperellum* with SA, T_5= Charcoal-based *T. asperellum* with TA, T_6= Charcoal-based *T. asperellum* with SA and TA, T_7= Charcoal-based *T. harzianum,* T_8= Charcoal-based *T. harzianum with* SA, T_9= Charcoal-based *T. harzianum* with TA, T_{10}= Charcoal-based *T. harzianum with* SA and TA and T_{11}= Carbendazim).

4.3.2 Efficacy of *Trichoderma* spp. and antioxidants against Fusarium wilt disease incidence (%) at 60 DAS

Triple combination of *T. asperellum*, salicylic acid and tartaric acid showed the statistically significant lowest Wilt disease incidence (7.33 %). These results were followed by the application of the triple combination of *T. harzianum*, salicylic acid and tartaric acid (8.31 %); the combination of *T. asperellum* with salicylic acid and *T. harzianum* with salicylic acid (10.39% and 11.22 %); the combination of *T. asperellum* with tartaric acid and *T. harzianum* with tartaric acid (13.14 % and 14.74 %); single use of salicylic acid and tartaric acid (16.97 % and 17.32 %); single application of *T.asperellum* and *T. harzianum* (19.72 % and 20.39 %). The control showed the highest result of disease incidence (6.18 %) (Table 7).

Table 7. Efficacy of *Trichoderma* spp. and antioxidants against Fusarium wilt disease incidence (%) at 60 DAS.

Treatments	**Wilt disease incidence (%)**	
	At 60 DAS	**Reduction over control (%)**
T_0	24.32a ± 1.30	00
T_1	19.72b ± 0.83	18.91
T_2	16.97c ± 0.80	30.22
T_3	17.32c ± 1.26	28.78
T_4	10.39f ± 0.77	57.28
T_5	13.14e ± 0.46	45.97
T_6	7.33gh ± 0.10	69.86
T_7	20.39b ± 0.77	16.16
T_8	11.22f ± 0.72	53.87
T_9	14.74d ± 0.22	39.39
T_{10}	8.31g ± 0.29	65.83
T_{11}	6.18 ± 0.53	74.59
LSD (0.05)	1.57	

*Means ± Standard deviation followed by the same letter did not differ at 5% level of probability (T_0= Control, T_1= Charcoal-based *T. asperellum*, T_2= Salicylic Acid (SA) (4.0 mM), T_3= Tartaric Acid (TA) (10.0 mM), T_4= Charcoal-based *T. asperellum* with SA, T_5= Charcoal-based *T. asperellum* with TA, T_6= Charcoal-based *T. asperellum* with SA and TA, T_7= Charcoal-based *T. harzianum*, T_8= Charcoal-based *T. harzianum with* SA, T_9= Charcoal-based *T. harzianum* with TA, T_{10}= Charcoal-based *T. harzianum with* SA and TA and T_{11}= Carbendazim).

4.4 Efficacy of *Trichoderma* spp. and antioxidants against Fusarium wilt disease severity (%) at 60 DAS

Triple combination of *T. asperellum*, salicylic acid and tartaric acid showed the statistically significant lowest Wilt disease incidence (16.33 %) with the triple combination of *T. harzianum*, salicylic acid and tartaric acid (19 %). These results were followed by the application of the combination of *T. asperellum* with salicylic acid and *T. harzianum* with salicylic acid (18.33 % and 20.66 %); the combination of *T. asperellum* with tartaric acid and *T. harzianum* with tartaric acid (23.33 % and 24.66 %); single use of salicylic acid and tartaric acid (25.66 % and 27.34 %); single application of *T. asperellum* and *T. harzianum* (33.66 % and 39 %). The control showed the highest result of disease incidence (84 %) (Table 8).

Table 8. Efficacy of *Trichoderma* spp. and antioxidants against Fusarium wilt disease severity (%) at 60 DAS

Treatments	Wilt disease severity (%)	
	At 60 DAS	**Reduction over control (%)**
T_0	84.00 a ± 3.60	00
T_1	33.66 c ± 1.15	59.93
T_2	25.66 d ± 1.15	69.45
T_3	27.34d± 1.52	67.07
T_4	18.33 f ± 0.57	78.18
T_5	23.33 e± 1.52	72.23
T_6	16.33 f ± 1.52	80.56
T_7	39.00 b ± 1	53.57
T_8	20.66 e ± 2.08	75.40
T_9	24.33 e ± 0.57	71.04
T_{10}	19.00 f ± 1	77.38
T_{11}	15.33 g ± 1.52	81.75
LSD (0.05)	3.14	

*Means ± Standard deviation followed by the same letter did not differ at 5% level of probability (T_0= Control, T_1= Charcoal-based *T. asperellum*, T_2= Salicylic Acid (SA) (4.0 mM), T_3= Tartaric Acid (TA) (10.0 mM), T_4= Charcoal-based *T. asperellum* with SA, T_5= Charcoal-based *T. asperellum* with TA, T_6= Charcoal-based *T. asperellum* with SA and TA, T_7= Charcoal-based *T. harzianum*, T_8= Charcoal-based *T. harzianum with* SA, T_9= Charcoal-based *T. harzianum* with TA, T_{10}= Charcoal-based *T. harzianum with* SA and TA and T_{11}= Carbendazim).

4.5 Effects of *Trichoderma* spp. and antioxidants on growth attributes of the yard long bean

The effect of all the treatments imposed for the management of Rust *(Uromyces phaseoli typica.)* and Fusarium wilt disease (*Fusarium oxysporum* f. sp. *phaseoli*) disease of yard long bean and their influence on plant growth parameters viz., plant height and leaf number in yard long bean was recorded at 30,45 and 60 DAT are as follows:

4.5.1 Effects of *Trichoderma* spp. and antioxidants on plant height (cm) at 30, 45, and 60 DAS

At 30 DAS, triple combination of *T. asperellum*, salicylic acid and tartaric acid showed the statistically significant highest plant height (54.83 cm). These results were followed by the application of the triple combination of *T.*

harzianum, salicylic acid and tartaric acid (51.63 cm); the combination of *T. asperellum* with salicylic acid and *T. harzianum* with salicylic acid (48.33 cm and 47.67 cm); the combination of *T. asperellum* with tartaric acid and *T. harzianum* with tartaric acid (46.73 cm and 44.60 cm); single use of salicylic acid and tartaric acid (44.27 cm and 42.60 cm); single application of *T. asperellum* and *T. harzianum* (39.53 cm and 33.57 cm). The control showed the lowest result of plant height (17.20 cm) (Table 9).

At 45 DAS, triple combination of *T. asperellum*, salicylic acid and tartaric acid showed the statistically significant highest plant height (146.00 cm) with the triple combination of *T. harzianum*, salicylic acid and tartaric acid (144.50 cm). These results were followed by the application of the combination of *T. asperellum* with salicylic acid and *T. harzianum* with salicylic acid (142.33 cm and 140.23 cm); the combination of *T. asperellum* with tartaric acid and *T. harzianum* with tartaric acid (46.73 cm and 44.60 cm); single use of salicylic acid and tartaric acid (134.17 cm and 131.83 cm); single application of *T. asperellum* and *T. harzianum* (113.17 cm and 111.00 cm). The control showed the lowest result of plant height (92.33 cm) (Table 9).

At 60 DAS, Triple combination of *T. asperellum*, salicylic acid and tartaric acid showed the statistically significant highest plant height (199.43 cm) with the triple combination of *T. harzianum*, salicylic acid and tartaric acid (198.10 cm). These results were followed by the application of the combination of *T. asperellum* with salicylic acid and *T. harzianum* with salicylic acid (194.40 cm and 193.03 cm); the combination of *T. asperellum* with tartaric acid and *T. harzianum* with tartaric acid (190.33 cm and 188.87 cm); single use of salicylic acid and tartaric acid (184.70 cm and 182.20 cm); single application of *T. asperellum* and *T. harzianum* (174.27 cm and 170.17 cm). The control showed the lowest result of plant height (148.8 cm) (Table 9).

Table 9. Effects of *Trichoderma* spp. and antioxidants on plant height (cm) at 30, 45, and 60 DAS

Treatments	**Plant height (cm) at 30 DAS**	**Plant height (cm) at 45 DAS**	**Plant height (cm) at 60 DAS**
T_0	21.20g ± 1.58	92.33g ± 1.46	148.87h ± 1.34
T_1	39.53e ± 3.93	107.10f ± 1.95	174.27f ± 1.35
T_2	44.27cde ± 3.74	113.17e ± 2.36	184.70e ± 1.31
T_3	42.60de ± 1.97	111.00e ± 3.60	182.20e ± 1.12
T_4	48.33bc ± 1.72	142.33bc ± 2.52	194.40c ± 0.78
T_5	46.73cd ± 1.90	134.17d ± 2.25	190.33d ± 1.57
T_6	54.83a ± 3.32	146.00ab ± 2.64	199.43b ± 1.14
T_7	33.57f ± 3.72	105.17f ± 2.00	170.17g ± 1.98
T_8	47.67bc ± 2.08	140.23c ± 1.75	193.03c ± 1.16
T_9	44.60cd ± 2.19	131.83d ± 1.89	188.87d ± 1.14
T_{10}	51.63ab ± 2.43	144.50ab ± 2.29	198.10b ± 1.10
T_{11}	55.90 ± 3.43	147.40a ± 0.79	202.33a ± 0.82
LSD (0.05)	4.76	3.75	2.63

*Means ± Standard deviation followed by the same letter did not differ at 5% level of probability (T_0= Control, T_1= Charcoal-based *T. asperellum*, T_2= Salicylic Acid (SA) (4.0 mM), T_3= Tartaric Acid (TA) (10.0 mM), T_4= Charcoal-based *T. asperellum* with SA, T_5= Charcoal-based *T. asperellum* with TA, T_6= Charcoal-based *T. asperellum* with SA and TA, T_7= Charcoal-based *T. harzianum,* T_8= Charcoal-based *T. harzianum with* SA, T_9= Charcoal-based *T. harzianum* with TA, T_{10}= Charcoal-based *T. harzianum with* SA and TA and T_{11}= Carbendazim).

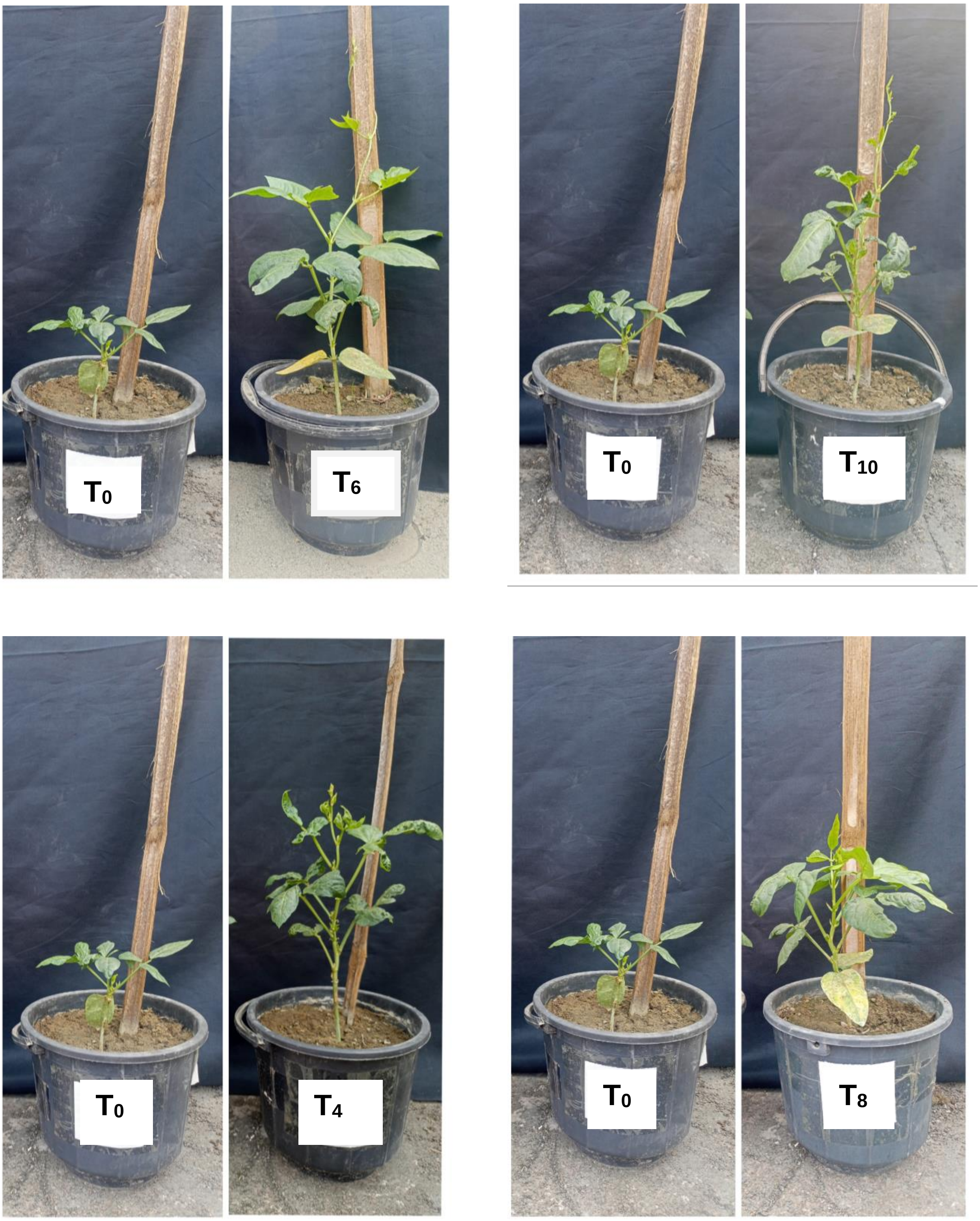


Figure 4. Effects of *Trichoderma* spp. and antioxidants on plant height (cm) at 30 DAS (T_0= Control, T_4= Charcoal-based *T. asperellum* with SA, T_6= Charcoal-based *T. asperellum* with SA and TA, T_8= Charcoal-based *T. harzianum with* SA and T_{10}= Charcoal-based *T. harzianum with* SA and TA)

4.5.2 Efficacy of different treatments on leaf number at 30,45 and 60 DAS

At 30 DAS, all the treatments showed a significant effect on increasing the number of leaves compared to the control. The highest number of leaves was observed from triple combination of *T. asperellum*, salicylic acid and tartaric acid (16). These results were followed by the application of the triple combination of *T. harzianum*, salicylic acid and tartaric acid (15.33); the combination of *T. asperellum* with salicylic acid and *T. harzianum* with salicylic acid (15 and 14.67); the combination of *T. asperellum* with tartaric acid and *T. harzianum* with tartaric acid (13.67 and 12.67); single use of salicylic acid and tartaric acid (12 and 11.33); single application of *T. asperellum* and *T. harzianum* (10.33 and 7.67). The control showed the lowest result of leaf number (4.33) (Table 12).

At 45 DAS, All the treatments showed a significant effect on increasing the number of leaves compared to the control. The highest number of leaves was observed from the triple combination of *T. asperellum*, salicylic acid and tartaric acid (39.33). These results were followed by the application of the triple combination of *T. harzianum*, salicylic acid and tartaric acid (38); the combination of *T. asperellum* with salicylic acid and *T. harzianum* with salicylic acid (37.33 and 35); the combination of *T. asperellum* with tartaric acid and *T. harzianum* with tartaric acid (32.33 and 29.33); single use of salicylic acid and tartaric acid (27 and 25.33); single application of *T. asperellum* and *T. harzianum* (21.33 and 20.67). The control showed the lowest result of leaf number (16.67) (Table 12).

At 60 DAS, all the treatments showed a significant effect on increasing the number of leaves compared to the control. The highest number of leaves was observed from the triple combination of *T. asperellum*, salicylic acid and tartaric acid (60.33). These results were followed by the application of the triple combination of *T. harzianum*, salicylic acid and tartaric acid (58.67); the combination of *T. asperellum* with salicylic acid and *T. harzianum* with salicylic acid (55.67 and 54); the combination of *T. asperellum* with tartaric acid and *T. harzianum* with tartaric acid (49.67 and 47.33); single use of salicylic acid and tartaric acid (41.67 and 39); single application of *T. asperellum* and *T.*

harzianum (33.33 and 31.67). The control showed the lowest result of leaf number (24) (Table 12).

Table 12. Efficacy of *Trichoderma* spp. and antioxidants on leaf number at 30, 45 and 60 DAS

Treatment	**Leaf number at 30 DAS**	**Leaf number at 45 DAS**	**Leaf number at 60 DAS**
T_0	4.33 h ± 0.47	16.67h ± 1.699	24.00h ± 1.63
T_1	10.33fg ± 1.25	21.33g ± 1.24	33.33g ± 1.69
T_2	12.00def ± 1.63	27.00ef ± 1.63	41.67e ± 1.24
T_3	11.33ef ± 1.69	25.33f ± 0.47	39.00f ± 0.82
T_4	15.00abc ± 1.63	37.33bc ± 1.24	55.67c ± 1.24
T_5	13.67bcde ± 1.69	32.33d ± 0.94	49.67d ± 0.81
T_6	16.00ab ± 1.63	39.33ab ± 1.24	60.33ab ± 0.47
T_7	7.67g ± 0.94	20.67g ± 0.94	31.67g ± 1.24
T_8	14.67abcd ± 0.94	35.00c ± 1.23	54.00c ± 0.81
T_9	12.67cdef ± 0.47	29.33e ± 1.47	47.33d ± 1.63
T_{10}	15.33abc ± 1.25	38.00b ± 0.81	58.67b ± 1.69
T_{11}	16.67a ± 2.05	41.67a ± 1.24	62.67a ± 0.94
LSD (0.05)	2.87	2.48	2.62

*Means ± Standard deviation followed by the same letter did not differ at 5% level of probability (T_0= Control, T_1= Charcoal-based *T. asperellum*, T_2= Salicylic Acid (SA) (4.0 mM), T_3= Tartaric Acid (TA) (10.0 mM), T_4= Charcoal-based *T. asperellum* with SA, T_5= Charcoal-based *T. asperellum* with TA, T_6= Charcoal-based *T. asperellum* with SA and TA, T_7= Charcoal-based *T. harzianum,* T_8= Charcoal-based *T. harzianum with* SA, T_9= Charcoal-based *T. harzianum* with TA, T_{10}= Charcoal-based *T. harzianum with* SA and TA and T_{11}= Carbendazim).

4.6 Relation between Rust incidence (%) and leaf number

In the regression, Rust incidence (%) was considered as independent and leaf number per plant as dependent variable. A strong negative relation was found between Rust incidence (%) of leaf and leaf number per plant. Due to Rust disease, (73.34 %) loss in yield per plant was observed. The regression equation also showed that for every one unit increase in Rust incidence, yield reduction of (0.92 %) will be occurred (Fig. 5).

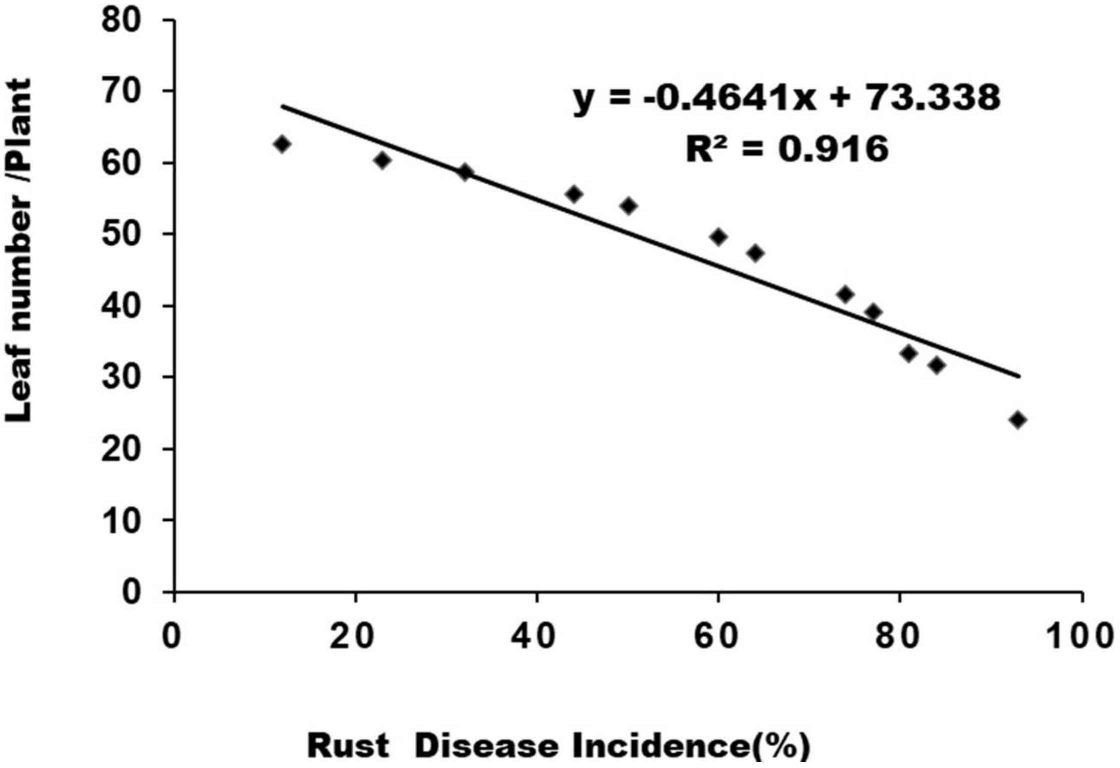


Figure 5. Correlation of co-efficient between Rust disease incidence (%) and leaf number in yard long bean.

4.6.1 Relation between Wilt disease incidence (%) and leaf number

In the regression, Fusarium wilt disease incidence (%) was considered as independent and leaf number per plant as dependent variable. A strong negative relation was found between Wilt disease incidence (%) of leaf and leaf number per plant. Due to Wilt disease, (77.45 %) loss in yield per plant was observed. The regression equation also showed that for every one unit increase in Wilt disease incidence, yield reduction of (0.99 %) will be occurred (Figure 5).

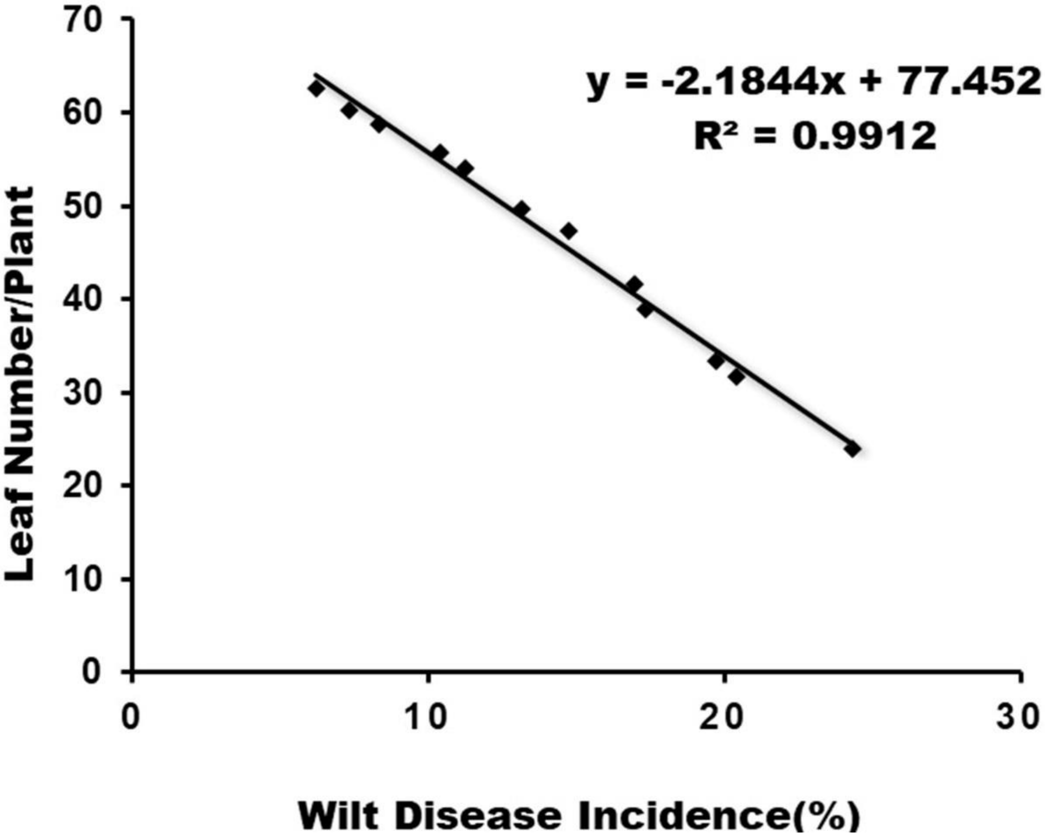


Figure 6. Correlation of co-efficient between Wilt disease incidence (%) and leaf number.

4.6.2 Relation between Rust severity (%) and leaf number

In the regression, Rust severity (%) was considered as independent and the leaf number per plant as dependent variable. A strong negative relation was found between Rust severity (%) of leaf and leaf number per plant. Due to Rust disease, (69.80 %) loss in leaf number per plant was observed. The regression equation also showed that for every one unit increase in Rust severity, yield reduction of (0.96 %) will be occurred (Fig. 6).

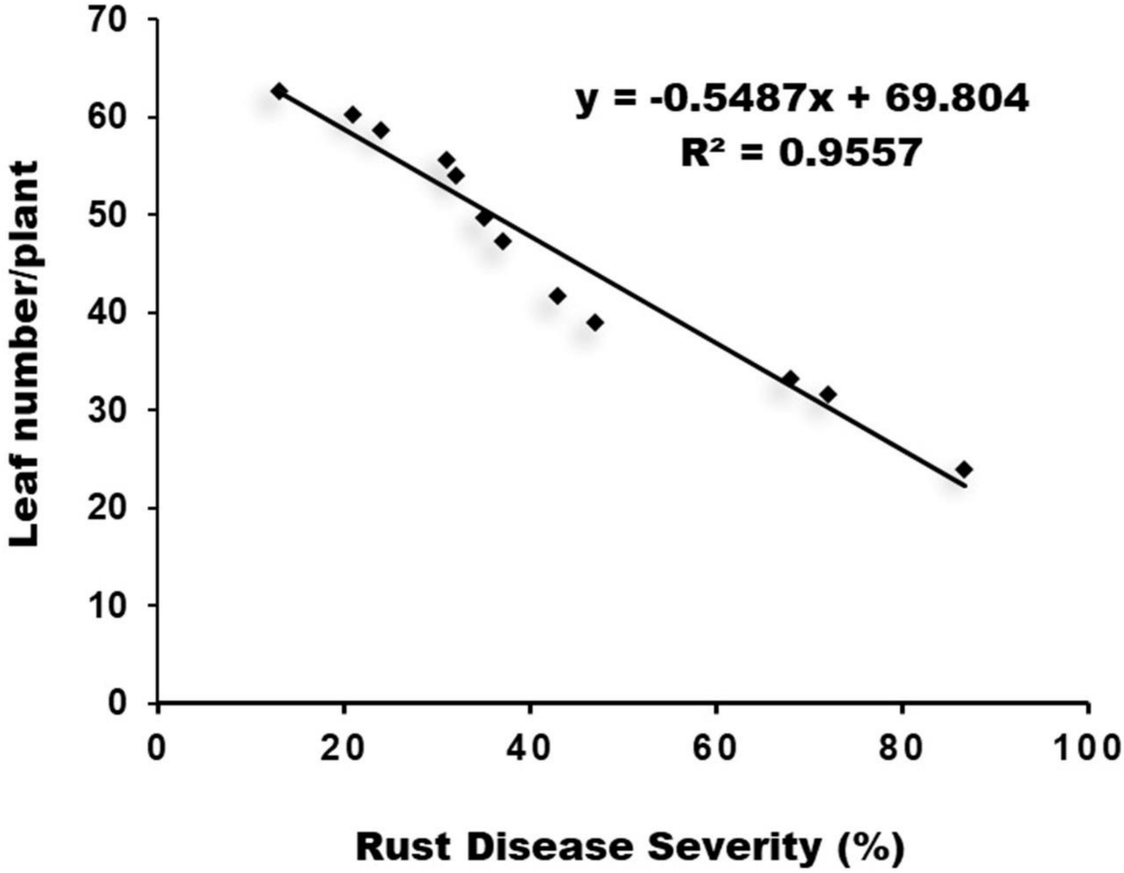


Figure 7. Correlation of co-efficient between Rust disease severity (%) and leaf number.

4.6.3 Relation between Wilt disease severity (%) and leaf number.

In the regression, Fusarium wilt disease severity (%) of leaf was considered as independent and the leaf number per plant as dependent variable. A strong negative relation was found between Wilt disease severity (%) of leaf and leaf number per plant. Due to Wilt disease, (69.80 %) loss in leaf number per plant was observed. The regression equation also showed that for every one unit increase in Wilt disease severity, yield reduction of (0.96 %) will be occurred (Figure 7).

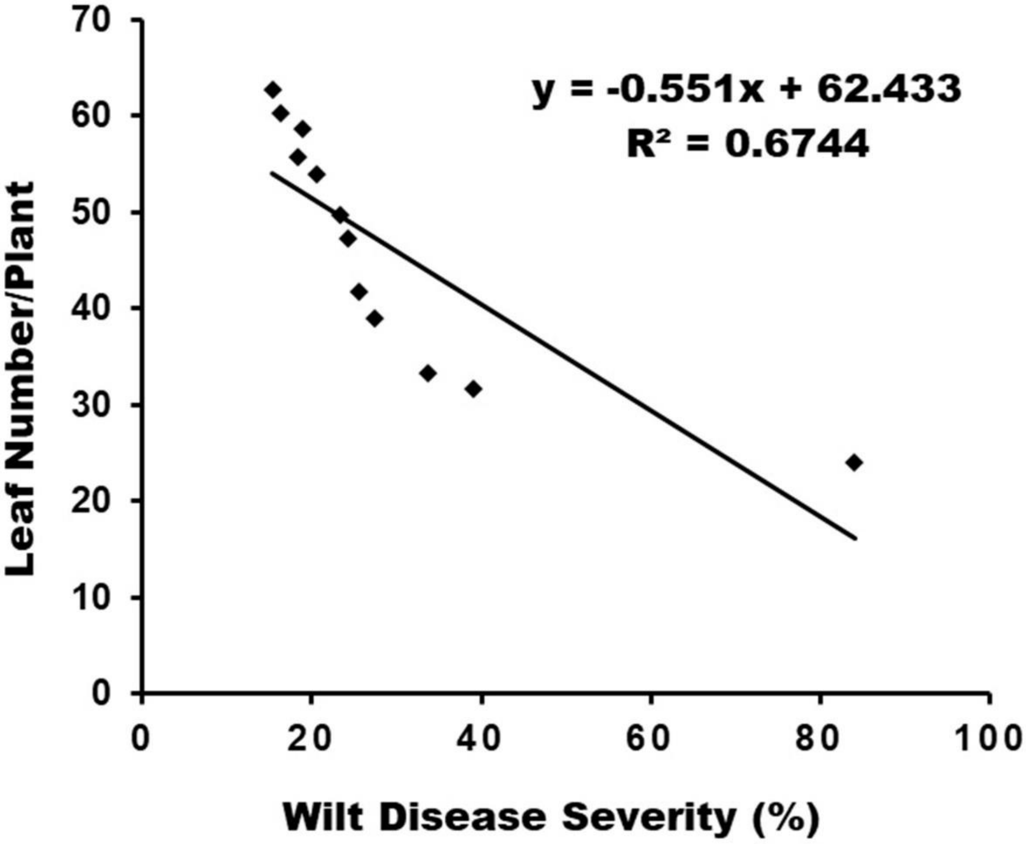
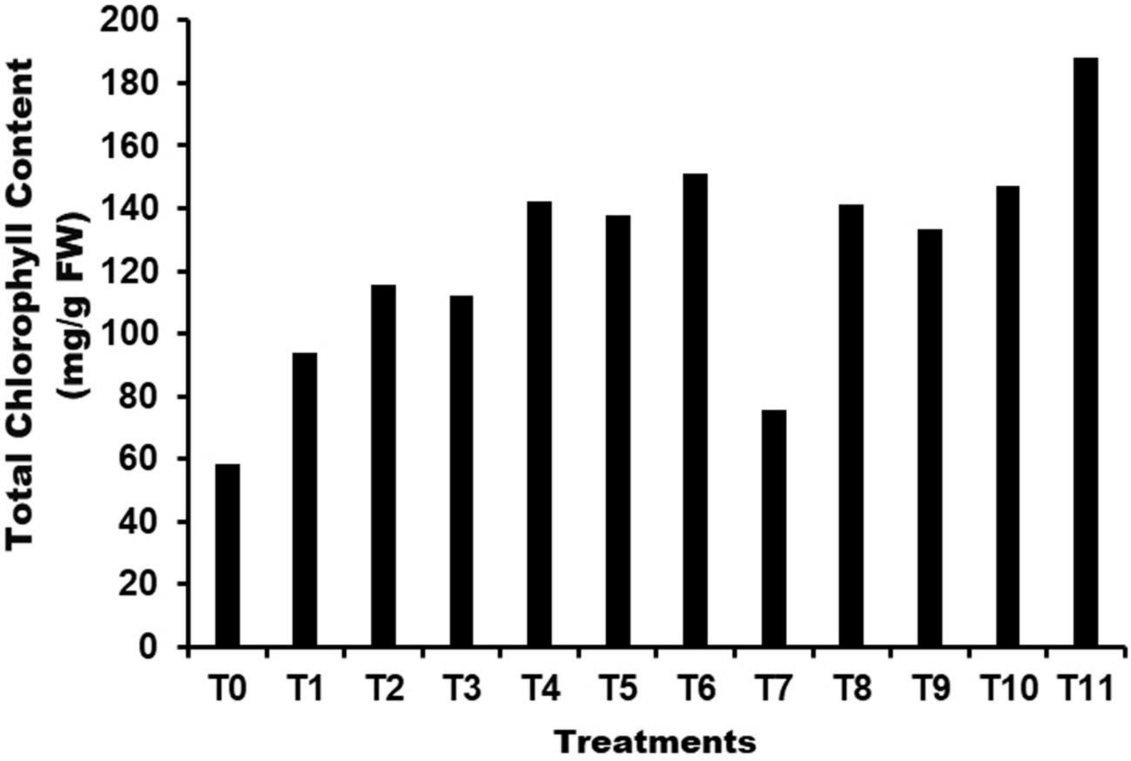


Figure 8. Correlation of co-efficient between Wilt disease severity (%) and leaf number.

4.7 Effects of *Trichoderma* spp. and antioxidants on total chlorophyll content in the leaves.

Triple combination of *T. asperellum*, salicylic acid and tartaric acid showed the statistically significant highest chlorophyll content (151.33 mg/g FW) with the triple combination of *T. harzianum*, salicylic acid and tartaric acid (147.31 mg/g FW). These results were followed by the application of the combination of *T. asperellum* with salicylic acid and *T. harzianum* with salicylic acid (142.15 mg/g FW and 141.36 mg/g FW); the combination of *T. asperellum* with tartaric acid and *T. harzianum* with tartaric acid (137.72 mg/g FW and 133.54 mg/g FW); single use of salicylic acid and tartaric acid (115.44 mg/g FW and 112.29 mg/g FW); single application of *T. asperellum* and *T. harzianum*

(93.89 mg/g FW and 75.47 mg/g FW). The control showed the lowest chlorophyll content (58.29 mg/g FW) (Fig. 8).



*Means ± Standard deviation followed by the same letter did not differ at 5% level of probability (T_0= Control, T_1= Charcoal-based *T. asperellum*, T_2= Salicylic Acid (SA) (4.0 mM), T_3= Tartaric Acid (TA) (10.0 mM), T_4= Charcoal-based *T. asperellum* with SA, T_5= Charcoal-based *T. asperellum* with TA, T_6= Charcoal-based *T. asperellum* with SA and TA, T_7= Charcoal-based *T. harzianum*, T_8= Charcoal-based *T. harzianum with* SA, T_9= Charcoal-based *T. harzianum* with TA, T_{10}= Charcoal-based *T. harzianum with* SA and TA and T_{11}= Carbendazim).

Figure 9. Effects of *Trichoderma* spp. and antioxidants on total chlorophyll content in the leaves.

4.8 Effects of *Trichoderma* spp. and antioxidants on yield contributing characters of yard long bean after 90 days of sowing

Triple combination of *T. asperellum*, salicylic acid and tartaric acid showed the statistically significant highest number of pod/plant and pod length (84.67, 65.63 cm). These results were followed by the application of the triple combination of *T. harzianum*, salicylic acid and tartaric acid (80.67, 64.30 cm); the combination of *T. asperellum* with salicylic acid and *T. harzianum* with salicylic acid (75.67, 63.53 cm and 72.67, 62.83 cm); the combination of *T.*

asperellum with tartaric acid and *T. harzianum* with tartaric acid (67.33, 61.23 cm and 65, 58.30 cm); single use of salicylic acid and tartaric acid (62.67, 56.50 cm and 588.33, 56.07 cm); single application of *T. asperellum* and *T. harzianum* (55.67, 53.77 cm and 51.67, 52.90 cm). The control showed the lowest number of pod/plant and pod length (49.33, 47.17 cm) (Table 13).

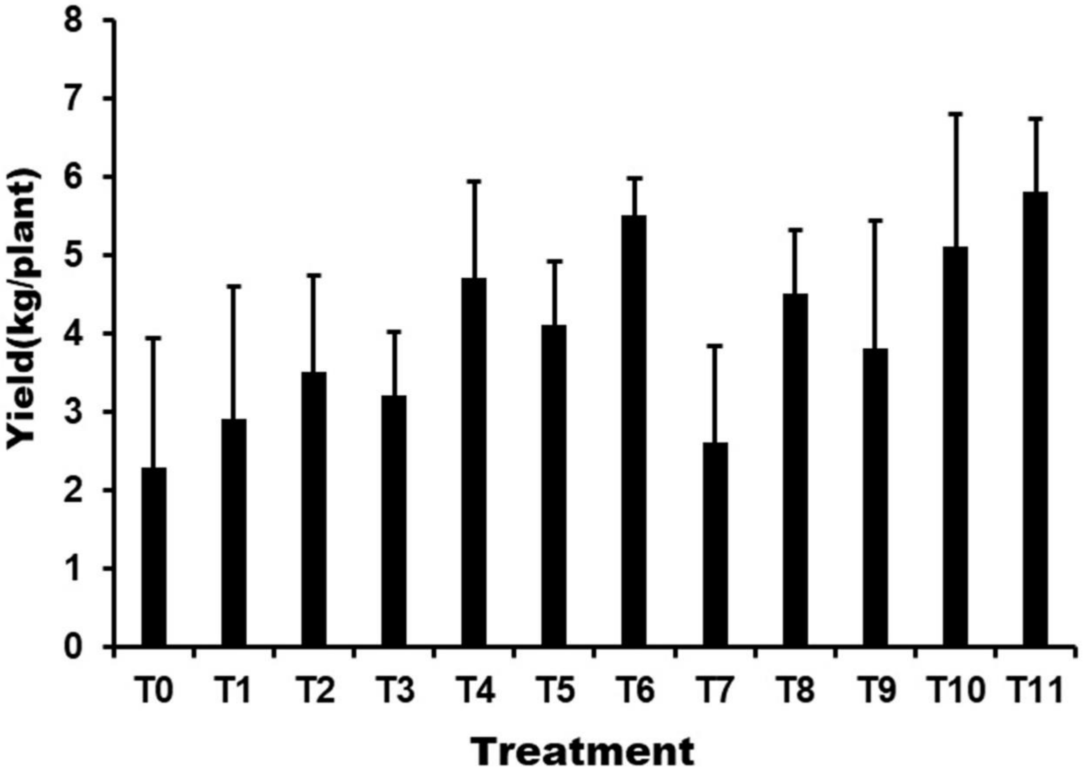
Table 13. Effects of *Trichoderma* spp. and antioxidants on yield contributing characters of yard long bean after 90 days of sowing.

Treatments	Pod number/plant	Pod length (cm)
T_0	49.33h	47.17h
T_1	55.67g	53.77g
T_2	62.67f	56.50ef
T_3	58.33g	56.07f
T_4	75.67d	63.53c
T_5	67.33e	61.23d
T_6	84.67b	65.63ab
T_7	51.67h	52.90g
T_8	72.67d	62.83cd
T_9	65.00ef	58.30e
T_{10}	80.67c	64.30bc
T_{11}	89.00a	66.50a

*Means ± Standard deviation followed by the same letter did not differ at 5% level of probability (T_0= Control, T_1= Charcoal-based *T. asperellum*, T_2= Salicylic Acid (SA) (4.0 mM), T_3= Tartaric Acid (TA) (10.0 mM), T_4= Charcoal-based *T. asperellum* with SA, T_5= Charcoal-based *T. asperellum* with TA, T_6= Charcoal-based *T. asperellum* with SA and TA, T_7= Charcoal-based *T. harzianum,* T_8= Charcoal-based *T. harzianum with* SA, T_9= Charcoal-based *T. harzianum* with TA, T_{10}= Charcoal-based *T. harzianum with* SA and TA and T_{11}= Carbendazim).

4.8.1 Effect of *Trichoderma* spp. and antioxidants on yield after 90 days after sowing

Triple combination of *T. asperellum*, salicylic acid and tartaric acid showed the statistically significant highest yield/pot (5.55 Kg) with the triple combination of *T. harzianum*, salicylic acid and tartaric acid (5.19 Kg). These results were followed by the application of the combination of *T. asperellum* with salicylic acid and *T. harzianum* with salicylic acid (4.78 Kg and 3.84 Kg); the combination of *T. asperellum* with tartaric acid and *T. harzianum* with tartaric acid (4.12 Kg and 3.84 Kg); single use of salicylic acid and tartaric acid (3.54 Kg and 3.29 Kg); single application of *T. asperellum* and *T. harzianum* (2.99 Kg and 2.64 Kg). The control showed the lowest yield (2.35 Kg) (Fig. 9).



*Means ± Standard deviation followed by the same letter did not differ at 5% level of probability (T_0= Control, T_1= Charcoal-based *T. asperellum*, T_2= Salicylic Acid (SA) (4.0 mM), T_3= Tartaric Acid (TA) (10.0 mM), T_4= Charcoal-based *T. asperellum* with SA, T_5= Charcoal-based *T. asperellum* with TA, T_6= Charcoal-based *T. asperellum* with SA and TA, T_7= Charcoal-based *T. harzianum,* T_8= Charcoal-based *T. harzianum with* SA, T_9= Charcoal-based *T. harzianum* with TA, T_{10}= Charcoal-based *T. harzianum with* SA and TA and T_{11}= Carbendazim).

Figure 10. Effect of *Trichoderma* spp. and antioxidants on yield after 90 days after sowing.

4.9 Relation between Rust incidence (%) and yield

In the regression, Rust incidence (%) was considered as independent and the yield Kg per plant as dependent variable. A strong negative relation was found between Rust incidence (%) of leaf and yield Kg per plant. Due to Rust disease, (6.54 %) loss in yield per plant was observed. The regression equation also showed that for every one unit increase in Rust incidence, yield reduction of (0.98 %) will be occurred (Fig. 10).

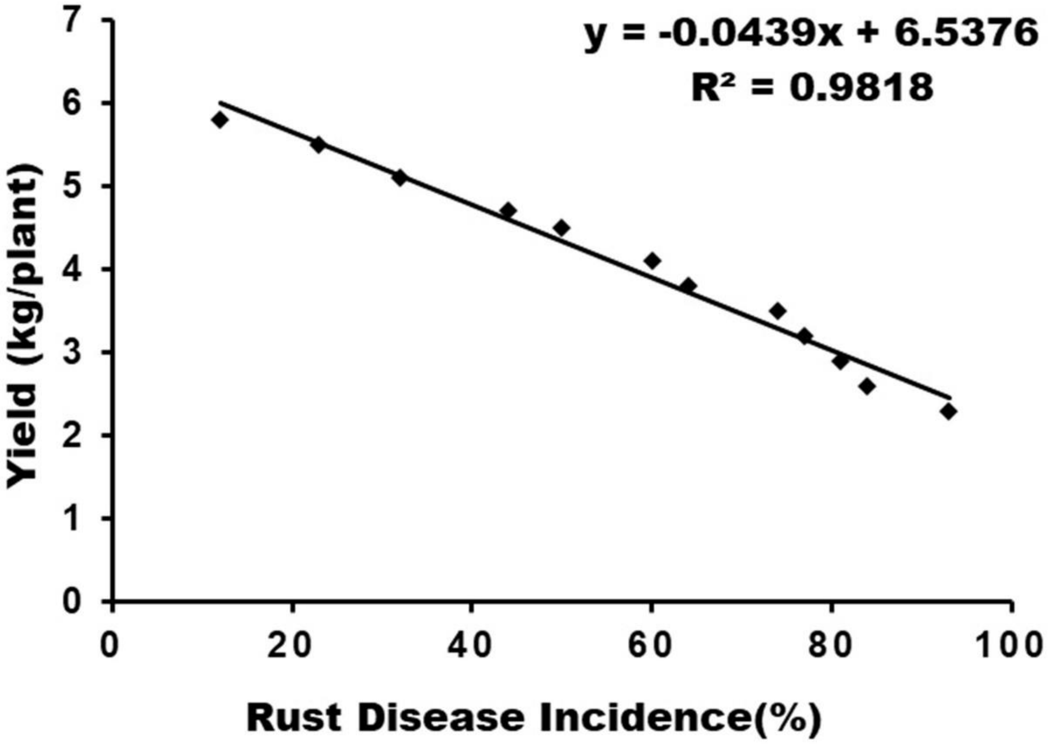


Figure 11. Correlation of co-efficient between Rust disease incidence (%) and yield in yard long bean.

4.9.1 Relation between Wilt disease incidence (%) and yield

In the regression, Fusarium wilt disease incidence (%) was considered as independent and the yield Kg per plant as dependent variable. A strong negative relation was found between Wilt disease incidence (%) of leaf and yield Kg per plant. Due to Wilt disease, (6.81 %) loss in yield per plant was observed. The regression equation also showed that for every one unit increase in Rust incidence, yield reduction of (0.98 %) will be occurred (Fig. 11).

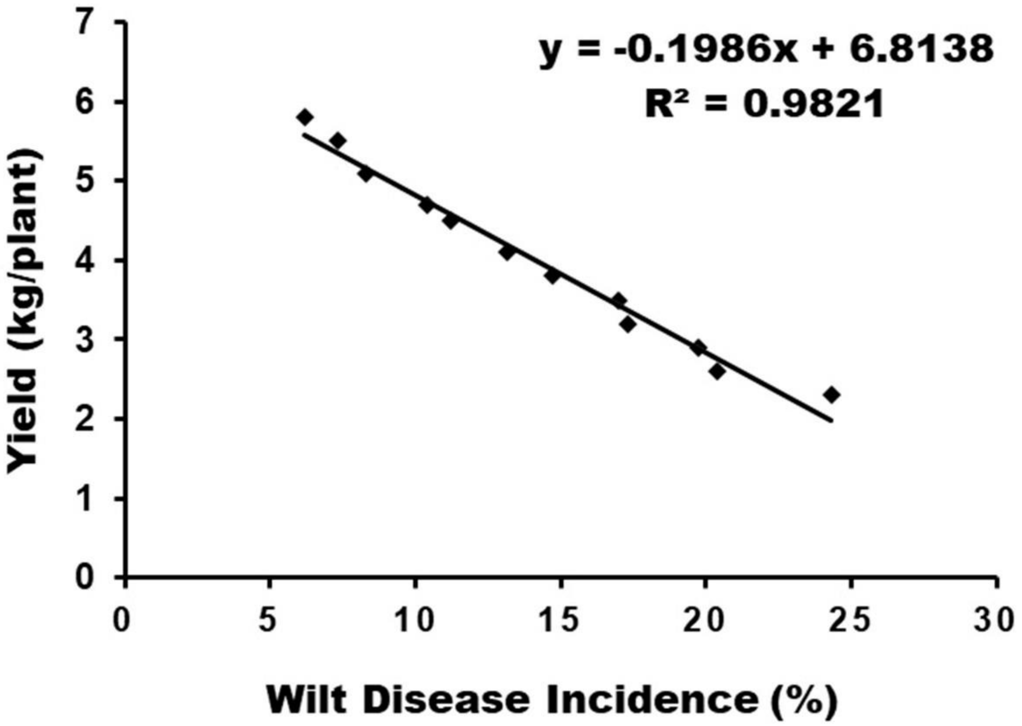


Figure 12. Correlation of co-efficient between Wilt disease incidence (%) and yield in yard-long bean.

4.9.2 Relation between Rust severity (%) of leaf and yield

In the regression, Rust severity (%) was considered as independent and the yield Kg per plant as dependent variable. A strong negative relation was found between Rust severity (%) of leaf and yield Kg per plant. Due to Rust disease, (6.06 %) loss in yield per plant was observed. The regression equation also showed that for every one unit increase in Rust severity, yield reduction of (0.89 %) will be occurred (Fig.12).

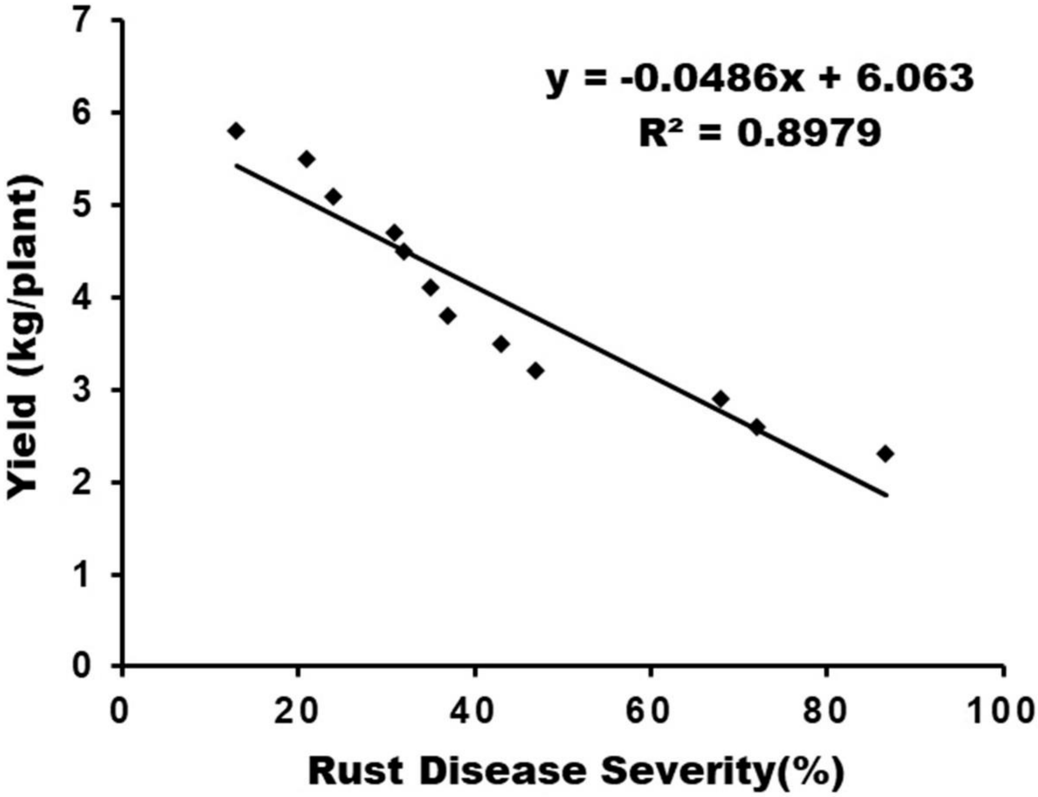


Figure 13. Correlation of co-efficient between Rust disease severity and yield in yard long bean.

4.9.3 Relation between Wilt disease severity (%) and yield

In the regression, Fusarium wilt disease severity (%) of leaf was considered as independent and the yield Kg per plant as dependent variable. A negative relation was found between Wilt disease severity (%) of leaf and yield Kg per plant. Due to Wilt disease, (5.32 %) loss in yield per plant was observed. The regression equation also showed that for every one unit increase in Wilt disease severity, yield reduction of (0.55 %) will be occurred (Fig. 13).

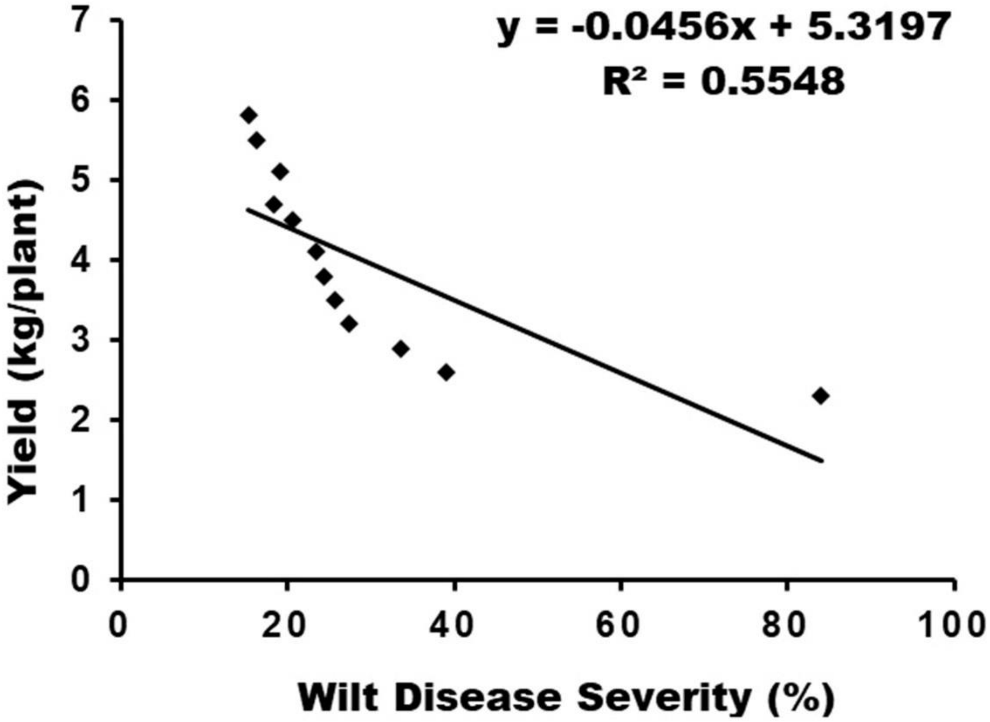


Figure 14. Correlation of co-efficient between Wilt disease severity (%) and yield

CHAPTER V

DISCUSSIONS

Rust and Wilt disease of yard-long beans caused by *Uromyces phaseoli typica* and *Fusarium oxysporum* f. sp. *phaseoli*, respectively both are devastating diseases in Bangladesh. Due to the pathogen's soil-borne origins, chemical control of the disease is both expensive and challenging. A contaminated environment and health risks for humans are further consequences of chemical control. People throughout every corner of the world have expressed concern regarding the detrimental impacts of chemical pesticides that are currently utilized in controlling many different diseases and pests (Meghla, 2023; Mazzola, 1998; Mark *et al*., 2006). To avoid the negative impact of chemical control, researchers around the world are searching for effective strategy alternative to chemicals. Beneficial microorganisms including *Trichoderma* spp. is an important element of the eco-friendly management of various crop diseases especially those caused by soil borne fungal pathogen.

The present research aimed to observe the efficacy of *Trichoderma* along with antioxidants in enhancing the systemic disease resistance of Rust and Wilt disease. In this study, all the treatments had a significant effect on reducing this disease either in vegetative and reproductive stage and increasing of yield. The triple combination of *T. asperellum*, salicylic acid and tartaric acid exhibited reduction in Rust and Wilt disease incidence and severity. Cheng *et al.* (2010) observed that *T. viride* T23 and *T. harzianum* T22 prevent yard-long bean disease, with an efficacy of 66.04 %; while Zhuang *et al*. (2005) and Roy (2023) demonstrated that upon treatment with *T. viride* T23 conidia and chlamydospores, cucumber seedlings exhibited a decrease in the disease index from 33.69 to 13.12 and 10.28, respectively. Bi *et al.* (2016) showed that *T. longibrachiatum* and *T. viride* prevent Brown rust and Fusarium wilt disease, with efficacies of 75.74 % and 70.76 %, respectively.

In general, combined effects of different approaches were more effective than the individual approach in reducing disease incidence and severity in plants. In addition to inhibiting several pathogenic fungi, *Trichoderma* also promoted the growth and yield of plants (Hyakumachi *et al.* 1994; Masunaka *et al.* 2011; Deng *et al.* 2013). For example, Yedidia *et al.* (2010) reported that root area like cucumber grew by 95 %, plant dry weight increased by 80 %, root length increased by 75 %, plant height increased by 45 %, and leaf area increased by 80 % when *T. harzianum* T203 was added to the soil. Zhang S W *et al.* (2016) demonstrated that *T. longibrachiatum* spore suspension significantly promoted the growth of *Meloidogyne incognita*-inoculated such as cucumbers. Furthermore, a number of *Trichoderma* strains enhanced the development and biomass accumulation of other plants, including cabbage (Xing *et al.* 2017) and *Sedum plumbizincicola* (Luo *et al.* 2016). Nitric acid level in leaves and plant chlorophyll concentration are crucial markers for assessing photosynthesis, as well as the uptake and application of nitrogen. Liu *et al.* (2013) reported that *T. viride* broth increased the activities of antioxidant enzymes in mangos and suppressed the growth of pathogens that cause mango anthracnose. Liu *et al.* (2014) demonstrated that *T. harzianum* T23 increased the activity of PAL, PPO, POD, and SOD and caused the production of phytoalexin and lignin in eggplants, increasing the plants' resistance to Fusarium wilt disease. Abdel-Monaim (2013), who discovered that *T. viride* and salicylic acid, either separately or together, suppressed the growth of pathogenic fungi that attack faba bean roots. Ismail (2006) discovered that tartaric acid inhibited the growth of fungi that cause Wilt disease, damping-off, and root rot in sesame (Sesamum indicum L). The in vivo findings confirmed that the triple combination of *T. asperellum*, salicylic acid (4 mM), and tartaric acid (10 mM) significantly affects the increase in photosynthetic pigment content in the yard-long bean plants. Fruit quality was also enhanced, including pod length(cm), number of pod/plants, and yield Kg/plant all showed a noteworthy increase.

Trichoderma spp. can suppress the growth of the fungal pathogen and enhance disease resistance in the host plant as well. *Trichoderma* spp. not only prevents Rust and Wilt disease but also promotes yard-long bean growth and yield. This antagonist is highly isolable and grows quickly on organic materials. *Trichoderma* have several modes of action, including mycoparasitism (Lumsden *et al*., 1995; Abada, 2002 and Ahmed, 2013), the synthesis of antifungal compounds (Robinson *et al*., 2009), and the possession of an enzymatic system that breaks down pathogens (Elad and Kapat, 1999 and Ziedan *et al*., 2005). Apart from these methods of operation, *Trichoderma* spp. additionally stimulate resistance in treated plants to protect them from specific diseases (Homer, 1993; Harman, 2006; Ahmed, 2005) develop under a broad range of environmental circumstances, including temperature (Bailey *et al*., 2008 and Singh and others, 2010). *Trichoderma* spp. have a beneficial impact on plant protection if colonized on roots, as they trigger the plant defence mechanism including the production of phytoalexins, Pathogenesis Related (PR) proteins, jasmonic acid, peroxidases, and chitinases (Djonovic *et al*., 2006). Salicylic acid (SA) is a phytohormone that is essential to defensive signalling (Vlot *et al*., 2009). SA activates defence responses through its downstream components NPR1 (Dong, 2004) and three redundant transcription factors, TGA2, TGA5, and TGA6 (C Y, Tessaro MJ, Lassner M, Li X, 2003). Increased SA levels induce redox changes and result in the reduction of NPR1 to a monomeric form that accumulates in the nucleus to activate defence gene expression (Mou *et al*., 2003). The antioxidants increase plant growth parameters, yield, and seed or fruit quality in several vegetables and field crops (Roy, 2023; El Wakil and El Metwally, 2000; El Wakil, 2003; El-Mougy *et al*., 2004; Karlidag *et al*., 2009; Abd El-Hai *et al*.,2009; Zahra *et al*., 2010).

CHAPTER VI

SUMMARY AND CONCLUSION

Yard-long bean is one of the most important vegetable crops not only in Bangladesh but also in many countries in the world. The yield of the vegetable is reduced by various factors where Rust and Wilt disease caused by *Uromyces phaseoli typica* and *Fusarium oxysporum* f. sp. *phaseoli* are considered as the most deleterious diseases. Traditionally farmers use chemical to control such kind of devastating diseases which are the most concern issue for their negative role in regards to the human health and environment. Therefore, researchers around the globe are focusing on the eco-friendly management of crop diseases using various beneficial micro-organisms. Hence, we aimed to the management of Rust and Wilt disease of yard-long bean, antagonistic microorganisms including formulated *T. asperellum* and *T. harzianum* along with salicylic acid (SA) and tartaric acid (TA) were selected to observe their efficacy against these diseases. As *T. asperellum* and *T. harzianum* showed potential antifungal efficacy against the causal agent of Fusarium Wilt and Brown Rust of yard-long bean, *T. asperellum* and *T. harzianum* were used in pot experiment for enhancing the systemic resistance in yard-long bean plant in order to eco-friendly management of Wilt disease. In addition to *T. asperellum, T. harzianum,* salicylic acid (SA) and tartaric acid (TA) were also used alone or in combination for achieving the desired objectives in yard-long bean. The combined application of *T. asperellum*, salicylic acid and tartaric acid showed minimum Rust and Wilt disease incidence followed by combined application of *T. harzianum,* SA, and TA; combined application of *T. asperellum* and SA; combined application of *T. harzianum* and SA; combined application of *T. asperellum* and TA; combined application of *T. harzianum* and TA; single application of SA; single application of TA; single application of *T. asperellum*; single application of *T. harzianum* and however, control showed the highest

Rust and Wilt disease incidence. The combined application of *T. asperellum*, salicylic acid and tartaric acid resulted the highest yield at 90 DAS in compared to other treatments and control. The combined application of *T. asperellum*, salicylic acid and tartaric acid also gave the best result in all parameters like plant height, leaf number, pod number, and pod length compared to all other treatments applied. The combined application of *T. asperellum*, salicylic acid and tartaric acid showed total chlorophyll compare to other treatments. The findings of the study demonstrated the potentiality of the combined application of *T. asperellum*, salicylic acid and tartaric acid for the enhanced systemic resistance in yard-long beans plant which ultimately offer eco-friendly management of Brown rust and Fusarium wilt disease of yard-long bean. However, it is necessary to perform on-farm study in several places prior to making a final suggestion to the farmers.

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