Liquid Formulation of Native *Trichoderma* spp. for the Biocontrol of Fusarium leaf spot of Bottle Gourd

A THESIS

BY

SIDRATUL MUNTAHA BINTE ANAM OTITHI

Student No. 2305009

Session: 2023-2024

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IN

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DEPARTMENT OF PLANT PATHOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY

UNIVERSITY, DINAJPUR

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Dedicated to My Beloved Parents and Sibling

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The Author

ABSTRACT

Fusarium leaf spot of Bottle Gourd caused by *Fusarium oxysporum* is a newly emerging disease in Bangladesh. The present study aimed to develop a Potato-dextrose and yeast-molasses-based upgraded *Trichoderma* liquid formulation for the eco-friendly and sustainable management of the disease. Therefore two native *Trichoderma* species (*Trichoderma* HSTUT-6 & *Trichoderma* HSTUT-8) were used to develop the liquid formulations. *In-vitro* tests demonstrated the antifungal efficacy of the selected *Trichoderma* against the isolated and molecular identified *Fusarium* sp., the causal agent of leaf spot disease. *Trichoderma* HSTUT-8 showed a maximum zone of inhibition (86.67 %) against *F. oxyxporum* in the dual culture method followed by *Trichoderma* HSTUT-6 (82.23 %). *Trichoderma* HSTUT-8 grown on potato-dextrose-based liquid formulation showed the highest reduction of leaf spot incidence (70.03 % & 73.86 %) and severity (90.19 % and 88.89 %) over control at 80 days after sowing (DAS). This upgraded liquid formulation also showed higher plant height (51.33 & 49 cm), total chlorophyll content (1.88 & 1.84 mg/g fresh weight, respectively), total phenols content in fruits (0.583 & 0.553 mg catechol/100 g fresh weight), number of fruits/plant (13.67 & 14.67, respectively), fruit length (51.33 & 49 cm, respectively), weight (2.24 & 3.093 Kg, respectively), diameter (32.33 & 39.33 cm, respectively), total soluble solids in fruits (6.23 & 5.7 %, respectively), pH (6.8 & 6.64, respectively), moisture content (97.2, 97.63 %) in fruits of Naaz Green and Diana Bottle Gourd. Furthermore, the combined application of potato-dextrose-based *Trichoderma* liquid formulation showed minimum firmness of fruits (20.53 & 20.33 Kg/m^2). The study explored the use of the developed liquid formulation of *Trichoderma* HSTUT-6 and *Trichoderma* HSTUT-8 for the field application to control Fusarium leaf spot of Bottle Gourd.

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CHAPTER I

INTRODUCTION

Bottle Gourd (*Lagenaria siceraria*) is a widely cultivated, high-yielding, versatile, and drought-tolerant crop that belongs to the Cucurbitaceae family. This family consists of 118 genera and 825 species, which have a wide distribution in the warmer areas of the world (Minocha, 2015). It is grown extensively during the kharif and summer seasons. The fruits of the Bottle Gourd have a long, round, or oval to oblong shape (Shinde *et al.,* 2014). The fruits are medicinal and used for cardio-tonic, aphrodisiac, hepatoprotective, analgesic, anti-inflammatory, expectorant, diuretic, and antioxidant (Mohan *et al.,* 2012). The mature dried fruit is used to make jars, utensils, and musical instruments for storage (Decker-Walters *et al.,* 2001). India, Sri Lanka, South Africa, Indonesia, and Malaysia are the main worldwide producers of Bottle Gourd (Barot *et al.,* 2018). In India, China, Sri Lanka, and Bangladesh, Bottle Gourd is widely cultivated for its edible parts and medicinal properties (R. P. Prajapati *et al.,* 2010) . The fruit has 96 % moisture and is high in nutrients like vitamin B complex, choline, Vitamin C minerals, antioxidants, and dietary fiber (Gajera *et al.,* 2017). The fruit has high nutritional value, with 0.2g fats, 1.2g proteins, 3.75g carbohydrates, 0.7g fiber, and 15 Cal per 100 g dry weight energy (Hanif *et al.,* 2006). It serves as a diuretic, cooling agent, analgesic, and anti-ulcer (Milind & Satbir, 2011). The seed contains high-quality proteins, fatty acids, and vitamins (A, E, and C) (Hegazy & El Kinawy, 2011).

In Bangladesh, the leaves and tender shoots of Bottle Gourd plants are part of the local cuisine. Bottle Gourd grows and yields well in Bangladesh's winter climate. For optimal growth and fruiting, the ideal temperature range is 20-27°C during the day and 18-23°C at night. However, they can also be grown year-round by the seed-planting method, especially during the summer and monsoon seasons. Bangladesh's average Bottle Gourd yield is about 9.38 tons

per hectare, which is low compared to other countries (Quamruzzaman *et al.*, 2017).

Various factors, such as a lack of high-yielding varieties and quality seeds, as well as biotic and abiotic stresses, hinder the production of Bottle Gourd each year. One significant constraint for the low output of Bottle Gourd in Bangladesh is diseases caused by fungi, bacteria, and viruses. Disease-induced economic yield losses range from 20-60 % (Bruce 2013). Leaf spot and leaf blight diseases resulting in reduced fruit yield are commonly caused by fungi like *Cercospora citrulina* and *Alternaria cucumerina* (Maheshwari *et al.*, 2015).

Fusarium leaf spot disease is a plant disease that causes characteristic leaf spot symptoms on Bottle Gourd plants. *Fusarium* species are primarily differentiated morphologically by their colony characteristics, macroconidia shape, presence or absence of microconidia and chlamydospores, and the formation of conidiogenous cells (Omar *et al.,* 2018). It ultimately affects the crop's overall health and growth and is commonly found in open-field cultivation areas.

Traditionally, Bangladeshi farmers depend on monoculture, which leads to dependency on chemical fertilizers, and pesticides. Imbalanced use of chemical fertilizers, and pesticides, and intensive use of land without application of organic fertilizers has led to the deterioration of both soil quality and fertility (Rahman & Thapa, 1999). Using inorganic fertilizers alone is insufficient for maintaining high productivity in vegetable crops (Dass *et al.,* 2008). Organic fertilizers can lead to low productivity in vegetable crops with high nutrient requirements due to limited nutrient availability for planting (Cavigelli *et al.,* 2008). The excessive use of systemic copper fungicides leads to the accumulation of copper, which has detrimental effects on micro-organisms (Wightwick *et al.,* 2010).

On the other hand, biological control is thought to be a promising alternative to pesticides and plant resistance for managing plant diseases. Biological control is an approach to plant disease management that inhibits plant pathogens, improves plant immunity, and/or modifies the environment using beneficial microorganisms, compounds, or healthy cropping systems (He *et al.,* 2021). Systemic Acquired Resistance (SAR) is a new strategy to control several diseases in different crops (Hoitink & Boehm, 1999; Vallad & Goodman, 2004). Some bacteria and fungi have been identified as potential organisms that can induce systemic resistance in plants (Van Loon *et al.*, 1998).

Different strains of *Trichoderma* spp. are used for biological control. These fungi have high adaptability, fast growth, and a broad antibiotic spectrum. In particular, they have demonstrated significant inhibitory effects on major pathogenic fungi as they are adaptable, can be easily derived from indigenous soils, and can be cultivated easily. As a result, numerous studies have explored the potential use of *Trichoderma* as a bio-control agent. Utilizing beneficial fungi for biological control presents a practical and eco-friendly alternative to chemical methods for managing plant diseases (O'Brien, 2017). *Trichoderma*, a filamentous fungus found in soil, is a commonly employed biocontrol agent that has proven effective against various plant pathogens, including *Sclerotium, Fusarium, Rhizoctonia, Pythium*, and more (Al-Ani & Mohammed, 2020). Through a range of mechanisms such as competition, antibiosis, mycoparasitism, host-plant resistance, chitinolytic enzyme secretion, and inhibitory compound production, *Trichoderma* effectively suppresses plant pathogens (Harman *et al.,* 2004; Braun *et al.,* 2018). Several studies have confirmed *Trichoderma* spp.'s antagonistic and biological control potential against soil-borne pathogens. These findings are supported by various research studies, including those conducted by Hanson & Howell (2004), and Afzal *et al.,* (2013). Additionally, Harman (2000) demonstrated the commercial viability

of *Trichoderma* spp. as a bio-fungicide for controlling various economically significant soil-borne fungal plant pathogens.

They are also known for their ability to increase reproductive capacity, modify the rhizosphere, grow in unfavorable conditions, utilize nutrients effectively, aggressively combat phytopathogenic fungi, and promote plant growth while enhancing defense mechanisms (Harman *et al.,* 2004; Schuster & Schmoll, 2010; Pandya *et al.*, 2011; Tripathi *et al.*, 2017; Dagurere *et al.,* 2014; Keswani *et al.,* 2014). These properties have made *Trichoderma* an omnipresent genus able to grow in more expansive habitats and at high population densities (Chet *et al.,* 1997; Chaverri *et al.,* 2011). *Trichoderma* can be applied in both solid and liquid forms to effectively control various types of diseases. The efficacy of the formulations may be influenced by several factors, including the composition and strength of the carrier, the strain and vitality of the fungus, the existing environmental conditions, and their compatibility with other fungicides. Several studies have indicated that liquid formulations of *Trichoderma* may offer certain benefits compared to solid formulations, including increased spore density, simplified application, and extended shelf life.The aim of the study was to isolate leaf spot pathogen and develop a liquid formulation of *Trichoderma* to induce systemic acquired resistance in order to control the disease. The specific objectives of the present investigation are as follows:

1. To isolate and characterize the leaf spot causing pathogen.
2. To determine the *in vitro* efficacy against the leaf spot pathogen and develop an upgraded liquid formulation using the collected *Trichoderma.*
3. To evaluate the efficacy of the developed liquid formulation in enhancing disease resistance and improving agronomic attributes of Bottle Gourd.

CHAPTER II
REVIEW OF LITERATURE

2.1 Identification of *Fusarium*

Aslam *et al.,* (2021) reported the occurrence of brown necrotic leaf spots in seven Bottle Gourd crops in Faisalabad, Pakistan. The pathogen causing this disease was identified as *Fusarium equiseti*. The article describes the morphology of the fungus, the results of molecular identification, and the testing of Koch's postulates. This is the first report of leaf spots caused by F. equiseti in Bottle Gourd from Pakistan.

Hossain *et al.,* (2021) identified four okra fungal isolates as *Fusarium oxysporum*. The isolates showed the highest radial growth rates at pH 5 and 25º C.

Hussain *et al.,* (2012) identified eleven *Fusarium* isolates out of sixteen using polymerase chain reaction (PCR) with species-specific markers. They confirmed the identification by examining the nature of conidiogenous cells and the morphology of microconidia, macroconidia, and chlamydospores.

Torbati *et al.,* (2021) in their study present an in-depth study of fungicolous *Fusarium* species and their hosts, identifying 80 isolates that are related to 36 different host species and 32 fungal genera. The isolates are classified into 24 species that have been divided into nine species complexes, with a predominant association with necrotrophic behavior. The main fungal hosts for these isolates were primarily microfungi from the sub-kingdom Dikarya, as well as other hosts from the sub-kingdom Mucoromyceta of the kingdom Fungi and the phylum Oomycota of kingdom Straminipila.

Vasic T. *et al.,* (2021) confirmed that daffodil's bulb rot is caused by *Fusarium oxysporum* f.sp. *narcissi*. The confirmation was made through molecular characterization, including sequence and phylogenetic analysis of genomic regions ITS and TFFI. The results obtained through molecular

characterization were consistent with those obtained through morphological identification.

2.2 Biological Control of *Fusarium*

Siddiqui *et al.* (2008) showed that there was a reduction of 85.04 % in Choanephora wet rot severity in 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.

Chaves *et al.,* (2016) performed in vitro tests that revealed 22 isolates inhibiting the growth of *F. oxysporum* f. sp. cubense (Foc, race 1), and *Trichoderma* spp. was found to be the most effective isolate for suppression of Foc in banana under greenhouse conditions.

Singh *et al.,* (1999) found that two chitinolytic bacterial strains, *Paenibacillus sp.* 300 and *Streptomyces sp.* 385, effectively suppressed *Fusarium* wilt in cucumber plants. A mixture of the two strains in a 1:1 or 4:1 ratio provided better control than individual strains or mixtures in other ratios. A zeolite-based, chitosan-amended formulation (ZAC) provided the best protection against the disease. The ZAC formulation was effective when added to the pathogen-infested medium 15 days before planting cucumber seeds and stored for 6 months. The combination of these two bacteria may involve the action of hydrolytic enzymes, as they produce chitinase and β-1,3-glucanase enzymes.

Bacon *et al.,* (2001) observed that, *Fusarium moniliforme* is a facultative fungal endophyte that produces fumonisins during biotrophic association with maize and saprophytic growth. It is transmitted vertically and horizontally to plants, with horizontal infection being contagiously spread and reduced by fungicides. Vertical transmission is crucial as it remains the reservoir for infection and toxin biosynthesis. A biological control system using Bacillus

subtilis and *Trichoderma* fungi has shown promise in reducing mycotoxin accumulation during the endophytic growth phase.

Larkin & Fravel (2002) evaluated the impact of various environmental and cropping conditions on the biological control of *Fusarium* wilt of tomato using nonpathogenic *Fusarium oxysporum* (CS-20 and CS-24) and F. solani (CS-1) isolates. The results showed that CS-20 significantly reduced disease incidence at all temperature regimes tested, with CS-20 being the most effective. Isolates CS-24 and CS-1 reduced disease incidence in greenhouses and at high temperatures but were less effective at the optimum temperature for disease development. Both isolates were equally effective against all three races of the pathogen and on eight different tomato cultivars with varying levels of inherent resistance to *Fusarium* wilt.

El-Desouky *et al.,* (2018) showed that *T. hamatum* gave 76.3 %, 9.6 %, and 70.4 %, *T. harzianum* gave 78.5 %, 76.6 % and 63.7 %, and *T viride* gave 5.5 %. 78.8 % and 61.8 %, while *C. minivans* gave 62.2 %, 60 %, and 71.8 % inhibition of *Fusarium oxysporum*, *F. solani* and *Sclerotium rolfsii*, respectively.

Amin *et al.,* (2010) tested Six *Trichoderma* spp. isolates for their ability to produce volatile metabolites against seven fungal plant pathogens. Results showed that *T. viride* (Tv-1) was most effective in reducing mycelial growth of *F. oxysporum* (41.88 %), while *T. viride* (Tv-2) reduced mycelial growth and sclerotial production in R. solani (30.58 %) and *S. rolfsii* and *S. sclerotiorum.*

Yang *et al.,* (2010) tested the biocontrol efficacy of *Trichoderma harzianum* SQR-T037 in cucumber plants using SQR-T037 conidia suspension, blended with organic fertilizer (TBF), or fermented organic fertilizer (TFF). Results showed that increasing the SQR-T037 number led to decreased Percent Disease Indexes (PDIs). TFF treatment showed the highest SQR-T037 population in the rhizosphere and bulk soil. TFF treatments were superior in

disease control, sustaining colonization, and decreasing *F. oxysporum* abundance.

Hamed *et al.*, (2011) found that the protective effect of Lactic acid bacteria (LAB) significantly increased after challenging inoculation by *Fusarium oxysporum*, mainly when LAB was applied as seed treatment. The number of roots increased to 358 % over the control, and the total fresh weight of tomato plants increased by 390 %.

Nosir *et al.*, (2011) observed that the presence of *T. harzianum* T22 appeared to prevent Fusaric acid (FA) (5-n-butylputidine 2-carboxyl acid) secretion secreted from *Fusarium oxysporum* f. sp. Gladioli (Massey) Synder and Hansen into the corms of Gladiolus grandiflorus. In the presence of A. migulanus, however, the amount of FA secreted into the corm tissues increased.

Dolatabadi *et al.*, (2012) mentioned that Lentil wilt, caused by *Fusarium oxysporum* f. sp. lentis, is a major challenge for successful cultivation. Four antagonistic fungi, including *Piriformospora indica, Sebacina vermifera, Trichoderma viride,* and *Trichoderma harzianum,* were tested against the pathogen. Results showed that these fungi effectively inhibited the pathogen. Pot culture experiments showed maximum plant height and minimum disease severity in pots treated with *S. vermifera+T. harzianum.*

Martínez-Medina *et al.*, (2014) in their study investigated the impact of *Trichoderma* strains on plant growth and biocontrol, focusing on their ability to influence the phytohormonal network of their host plants. Four *Trichoderma* isolates were tested for their antagonistic activity against *Fusarium oxysporum* f. sp. melonis and their growth-promoting and biocontrol activity against *Fusarium* wilt on melon plants. Principal component analysis (PCA) showed a strong association between auxin induction and plant growth stimulation by *Trichoderma*. The study found that the disease-protectant ability of Trichoderma strains against *Fusarium oxysporum* infection is more related to alterations in hormone content.

Prabhakaran *et al.,* (2015) showed that *Trichoderma* isolates from soil samples from India were grouped into four species: *Trichoderma asperellum*, *T. harzianum*, *T. pseudokoningii*, and *T. longibrachiatum*. These isolates were tested for in vitro biological control against diseases like *Alternaria solani*, *Bipolaris oryzae*, *Pyricularia oryzae*, and *Sclerotinia sclerotiorum.* All four species showed 100 % potential inhibition against rice blast pathogen *Pyracularia oryzae*, while *T. harzianum* showed higher inhibition against *S. sclerotiorum*, causing white mold disease.

Bahroun (2018) isolated three strains of endophytic bacteria from Faba bean (*Vicia faba*) and chickpea (*Cicer arietinum*) nodules *Rahnella aquatilis* B16C, Pseudomonas yamanorum B12 and Pseudomonas fluorescens B8P significantly reduced the pathogen symptom severity of inhibit phytopathogenic *F. solani* on *In-vitro* antibiosis test *R. aquatilis* B16C showed the best-protecting potentiality with three Faba bean cultivars.

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

Chen *et al.,* (2020) showed that bioorganic fertilizers developed using *Bacillus licheniformis* (X-1) and *Bacillus methylotrophic* (Z-1) exhibited a strong inhibition ability against the pathogen *Fusarium* compared with the chemical and organic fertilizers. These fertilizers allowed 80 % disease-free strawberry production together with improved physical and biochemical indexes in the pot experiment.

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 yields compared to half dose and standard dose of N-P-K, respectively. Eliciting an increase in mineral contents, total soluble solids, and bioactive molecules such as lycopene and ascorbic acid thereby increased the nutritional and functional quality of the tomato fruits.

Chaudhari *et al.,* (2022) evaluated the in vitro efficacy of various bio-agents against Bottle Gourd wilt. *Trichoderma viride* (Sardarkrushinagar) was found to be the most effective, followed by *T. harzianum*, *viride*, *Bacillus subtilis*, and *Pseudomonas fluorescens*. The maximum growth inhibition of *Fusarium oxysporum* was recorded with neem extract (52.38 %) and nilgiri extract (41.62 %).

Pradhan *et al.,* (2022) evaluated that, two formulations of *Trichoderma viride (T. viride)* as a biocontrol agent for *Fusarium* wilt disease in chickpeas. The *T. viride* ITCC 7764 strain showed the most potential for controlling wilt. The optimized TvP formulation was found to be more effective in controlling wilt incidence and seed germination in chickpeas. However, both formulations showed greater bio-efficacy compared to synthetic fungicides and conventional talc-based formulations. Further research is needed to determine the compatibility of these products with other agrochemicals for an integrated disease management schedule in chickpeas.

Ruchika *et al.,* (2022) observed that, Bottle Gourd, a crucial vegetable crop, is significantly affected by diseases, including wilt (*Fusarium oxysporum* f.sp. *lagenariae*). Biocontrol is a promising approach to manage this problem, as chemical fungicides have limitations such as fungicide resistance and environmental hazards. This study evaluated potential biocontrol agents against Fusarial wilt of Bottle Gourd. Two isolates of *Trichoderma* and *Pseudomonas* were isolated from wilt-affected regions in Punjab and tested under in vitro and in vivo conditions. The results showed that *Trichoderma asperellum* (T2 and T2) and *Pseudomonas aeruginosa* (P1) had superior antagonistic potential. In vivo, *Trichoderma asperellum* (T2) showed maximum disease inhibition of 84.62 and 80.00 % in March and June sown Bottle Gourd crops, respectively. *Trichoderma asperellum* also exhibited the highest plant growth promotion and enhanced yield. The findings suggest that these biocontrol agents have greater

potential in managing the wilt of Bottle Gourd and can be used commercially to manage the disease in farmer's fields.

Nezu *et al.*, (2023) aimed to study the efficacy of medicinal plants like neem and mahogany in controlling Anthracnose of chili, a disease causing significant crop loss in Bangladesh. The combination of mahogany leaf extract and peat soil-based Trichoderma resulted in the highest reduction of disease incidence and severity, as well as the highest plant height and fruit weight.

Qulsum *et al.*, (2023) evaluated the management of bacterial wilt caused by *Ralstonia solanacearum* in brinjal plants. Twenty bacterial strains were isolated, and HSTUB 17 showed the most significant zone of inhibition against *R. solanacearum*. When combined with *B. cereus* HSTUB 17, *T. harzianum*, and *C. gigantea*, the consortia reduced bacterial wilt incidence by 74.87, 66.67, and 66.67 % at 30, 50, and 70 days after transplanting. The consortia also increased plant height, branches, fruits, and fruit yield compared to R. solanacearum-only treatments.

2.3 Beneficial effect of *Trichoderma* spp.

Abdel-Fattah *et al.*, (2007) mentioned that Rice brown spot, caused by *Bipolaris oryzae*, is a serious disease, causing significant yield loss. *Trichoderma harzianum*, an effective biocontrol agent, was investigated for its in vitro and field application. Results showed that *T. harzianum* antagonizes Bipolaris oryzae, overgrowing it, and preventing linear growth. Under field conditions, spraying a spore suspension reduced disease severity and incidence, increased grain yield, and increased photosynthetic pigments in rice leaves.

Azarmi (2011) examines the effects of *Trichoderma* isolates T969 and T447 on tomato seedling vigor and nutrient uptake. Seed germination rate is not affected by Trichoderma application, but shoot height, shoot diameter, shoot fresh and dry weight, and root fresh and dry weight increase when sown in

Trichoderma sp. *T* and *T. harzianum T969* fortified soil. Chlorophyll content also increases in seedlings grown in *Trichoderma sp. T* amended soil.

Amira *et al.,* 2017 described that *Fusarium* root rot, a cryptogamic disease in olive trees, can be controlled using biological control agents (BCAs). A Tunisian strain of *T. harzianum* (Ths97) was found to inhibit Fso14 growth in vitro and develop a strong protective role against root infestation. Ths97 up-regulated defense-related genes when inoculated with Fso14, suggesting primed-plant events. These promising results suggest Ths97 could be a beneficial agent for controlling *Fusarium* root rot disease in olive trees.

Bidellaoui *et al.,* (2018) in their study compared the effects of *Rhizophagus irregularis* and *Trichoderma asperellum* strain T34 on *Fusarium* tomato wilt and plant growth. Both fungi reduced disease incidence and increased plant height. *R. irregularis* provided the highest levels of chlorophyll, while T34 had better measures for infected plants. *R. irregularis* induced greater accumulation of P, K, Zn, Cu, and Mo. The study found that substrate depletion was lower for plants inoculated with either fungus or T34 compared to control plants.

Elad, (2000) proved that biocontrol of foliar diseases is an alternative method for managing pathogens. Isolate T39 of *Trichoderma harzianum* is a well-studied commercial biocontrol agent that effectively controls foliar pathogens in cucumbers under commercial greenhouse conditions. The agent's efficacy is comparable for different rates and involves local and systemic resistance. BCA suppresses enzymes of *B. cinerea* through protease secretion on plant surfaces, but it doesn't require antibiotics or mycoparasitism.

Guzmán-Guzmán *et al.,* (2023) stated that, *T. harzianum, T. asperellum, T. atroviride, T. longibrachiatum, T. viride*, and *T. virens* are the most studied *Trichoderma* species. The first three species are commonly used as bio-control agents, using mycoparasitism and competition against fungal phytopathogens

Inbar *et al.,* (1994) examined *Trichoderma harzianum,* a peat-bran preparation applied to cucumber and pepper seedlings in a commercial nursery. The treatment significantly increased height, leaf area, and plant dry weight. The seedlings were more developed and vigorous, with higher chlorophyll content. After two growth cycles, *Trichoderma*-treated plants were found to be more resistant to damping-off disease, with significant reductions in damping-off in middle and border beds.

Islam *et al.,* (2021) found that the chickpea-based *Trichoderma harzianum* and its spore suspension can effectively control damping off and foot rot diseases in chilli plants. The combination treatment reduced damping off by 75.02-86.96 % and foot rot by 74.29-89.28 % compared to both fungi. It also increased seed germination by 66.23-90.57 % and resulted in higher shoot length, root length, and vigor index.

Kapoor AS. (2008) described that *Trichoderma* spp., a bioagent, effectively inhibited mycelial growth against various soilborne pathogens, including *Rhizoctonia solani, Pythium debaryanum, Sclerotinia minor*, and *Fusarium oxysporum* f. sp. *pisi*. It also significantly inhibited conidial and sclerotial germination of *F. oxysporum* f.sp. *pisi* and *S. rolfsii*. Wheat bran, combined with FYM, supported the maximum multiplication of *T. harzianum*. Wheat bran-based formulations were found effective in controlling the root rot of peas and collar rot of tomatoes.

Kumar *et al.,* (2021) identified a promising biocontrol agent with multi-trait properties. Twelve isolates were tested against *Fusarium solani* and their biochemical responses were assessed. *T. harzianum* showed the highest percent inhibition, specific activity of defense enzymes, and growth hormones. These isolates could be used as bio inoculants in subtropical India for biotic stress management and plant growth promotion.

Lee *et al.,* (2006) mentioned that *Trichoderma harzianum YC459* (Th 459), a fungicide from sawdust compost, effectively controls cucumber and

tomato gray mold caused by *Botrytis cinerea*. Its wettable powder formulation reduces cucumber gray mold severity through foliar spraying, maintaining control efficacy for at least seven days. Mixing Th 459 into nursery potting mix at seeding also reduces mold severity. Foliar spraying reduces tomato fruit infection by *B. cinerea* as effectively as dichlofluanid.

LI *et al.,* (2019) evaluated that, the effects of three *Trichoderma* strains, *Trichoderma asperellum, and T. asperellum-8525, T. harzianum 610*, and *T. pseudokoningii 886*, on preventing cucumber *Fusarium* wilt. All three strains showed higher control effects than previous studies, with efficacies over 78 %. *Trichoderma 866* was the most effective, with 78.64 % disease control efficacy and a 33 % cucumber yield increase. Seedlings inoculated with *Trichoderma* increased plant height, stem diameter, leaf area, chlorophyll content, and nitric nitrogen content. These strains also promoted cucumber growth, preventing wilt, and improving cucumber yield and quality.

Rubio *et al.,* (2014) mentioned that *Trichoderma parareesei* and *Trichoderma reesei* produce cellulases and xylanases. An anamorphic strain, T6 (formerly *T. reesei*), shows biocontrol potential against phytopathogens and enhances hyphal growth in tomato plants. T6 improves defense against *Botrytis cinerea* and promotes growth under salt stress.

Sallam *et al.,* (2019) tested Seven *Trichoderma* spp. isolates from Egypt for their ability to antagonize *Fusarium oxysporum* f.sp. *lycopersici*, the cause of tomato wilt disease. The highest percentage of inhibition was achieved with the T7 isolate. In greenhouse experiments, the isolates significantly decreased disease severity compared to untreated control. Real-time RT-PCR assessed the defense-related gene expression in tomato plants, with the highest degree of gene expression found in T3&FOL treatments. Two species of antagonistic *Trichoderma* were identified.

Sharma *et al.,* (2020) examined the ecology of four *Trichoderma* harzianum isolates against *Sclerotinia sclerotiorum*, focusing on their potential

for bio-formulations. The isolates grew well in various conditions, with the highest colony-forming units observed for the *T. harzianum-P* isolate. Field trials tested the efficacy of these bio-formulations against Sclerotinia rot disease in Indian mustard. The highest reduction was achieved by the *T. harzianum-GR* isolate, followed by soil application and foliar spray.

Studholme *et al.,* (2013) in their study explore the unique properties of *Trichoderma hamatum strain GD12*, which promotes plant growth, activates biocontrol against soil pathogens, and induces systemic resistance to foliar pathogens. It also demonstrates that GD12 can confer beneficial agronomic traits to other plants, such as lettuce and rice. The study also reveals that GD12 mycoparasitises pre-emergence soil pathogens, increasing plant growth promotion. The study presents de novo genome sequence data to understand its genetic potential.

Sudantha & Suwardji, (2021) investigated Sembalun Bumbung Village, East Lombok, the effectiveness of *Trichoderma* spp. bio-fungicide in managing *Fusarium* wilt disease in many shallot cultivars was evaluated. Findings indicated that liquid, tablet, and powder bio fungicides prevented *Fusarium* wilt disease by up to 0 %, compared to a control population with a 60 % disease percentage. The cultivars of Bali Karet had the highest production and growth.

Tucci *et al.* (2010) showed how genetic variability in tomato lines can affect their interaction with biocontrol strains of *T. atroviride* and *T. harzianum*. Some lines showed enhanced growth and resistance to *Botrytis cinerea*, while others had no or even a detrimental effect. Gene expression studies revealed that *Trichoderma* can induce long-lasting up-regulation of defense mechanisms in responsive lines, enhancing systemic resistance to pathogens.

Zeilinger *et al.,* (2016) stated that Mycoparasitism is highly efficient in decreasing the amount of pathogen inoculum, particularly for soil-borne diseases, for which *Trichoderma* spp. are frequently used.

CHAPTER III

MATERIALS AND METHODS

This study aimed to identify and analyze a potential type of *Trichoderma* that could help manage *Fusarium* leaf spot disease in Bottle Gourds. The focus was investigating how this type of *Trichoderma* could evoke resistance mechanisms against the disease. The study followed a systematic approach.

3.1 Sampling

Leaves showing signs of leaf spot disease from Bottle Gourd plants grown in open fields were collected carefully and brought to the Plant Pathology Laboratory of Hajee Mohammad Danesh Science and Technology Dinajpur. The samples were washed thoroughly with running tap water and left to dry in the shade.

3.2 Isolation and Identification of *Fusarium*

The process involved sectioning infected Bottle Gourd leaves into smaller fragments, which were then sterilized for one minute using a 0.5 % sodium hypochlorite (NaOCl) solution. The fragments were then washed three times with sterilized distilled water. The next step was to place these fragments on autoclaved petri dishes containing Potato Dextrose Agar (PDA) to obtain a pure culture. The isolated fungi were identified by examining the morphological and cultural characteristics of the mycelium, conidiophores, conidia, and colony morphology.

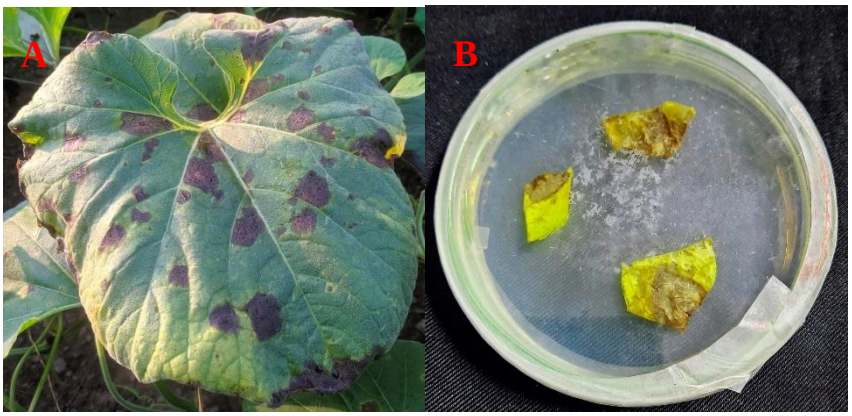

Figure 1. A) Collected leaf Sample B) Pathogen Isolation from diseased leaf

3.3 Pathogenicity test (Verification of KOCH's postulates)

Koch's postulate was used to test the disease-causing ability of each fungal isolate *in vivo* in the study area. Initially, healthy leaves were selected from the cultivation area and sterilized with 75 % ethanol to remove any surface contaminants. In the pathogenicity test, a pure culture of *Fusarium* was introduced to Bottle Gourd plants to evaluate its impact on plant health. A suspension was made using 500 mL of distilled water and a 1 % solution of the fungi, which was then sprayed over the leaves of healthy Bottle Gourd plants. Inoculation into the leaves via foliar application was done carefully. The inoculated and control leaves were covered separately with sterilized polythene bags with small holes for air passage and to resist insect attacks. The plants were observed for characteristic symptoms such as yellowing, browning, rotting, spots, and stunted growth. Over the test duration, the progression of symptoms was monitored and compared to control plants, providing insights into *Fusarium* pathogenicity.

Figure 2. Pathogenecity test

3.4 Antagonistic effects of *Trichoderma* spp. against *Fusarium*

The potential antagonistic efficacy of two species of *Trichoderma* including *Trichoderma* HSTUT-6 and *Trichoderma* HSTUT-8 in inhibiting the growth of the tested fungi was investigated using the dual-culture technique. Discs of 0.6 cm, derived from the growing edge of a 10-day-old culture of *Fusarium*, were placed at a 1 cm distance from the edge of sterilized Petri dishes containing PDA media. Simultaneously, 0.6 cm diameter discs of a 7-day-old culture of *Trichoderma* HSTUT-6 and *Trichoderma* HSTUT-8 were placed on the opposite side. The interaction was observed, measuring inward linear growth after 2, 4, 6, and 8 days. Results regarding inhibition zones or the overgrowth of *Trichoderma* HSTUT-6 and *Trichoderma* HSTUT-8 on *Fusarium oxysporum* were recorded.

3.5 Application of *Trichoderma* spp. to enhance systemic resistance in Bottle Gourd

3.5.1 Experimental site

The experimental site, situated at 25038/N latitude and 88041/E longitude, with an elevation of 35 m above sea level, falls within the old Himalayan Piedmont plain (AEZ-1). This specific location provided the geographic context for the experimentation.

3.5.2 Preparation of pot soil and potting

Sandy loam soil and well-decomposed cow dung were collected and mixed in a ratio of 2:2:1. The mixture was then sterilized with formalin at a rate of 3 % per cubic foot of soil. The treated soil was covered with brown paper for 72 hours without interference. After 72 hours, the brown paper was removed, and the sterilized soil was exposed to air drying to remove excess formalin vapor for 48 hours. A total of 36 pots, each with a diameter of 10 cm, were washed with sterilized water and filled with 1 Kg of the sterilized mixed soil for seed germination.

3.5.3 Collection of seeds

Seeds of Bottle Gourd were collected from the renowned Bangladeshi seed company Laltir Seed Company. Two varieties, Naaz green and Diana, were chosen for the experiment.

3.5.4 Sowing of seeds

Before sowing the seeds, the surface was sterilized with a 70% Ethanol solution for 1 minute. For germination, the seeds were washed three times with distilled water before each seed was sown in an individual pot.

3.5.5 Treatment Combinations

The developed liquid formulations were applied to enhance the systemic acquired resistance in Bottle Gourd against *Fusarium* spot leaf disease caused by *F. oxysporum* f.sp. *cucumerinum*. However, the experiments comprised of four (4) treatments were as follows:

T_0: Control (*Fusarium oxysporum* only)

T_1: *Trichoderma* HSTUT-6 grown on Potato dextrose broth

T_2: *Trichoderma* HSTUT-6 grown on Molasses yeast solution

T_3: *Trichoderma* HSTUT-8 grown on Potato dextrose broth

T_4: *Trichoderma* HSTUT-8 grown on Molasses yeast solution

T_5: Turbo 50 WP (50 % Carbendazim)

3.5.6 Experimental design and layout

The experiment followed a Randomized Complete Block Design with three replications. There were 36 plants in total, with a plant-to-plant distance of 2.5 x 2 m.

3.5.7 Preparation and incubation of *Fusarium oxysporum*

Pure cultures were prepared on PDA (200 g potato, 20 g dextrose, 17 g agar, 1000 mL distilled water, and neutral pH) for ten days in the dark at 28 ºC from the previously obtained cultures.

3.5.8 Field Preparation

A well-drained field with fertile soil was selected for planting. The field was ploughed to a depth of 8 inches, and all unwanted weeds and plants were removed. For transplanting 36 seedlings, 36 pits were dug, each measuring 30 x 30 cm.

3.5.9 Stacking and trellis

Seedlings were initially supported using bamboo, and as they grew longer, horizontal bamboo trellises were used to support the heavy fruit of the Bottle Gourd.

Figure 3. Stacking & Trellis

3.5.10 Preparation of upgraded *Trichoderma* spp. liquid formulation

For potato-dextrose-based liquid formulation, 200 g of peeled and diced potatoes were boiled in 1 liter of distilled water until soft, after which the mixture was filtered to obtain the potato extract. Next, 20 g of dextrose were added to the extract and mixed thoroughly. The resulting medium was autoclaved and after cooling to room temperature, the spores from pure cultures of *Trichoderma* HSTUT-6 and *Trichoderma* HSTUT-8 were inoculated into the sterile medium under aseptic conditions. The inoculated medium was then incubated at 25-28 °C for 10 days to promote fungal growth.

For yeast-molasses-based liquid formulation, 10 g of yeast extract and 30 g of molasses were dissolved in 1 liter of warm, distilled water with thorough stirring. The mixture was then sterilized by autoclaving. After the medium was cooled to room temperature, it was inoculated with *Trichoderma* HSTUT-6 and *Trichoderma* HSTUT-8 spores under aseptic conditions. The inoculated

medium was incubated at 25-28 °C for 7-10 days to promote fungal growth. Both the formulation of cultured *Trichoderma* was diluted with sterile water for foliar application to achieve a spore concentration of 10^6-10^7 spores/mL.

3.5.11 Application of fertilizers

Well-decomposed cow dung (10 Kg/pit) was applied at the time of final soil preparation. The sources of nutrition were $ZnSo_4$ (10 g/pit) and TSP (50 g/pit). Urea (4 Kg/pit) was applied in two equal installments 20 and 30 days after Sowing.

3.5.12 Application of developed liquid formulation of *Trichoderma* spp.

In fifteen-days intervals, the liquid formulation of *Trichoderma* spp. (*Trichoderma* HSTUT-6 & *Trichoderma* HSTUT-8) was applied in the field for four times in the overall growth period. The first application was done at the time of field preparation. The rest of the three were foliar applications and applied after sowing of the Bottle Gourd plants.

3.5.13 Application of pesticides

Ripcord, and Ecomec were applied when needed to prevent insect infestation of Thrips.

3.5.14 Intercultural operations

Weeding, thinning, pruning, and irrigation were done as necessary. Yellow sticky traps and Pheromone traps were installed to control hoppers and fruit flies.

3.5.15 Cross-pollination

Bottle Gourd plants are typically monoecious, meaning they have separate male and female flowers on the same plant. In conducting cross-

pollination for Bottle Gourd plants, male and female flowers on the same plant were carefully identified. With the help of a soft brush or cotton swab, pollen from a male flower's stamen to the female flower's stigma was transferred, ensuring successful fertilization. This hands-on approach allows for controlled breeding.

3.6 Data Collection

3.6.1 Recording of disease

The disease resistance indexes included plant survival rate, disease incidence rate, and disease index and control effect. The grading system of Bottle Gourd disease severity was according to Zhang S P's standard (2016), and the disease index was calculated according to the method of Zong and Kang (2002).

Grade	Symptoms
0	No symptoms
1	The browning spots or rotting area of true leaves and cotyledon does not exceed 50 % of the total area;
2	The browning spots or rotting of true leaves and cotyledons exceeds 50 % of the total area
3	Leaves are rotted and dead with only growing points surviving
4	The entire plant is severely rotted or dead

Disease index = Σ (Plant number in the grade × Grade number) ÷ (Total plant number × The highest-grade number) × 100 %

Control effect (%) = (Disease index in the control group – Disease index in the treated group) ÷ Disease index in the control group × 100

Disease severity (%) = (Area of plant tissue infected ÷ Total area of tissue) ×100

3.6.2 Plant height (cm)

The plant height (cm) was measured by scale from ground level to the tip of the plant, expressed in centimeters, and the mean was computed 30 days after the seeds were sown.

3.6.3 Number of fruits per plant

Total numbers of marketable fruits were harvested. The total number of fruits was counted, and the average number of fruits per plant was calculated. Fruits were picked based on horticultural maturity, size, color, and age, which were determined for consumption as the fruit grew and soon reached the marketable stage. Picking was done throughout the harvesting period.

3.6.4 Weight (Kg) of fruit

The fruit's weight was recorded from the randomly selected plant from each replication, and the total yield was calculated by multiplying the plant population by the average plant yield.

3.6.5 Estimation of chlorophyll content of leaves (mg/g)

The chlorophyll content of the third leaf from the top of each treatment was estimated according to Whitman *et al.* (1971). In brief, 0.25 g leaf tissue from the middle of the leaf was taken in brown bottles containing 25 mL of 80 % aqueous acetone. The bottles were kept in the dark for 48 h. The optical density of the colored solutions was determined against 80 % acetone as blank using a SPECTROPHOTOMETER model T60 at 470, 646 nm, and 663 nm. Total chlorophyll was measured using the following formula:

$$Total\ chlorophyll = [20.2 * D\ (^{645}{}_{nm}) + 8.02 * D\ (^{663}{}_{nm})] * [V/W1000]$$

Here, V= Volume of 80 % aqueous acetone (mL); W= Weight of fresh leaf

D_{645}= Absorbance at 645nm wavelength; D_{663}= Absorbance at 663nm wavelength

3.6.6 Estimation of total phenol content of fruits (mg/100g)

Total phenol content was estimated according to Singleton and Rossi (1965) and Saikia *et al*. (2012). In brief, a 0.5 mL filtrated sample was thoroughly mixed with 0.5 milliliters of Folin-Ciocalteu's reagent in a 25-mL falcon tube. Subsequently, 1 mL of 7.5 % saturated sodium carbonate (Na_2CO_3) was added to the falcon tube to neutralize the solution, followed by vortexing for 30 seconds. The mixture was then left to react in a dark place for 35 minutes at room temperature and subsequently centrifuged at 4000 revolutions per minute for 10 minutes. The sample's absorption at 725nm was measured using a visible spectrophotometer T60, with gallic acid utilized to establish a standard (calibration) curve. The results are expressed as mg/g of Gallic acid per 100g of juice.

%Total phenol (mg/g sample) = × 100

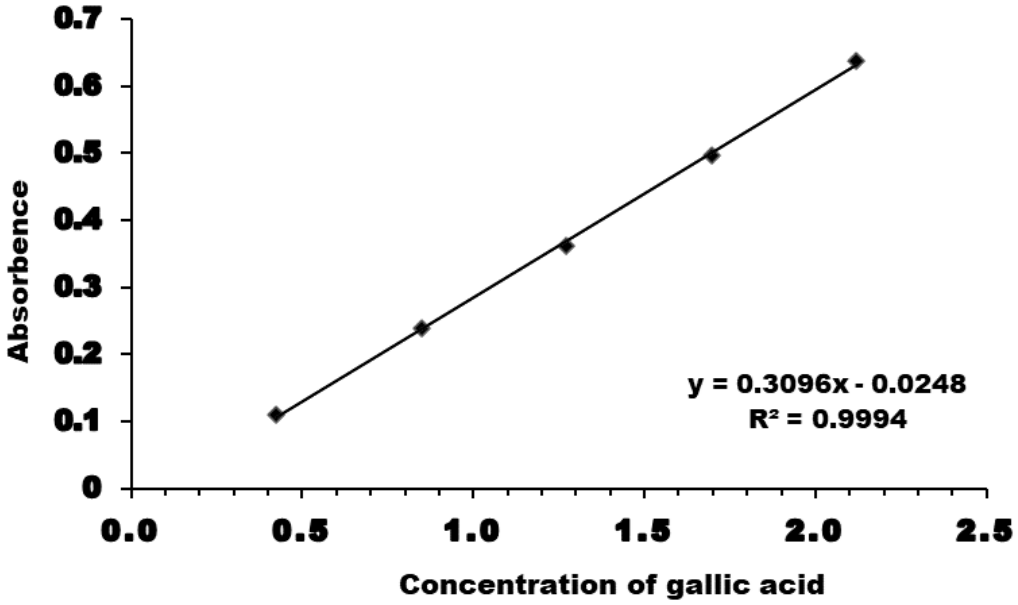

Figure 4. Estimation curve of gallic acid for estimation of Phenol content (mg/g)

3.6.7 Estimation of total soluble solids (TSS) (°Brix)

Total soluble solids (TSS) were determined using a refractometer and expressed as % °Brix.

3.6.8 Measurements Firmness (Kg/m^2)

Farmness of fruits was measured using a penetrometer and expressed as Kg/m^2.

3.6.9 Measurements of pH

Fruit pH was measured by a digital pH meter.

3.6.10 Statistical analysis

Various parameters were used to obtain data to determine the effectiveness of different treatments for controlling *Fusarium* leaf spot disease in Bottle Gourd. The statistical analysis was performed using Statistix10 software, and the mean difference among the treatments was estimated by the DMRT (Duncan's Multiple Range test) at a 5 % probability level (Gomez and Gomez 1948).

CHAPTER IV

RESULTS

The process of identifying a strain of pathogenic fungi responsible for leaf spot disease in Bottle Gourd through molecular characterization and controlling it with the upgraded liquid formulation of *Trichoderma* spp. in the field condition.

4.1 Isolation and Identification of *F. oxysporum*

Pathogenic fungi were isolated from the infected Bottle Gourd leaves and morphologically identified as *F. oxyxporum*. The fungus proudce both macroconidia and microconidia, along with colorless, extensively branched hyphae containing chlamydospores (Fig. 5). The isolated *Fusarium* strain displayed finer conidial ornamentation, slightly ovoidal conidia, a faster growth rate, mostly paired branches, ampulliform phialides, and a consistent presence of chlamydospores.

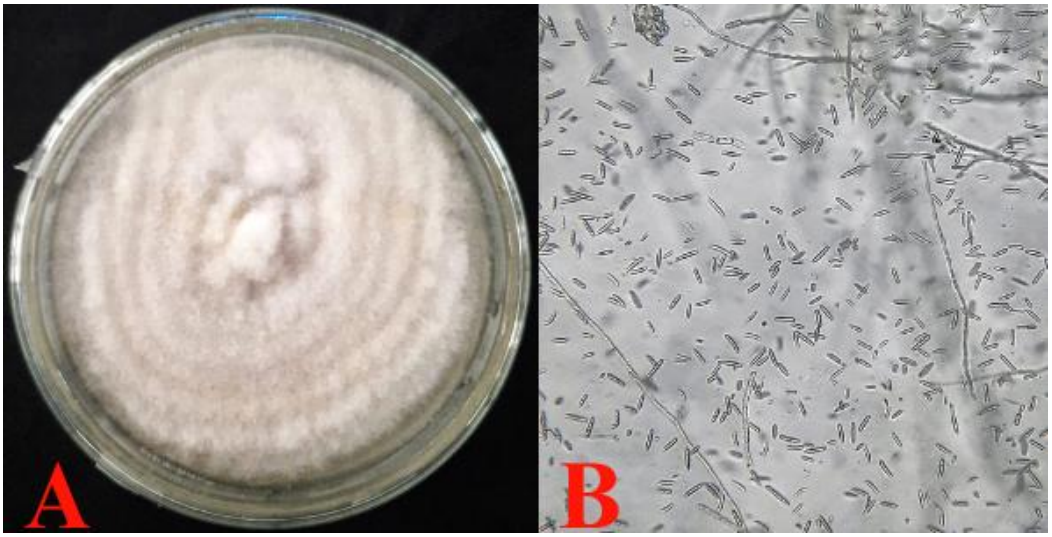

Figure 5. A) Colony morphology and **B)** Microscopic view of *F. oxyxporum*

4.2 *In-vitro* antifungal efficacy of *Trichoderma* against *Fusarium*

A total of two previously isolated *Trichoderma* were collected and tested against *Fusarium* for their antifungal efficacy. Both the isolates namely *Trichoderma* HSTUT-8 and *Trichoderma* HSTUT-6 showed inhibitory efficacy against *Fusarium* (86.67 %) (Table 1).

Table 1: Inhibitory effect of the collected *Trichoderma* against *Fusarium*

Serial No.	***Trichoderma* Isolates**	**Suppression of *Fusarium* Radial Growth**
1	*Trichoderma* HSTUT-6	82.23 %
2	*Trichoderma* HSTUT-8	86.67 %

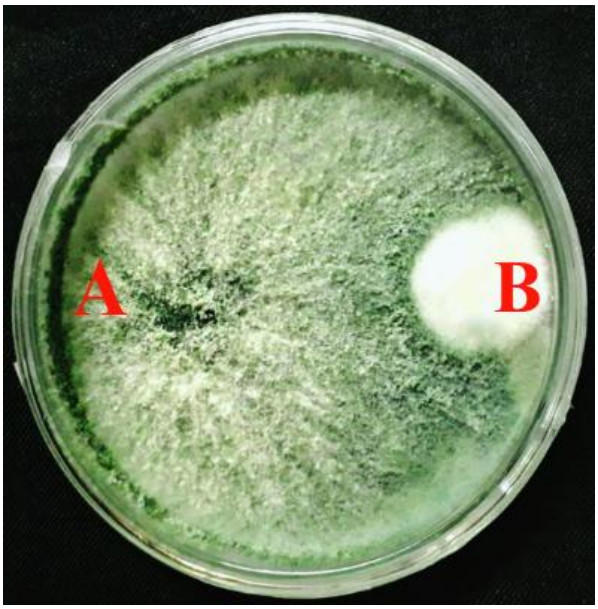

Figure 6. Inhibitory effect of the used *Trichoderma* against *F. oxysporum* by Dual Culture Method

4.3 Molecular characterization of the *F. oxysporum*

4.3.1 Gel Electrophoresis

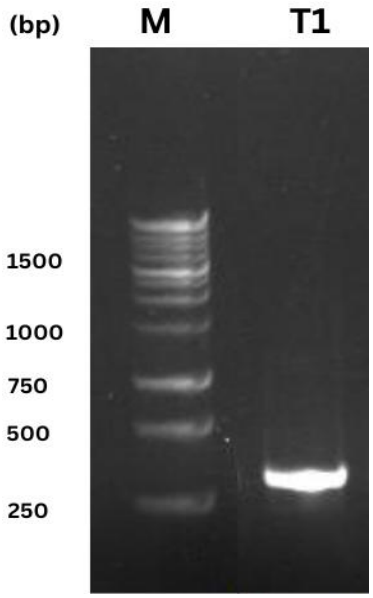

Figure 7. Gel electrophoresis profiles of amplified DNA of *Fusarium oxysporum*

In gel electrophoresis, the ITS gene sequences showing the isolated pathogen *Fusarium* were shown at around 350 bp.

4.4 Efficacy of developed liquid formulation of *Trichoderma* spp. against Fusarium leaf spot incidence (%) in Naaz Green Bottle Gourd at 60 DAS

In Naaz Green Bottle Gourd, the application of *Trichoderma* HSTUT-8 potato-dextrose-based liquid formulation showed the lowest disease incidence (27.18 %); followed by *Trichoderma* HSTUT-8 yeast-molasses-based formulation (31.35 %); *Trichoderma* HSTUT-6 potato-dextrose-based formulation (45.34 %); *Trichoderma* HSTUT-6 yeast-molasses-based formulation (49.33 %). However, plants inoculated with *F. oxyxporum* (control) only showed the highest disease incidence (73.42 %) (Table 2).

Table 2: Efficacy of the developed liquid formulation of *Trichoderma* spp. against Fusarium leaf spot incidence (%) in Naaz Garden Bottle Gourd at 60 DAS

Treatments	Disease incidence (%)	
	At 60 DAS	Reduction over control (%)
T_0	73.43a ± 5.5	0.00
T_1	45.34c ± 0.17	38.24
T_2	27.18def ± 2.55	62.97
T_3	49.33bc ± 7.02	32.81
T_4	31.35de ± 3.9	57.30
T_5	20.25f ± 3.9	72.41

*Means followed by different letter(s) in the column are significantly different according to Duccans's multiple range test at p= 0.05. (T0= Control; T1= *Trichoderma* HSTUT-6 potato dextrose formulation T2 = *Trichoderma* HSTUT-8 potato dextrose formulation; T3 = *Trichoderma* HSTUT-6 yeast molasses formulation; T4 = *Trichoderma* HSTUT-8 yeast molasses formulation; T5 = Turbo 50WP).

4.5 Efficacy of developed liquid formulation of *Trichoderma* spp. against *Fusarium* leaf spot incidence (%) in Diana Bottle Gourd at 60 DAS

In Diana Bottle Gourd, the application of *Trichoderma* HSTUT-8 *Trichoderma* potato-dextrose-based formulation showed the lowest disease incidence (25.53 %); followed by *Trichoderma* HSTUT-8 yeast-molasses-based formulation (32.43 %); *Trichoderma* HSTUT-6 potato-dextrose-based formulation (55.48 %); *Trichoderma* HSTUT-6 yeast-molasses-based formulation (50.79 %). However, plants inoculated with *F. oxyxporum* only (control) showed the highest disease incidence (82.23 %) (Table 3).

Table 3: Efficacy of developed liquid formulation of *Trichoderma* spp. against Fusarium leaf spot incidence (%) in Diana Bottle Gourd at 60 DAS

Treatments	Disease incidence (%)	
	At 60 DAS	Reduction over control (%)
T_0	82.22a ± 3.64	0.00
T_1	55.48b ± 3.46	32.52
T_2	25.53def ± 3.91	68.94
T_3	50.79bc ± 2.74	38.22
T_4	32.43d ± 1.97	60.54
T_5	21.68ef ± 5.10	73.63

*Means followed by different letter(s) in the column are significantly different according to Duccans's multiple range test at p= 0.05. (T0= Control; T1= *Trichoderma* HSTUT-6 potato dextrose formulation T2 = *Trichoderma* HSTUT-8 potato dextrose formulation; T3 = *Trichoderma* HSTUT-6 yeast molasses formulation; T4 = *Trichoderma* HSTUT-8 yeast molasses formulation; T5 =Turbo 50WP).

4.6 Efficacy of developed liquid formulation of *Trichoderma* spp. against Fusarium leaf spot incidence (%) in Naaz Green Bottle Gourd at 80 DAS

In Naaz Green Bottle Gourd, the application of *Trichoderma* HSTUT-8 potato-dextrose-based formulation showed the lowest disease incidence (23.82 %); followed by *Trichoderma* HSTUT-8 yeast-molasses-based formulation (31.76 %); *Trichoderma* HSTUT-6 yeast-molasses-based formulation (38.39 %); *Trichoderma* HSTUT-6 potato-dextrose--based formulation (40.11 %). However, plants inoculated with *F. oxyxporum* only (control) showed the highest disease incidence (79.51 %) (Table 4).

Table 4: Efficacy of developed liquid formulation of *Trichoderma* spp. against Fusarium leaf spot incidence % in Naaz Green at 80 DAS

Treatments	Disease incidence (%)	
	At 80 DAS	Reduction over control (%)
T_0	79.51a ± 1.26	0.00
T_1	40.11b ± 1.54	49.54
T_2	23.82e ± 1.59	70.03
T_3	38.39bc ± 0.94	51.71
T_4	31.76cd ± 3.30	60.05
T_5	21.62e ± 0.89	72.80

*Means followed by different letter(s) in the column are significantly different according to Duccans's multiple range test at p= 0.05. (T0= Control; T1= *Trichoderma* HSTUT-6 potato dextrose formulation T2 = *Trichoderma* HSTUT-8 potato dextrose formulation; T3 = *Trichoderma* HSTUT-6 yeast molasses formulation; T4 = *Trichoderma* HSTUT-8 yeast molasses formulation; T5 =Turbo 50WP).

4.7 Efficacy of developed liquid formulation of *Trichoderma* spp. against Fusarium leaf spot incidence (%) in Diana Bottle Gourd at 80 DAS

In Diana Bottle Gourd, the application of HSTUT-8 *Trichoderma* potato-dextrose-based formulation showed the lowest disease incidence (25.53 %); followed by *Trichoderma* HSTUT-8 yeast-molasses-based formulation (32.43 %); *Trichoderma* HSTUT-6 yeast-molasses-based formulation (50.79 %); *Trichoderma* HSTUT-6 potato-dextrose-based formulation (55.48 %). However, plants inoculated with *F. oxyxporum* only (control) showed the highest disease incidence (84.86 %) (Table 5).

Table 5: Efficacy of developed liquid formulation of *Trichoderma* spp. against Fusarium leaf spot incidence % in Diana Bottle Gourd at 80 DAS

Treatments	Disease incidence (%)	
	At 80 DAS	Reduction over control (%)
T_0	84.86a ± 3.64	0.00
T_1	39.18b ± 3.46	53.82
T_2	22.18e ± 3.91	73.86
T_3	38.81bc ± 2.74	54.25
T_4	26.91de ± 1.97	64.75
T_5	21.93e ± 5.10	74.15

*Means followed by different letter(s) in the column are significantly different according to Duccans's multiple range test at p= 0.05. (T0= Control; T1= *Trichoderma* HSTUT-6 potato dextrose formulation T2 = *Trichoderma* HSTUT-8 potato dextrose formulation; T3 = *Trichoderma* HSTUT-6 yeast molasses formulation; T4 = *Trichoderma* HSTUT-8 yeast molasses formulation; T5 =Turbo 50WP).

4.8 Efficacy of developed liquid formulation of *Trichoderma* spp. against Fusarium leaf spot disease severity (%) of Naaz green Bottle Gourd at 60 DAS

In Naaz Green Bottle Gourd, the application of *Trichoderma* HSTUT-8 potato-dextrose-based formulation showed the lowest disease incidence (8.33 %); followed by *Trichoderma* HSTUT-8 yeast-molasses-based formulation (10 %); *Trichoderma* HSTUT-6 yeast-molasses-based formulation (20 %); *Trichoderma* HSTUT-6 potato-dextrose--based formulation (35 %). However, plants inoculated with *F. oxyxporum* only (control) showed the highest disease severity (81.67 %) (Table 6).

Table 6: Efficacy of *Trichoderma* spp. against Fusarium leaf spot (disease severity) in Naaz Green Bottle Gourd at 60 DAS

Treatments	Disease severity (%)	
	At 60 DAS	Reduction over control (%)
T_0	81.66a ± 4.40	0.00
T_1	35.00b ± 2.88	57.14
T_2	8.33d ± 1.67	89.79
T_3	20.00c ± 2.88	75.51
T_4	10.00d ± 0	87.75
T_5	8.33d ± 1.67	89.79

*Means followed by different letter(s) in the column are significantly different according to Duccans's multiple range test at p= 0.05. (T0= Control; T1= *Trichoderma* HSTUT-6 potato dextrose formulation T2 = *Trichoderma* HSTUT-8 potato dextrose formulation; T3 = *Trichoderma* HSTUT-6 yeast molasses formulation; T4 = *Trichoderma* HSTUT-8 yeast molasses formulation; T5 =Turbo 50WP).

4.9 Efficacy of developed liquid formulation of *Trichoderma* spp. against Fusarium leaf spot disease (disease severity) in Diana Bottle Gourd at 60 DAS

In Diana Bottle Gourd, Turbo 50WP showed the lowest disease severity (6.67 %). When it comes to the *Trichoderma* treatments, the application of *Trichoderma* HSTUT-8 potato-dextrose-based formulation showed the lowest disease incidence (8.33 %); followed by *Trichoderma* HSTUT-8 yeast-molasses-based formulation (13.33 %); *Trichoderma* HSTUT-6 yeast-molasses-based formulation (18.33 %); *Trichoderma* HSTUT-6 potato-dextrose--based formulation (41.67 %). However, plants inoculated with *F. oxyxporum* only (control) showed the highest disease severity (85 %) (Table 7).

Table 7: Efficacy of developed liquid formulation of *Trichoderma* spp. against Fusarium leaf spot disease (disease severity) in Diana Bottle Gourd at 60 DAS

Treatments	Disease severity (%)	
	At 60 DAS	Reduction over control (%)
T_0	85.00a ± 2.88	0.00
T_1	41.67b ± 1.67	51.76
T_2	8.33d ± 1.67	90.19
T_3	18.33c ± 1.67	78.43
T_4	13.33cd ± 1.67	84.31
T_5	6.67d ± 1.67	92.15

*Means followed by different letter(s) in the column are significantly different according to Duccans's multiple range test at p= 0.05. (T0= Control; T1= *Trichoderma* HSTUT-6 potato dextrose formulation T2 = *Trichoderma* HSTUT-8 potato dextrose formulation; T3 = *Trichoderma* HSTUT-6 yeast molasses formulation; T4 = *Trichoderma* HSTUT-8 yeast molasses formulation; T5 =Turbo 50WP).

4.10 Efficacy of developed liquid formulation of *Trichoderma* spp. against Fusarium leaf spot (disease severity) in Naaz Green Bottle Gourd at 80 DAS

In Diana Bottle Gourd, the application of *Trichoderma* HSTUT-8 potato-dextrose-based formulation showed the lowest disease incidence (8.33 %); followed by *Trichoderma* HSTUT-8 yeast-molasses-based formulation (18.33 %); *Trichoderma* HSTUT-6 yeast-molasses-based formulation (26.67 %); *Trichoderma* HSTUT-6 potato-dextrose-based formulation (38.33 %). However, plants inoculated with *F. oxyxporum* only (control) showed the highest disease severity (85 %) (Table 8).

Table 8: Efficacy of developed liquid formulation of *Trichoderma* spp. against Fusarium leaf spot (disease severity) in Naaz Green Bottle Gourd at 80 DAS

Treatments	Disease severity (%)	
	At 80 DAS	Reduction over control (%)
T_0	85.00a ± 5	0.00
T_1	38.33b ± 4.40	54.90
T_2	8.33e ± 1.67	90.19
T_3	26.67c ± 1.67	68.62
T_4	18.33d ± 1.67	78.43
T_5	6.67e ± 1.67	92.15

*Means followed by different letter(s) in the column are significantly different according to Duccans's multiple range test at p= 0.05. (T0= Control; T1= *Trichoderma* HSTUT-6 potato dextrose formulation T2 = *Trichoderma* HSTUT-8 potato dextrose formulation; T3 = *Trichoderma* HSTUT-6 yeast molasses formulation; T4 = *Trichoderma* HSTUT-8 yeast molasses formulation; T5 =Turbo 50WP).

4.11 Efficacy of developed liquid formulation of *Trichoderma* spp. against Fusarium leaf spot (disease severity) in Diana Bottle Gourd at 80 DAS

In Diana Bottle Gourd, the application of *Trichoderma* HSTUT-8 potato-dextrose-based formulation showed the lowest disease incidence (10 %); followed by *Trichoderma* HSTUT-8 yeast-molasses-based formulation (10 %); *Trichoderma* HSTUT-6 yeast-molasses-based formulation (21.67 %); *Trichoderma* HSTUT-6 potato-dextrose--based formulation (28.33 %). However, plants inoculated with *F. oxyxporum* only (control) showed the highest disease severity (90 %) (Table 9).

Table 9: Efficacy of developed liquid formulation of *Trichoderma* spp. against Fusarium leaf spot (disease severity) in Diana Bottle Gourd at 80 DAS

Treatments	Disease severity (%)	
	At 80 DAS	Reduction over control (%)
T_0	90.00a ± 2.88	0.00
T_1	21.67cd ± 1.67	75.92
T_2	10.00e ± 0	88.89.
T_3	28.33c ± 2.88	68.51
T_4	10.00e ± 2.88	88.89
T_5	6.67e ± 1.67	92.59

*Means followed by different letter(s) in the column are significantly different according to Duccans's multiple range test at p= 0.05. (T0= Control; T1= *Trichoderma* HSTUT-6 potato dextrose formulation T2 = *Trichoderma* HSTUT-8 potato dextrose formulation; T3 = *Trichoderma* HSTUT-6 yeast molasses formulation; T4 = *Trichoderma* HSTUT-8 yeast molasses formulation; T5 =Turbo 50WP)

4.12 Effects of developed liquid formulation of *Trichoderma* spp. on plant height (cm) of Bottle Gourd at 120 DAS

In Naaz Green Bottle Gourd, *Trichoderma* HSTUT-8 grown on potato-dextrose liquid formulation showed the maximum plant height (485.21 cm). It was followed by the application of the *Trichoderma* HSTUT-8 grown on yeast-molasses liquid formulation as well as *Trichoderma* HSTUT-6 yeast molasses liquid formulation and *Trichoderma* HSTUT-6 potato-dextrose liquid formulation (425 cm, 421.67 cm & 413.33 cm respectively). The treated plant with pathogenic fungi *F. oxyxporum* showed the lowest plant height (240 cm).

In Diana Bottle Gourd, *Trichoderma* HSTUT-8 grown on potato-dextrose liquid formulation showed the maximum plant height (601.67 cm) with *Trichoderma Trichoderma* HSTUT-6 grown on potato-dextrose liquid formulation (581.67 cm) *Trichoderma* HSTUT-8 yeast-molasses liquid formulation (500 cm), and *Trichoderma* HSTUT-6 yeast molasses liquid formulation (480 cm). These results were followed by the effects of Turbo

50WP (529.33 cm). The pathogenic fungi *F. oxyxporum* inoculated plant showed the lowest result of plant height (395.67 cm) (Table 10).

Table 10: Efficacy of developed liquid formulation of *Trichoderma* spp. on yield contributing characters of Naaz Green and Diana Bottle Gourd plant at 120 DAS

Treatments	Plant height (cm) at 120 DAS	
	Naaz Green	Diana
T_0	240.00f ± 1.45	395.67e ± 0.88
T_1	413.33de ± 1.67	581.67b ± 2.18
T_2	485.21b ± 1.85	601.67a ± 2.08
T_3	421.67cd ± 3.38	480.00de ± 1.52
T_4	425.00c ± 2.08	500.00cd ± 1.15
T_5	501.67a ± 2.02	529.33c ± 3.28

*Means followed by different letter(s) in the column are significantly different according to Duccans's multiple range test at p= 0.05. (T0= Control; T1= *Trichoderma* HSTUT-6 potato dextrose formulation T2 = *Trichoderma* HSTUT-8 potato dextrose formulation; T3 = *Trichoderma* HSTUT-6 yeast molasses formulation; T4 = *Trichoderma* HSTUT-8 yeast molasses formulation; T5 =Turbo 50WP).

4.13 Effects of the developed liquid formulation of *Trichoderma* spp. on Yield Contributing Characters of Naaz Green Bottle Gourd

In Naaz Green Bottle Gourd, *Trichoderma* HSTUT-8 grown on potato-dextrose liquid formulation showed the maximum fruit diameter (32.33 cm). These results were followed by *Trichoderma* HSTUT-8 molasses-yeast liquid formulation, *Trichoderma* HSTUT-6 yeast-molasses liquid formulation, and *Trichoderma* HSTUT-6 grown on the potato-dextrose liquid formulation (29 cm, 28.67 cm & 26.67 cm respectively). The pathogenic fungi *F. oxyxporum* showed the lowest result of fruit diameter of (24.67 cm). *Trichoderma* HSTUT-8 grown on potato-dextrose liquid formulation showed the maximum fruit weight (2.24 Kg). These results were followed by *Trichoderma* HSTUT-6 yeast-molasses liquid formulation (1.55 Kg), *Trichoderma* HSTUT-8 yeast-molasses liquid formulation (1.51 Kg), and *Trichoderma* HSTUT-6 potato-

dextrose liquid formulation (1.27 Kg). The pathogenic fungi *F. oxyxporum* showed the lowest result in fruit weight (1.21 Kg). The *Trichoderma* HSTUT-8 potato dextrose liquid formulation showed the highest fruit height (51.33 cm) followed by the *Trichoderma* HSTUT-8 yeast-molasses liquid formulation (41 cm). These results were followed by *Trichoderma* HSTUT-6 yeast-molasses liquid formulation (39.33 cm), and *Trichoderma* HSTUT-6 potato-dextrose liquid formulation (36.67 cm). The pathogenic fungi *F. oxyxporum* inoculated plant showed the lowest result in fruit height (32.33 cm) (Table 11).

Table 11: Efficacy of developed liquid formulation of *Trichoderma* spp. on yield contributing characters of Naaz Green Bottle Gourd

Treatment	Number of Fruit/Plant	Fruit Height (cm)	Fruit Diameter (cm)	Fruit Weight (Kg)
T_0	4.67e ± 0.88	32.33d ± 1.45	24.67fg ± 1.45	1.21d ± 0.10
T_1	8.67c ± 0.67	36.67cd ± 1.67	26.67efg ± 0.67	1.27d ± 0.10
T_2	13.67a ± 0.33	51.33a ± 1.85	32.33cd ± 1.45	2.24a ± 0.25
T_3	7.33cd ± 0.33	39.33bcd ± 3.38	28.67def ± 2.02	1.55bcd ± 0.21
T_4	10.33b ± 0.33	41.00bc ± 2.08	29.00def ± 0.57	1.51bcd ± 0.23
T_5	12.67a ± 0.67	45.33ab ± 2.02	31.58cd ± 0.74	1.96abc ± 0.29

*Means followed by different letter(s) in the column are significantly different according to Duccans's multiple range test at p= 0.05. (T0= Control; T1= *Trichoderma* HSTUT-6 potato dextrose formulation T2 = *Trichoderma* HSTUT-8 potato dextrose formulation; T3 = *Trichoderma* HSTUT-6 yeast molasses formulation; T4 = *Trichoderma* HSTUT-8 yeast molasses formulation; T5 =Turbo 50WP).

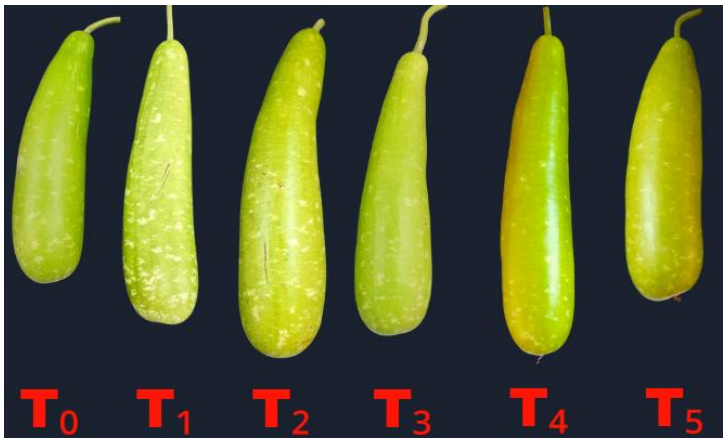

Figure 8. Fruits of Naaz Green as affected by different treatments

4.14 Effects of developed liquid formulation of *Trichoderma* spp. on yield and yield contributing characters of Diana Bottle Gourd

In Diana Bottle Gourd, *Trichoderma* HSTUT-8 grown on potato-dextrose liquid formulation showed the maximum fruit diameter (39.33 cm) statistically similar to Turbo 50WP (43.50 cm). These results were followed by *Trichoderma* HSTUT-8 molasses-yeast liquid formulation, *Trichoderma* HSTUT-6 yeast-molasses liquid formulation, and *Trichoderma* HSTUT-6 grown on the potato-dextrose liquid formulation (38.93 cm, 34.67 cm & 29.67 cm respectively). The pathogenic fungi *F. oxyxporum* showed the lowest result of fruit diameter of (23.53 cm). *Trichoderma* HSTUT-8 grown on potato-dextrose liquid formulation showed the maximum fruit weight (3.093 Kg). These results were followed by *Trichoderma* HSTUT-8 yeast-molasses liquid formulation (1.87 Kg), *Trichoderma* HSTUT-6 potato-dextrose liquid formulation (1.74 Kg), and *Trichoderma* HSTUT-6 yeast-molasses liquid formulation (1.67 Kg). The pathogenic fungi *F. oxyxporum* showed the lowest result in fruit weight (1.37 Kg). The *Trichoderma* HSTUT-8 potato dextrose liquid formulation showed the highest result in fruit height (49 cm) followed by the *Trichoderma* HSTUT-8 yeast-molasses liquid formulation (45 cm). These results were followed by *Trichoderma* HSTUT-6 potato-dextrose liquid formulation (37.33), and *Trichoderma* HSTUT-6 yeast-molasses liquid

formulation (37 cm). The pathogenic fungi *F. oxyxporum* showed the lowest result in fruit height (33.67 cm) (Table 12).

Table 12: Efficacy of developed liquid formulation of *Trichoderma* spp. on yield contributing characters of Diana Bottle Gourd

Treatment	Number of Fruit/Plant	Fruit height (cm)	Fruit diameter (cm)	Fruit weight (Kg)
T_0	7.00de ± 0.57	33.67b ± 0.88	23.53d ± 0.86	1.37b ± 0.04
T_1	9.33d ± 0.33	37.33b ± 2.18	29.67c ± 0.88	1.74ab ± 0.25
T_2	14.67a ± 0.33	49.00a ± 2.08	39.33ab ± 0.88	3.09a ± 0.02
T_3	8.33d ± 0.33	37.00b ± 1.52	34.67bc ± 3.17	1.67ab ± 0.23
T_4	11.00c ± 0.57	45.00a ± 1.15	38.93ab ± 0.96	1.87ab ± 0.15
T_5	13.67ab ± 0.33	34.67b ± 3.28	43.50a ± 3.32	2.10a ± 0.4

*Means followed by different letter(s) in the column are significantly different according to Duccans's multiple range test at p= 0.05. (T0= Control; T1= *Trichoderma* HSTUT-6 potato dextrose formulation T2 = *Trichoderma* HSTUT-8 potato dextrose formulation; T3 = *Trichoderma* HSTUT-6 yeast molasses formulation; T4 = *Trichoderma* HSTUT-8 yeast molasses formulation; T5 =Turbo 50WP).

Figure 9. Fruits of Diana Bottle Gourd as affected by different treatments

4.15 Correlation of co-efficient between yield and disease severity

In the regression, disease severity (%) was considered independent, and yield g/plant was the dependent variable. A strong negative correlation existed between the disease severity and yield Kg/plant. The regression equation also

showed that, for each unit increase in wilt severity, yield (Kg/plant) reduction of (0.91 and 0.94 %) occurred (Fig. 10).

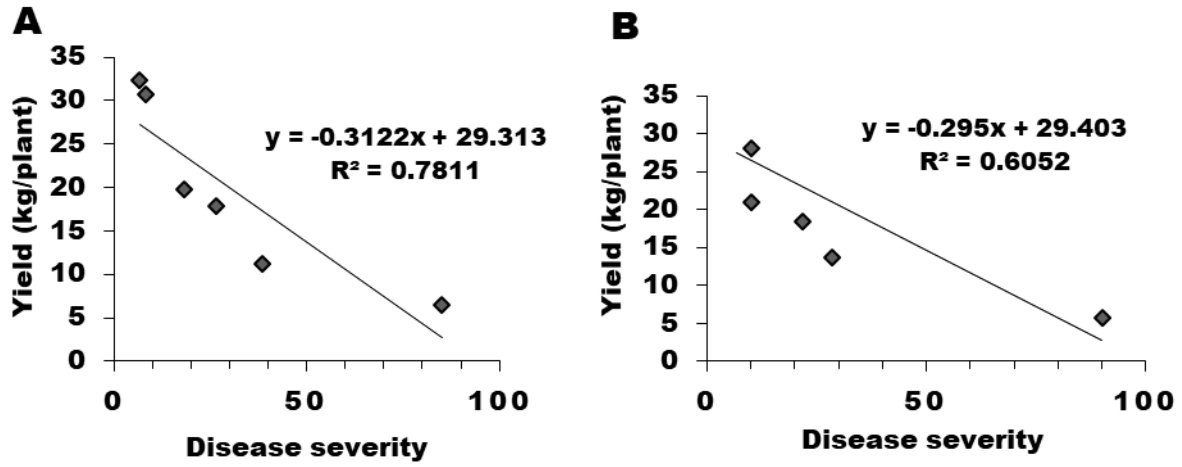

Figure 10. Correlation of co-efficient between yield and disease severity in A. Naaz Green and B. Diana Bottle Gourd

4.16 Interaction effect of treatments and variety on leaf spot severity against *F. oxyxporum*

Interaction of the variety and treatments revealed that statistically similar and significant disease severity reduction (90.19 and 89.89 %) was obtained in both the varieties with the application of *Trichoderma* HSTUT-8 grown on potato-dextrose liquid formulation combined over control (Table 13).

Table 13. Interaction effect of treatments and variety on Fusarium leaf spot severity against *F. oxyxporum*

Treatment×Variety	Severity (%)	Reduction over control	Treatment×Variety	Severity (%)	Reduction over control
T_0V_1	85.00a	0.00	T_0V_2	90.00a	0.00
T_1V_1	38.33b	54.90	T_1V_2	21.67cd	75.92
T_2V_1	8.33e	90.19	T_2V_2	10.00e	88.89
T_3V_1	26.67c	68.62	T_3V_2	28.33c	68.51
T_4V_1	18.33d	78.43	T_4V_2	10.00e	88.89
T_5V_1	6.67e	92.15	T_5V_2	6.67e	92.59

*Means followed by different letter(s) in the column are significantly different according to Duccans's multiple range test at p= 0.05. (T0= Control; T1= *Trichoderma* HSTUT-6 potato dextrose formulation T2 = *Trichoderma* HSTUT-8 potato dextrose formulation; T3 = *Trichoderma* HSTUT-6 yeast molasses formulation; T4 = *Trichoderma* HSTUT-8 yeast molasses formulation; T5 =Turbo 50WP).

4.17 Effect of developed liquid formulation of *Trichoderma* spp. on the content of photosynthetic pigments (Total Chrolophyll) in the leaves of Bottle Gourd

In Naaz Green, *Trichoderma* HSTUT-8 grown on potato-dextrose liquid formulation showed the highest total chlorophyll content (1.88 mg/g FW) followed by *Trichoderma* HSTUT-8 grown on yeast-molasses formulation (1.79 mg/g FW). These results were followed by the effects of the *Trichoderma* HSTUT-6 grown on yeast-molasses liquid formulation (1.67 mg/g FW); *Trichoderma* HSTUT-6 grown on potato-dextrose formulation showed the lowest result (0.93 mg/m^2 FW); The pathogenic fungi *F. oxyxporum* showed result of chlorophyll content (1.64 mg/g FW) (Fig. 11).

In Diana, *Trichoderma* HSTUT-8 grown on potato-dextrose liquid formulation again showed the highest total chlorophyll content (1.84 mg/g FW) and the *Trichoderma* HSTUT-8 grown on yeast-molasses solution gave the second total chlorophyll (1.65 mg/g FW). These results were followed by the

application of *Trichoderma* HSTUT-8 yeast-molasses liquid formulation (1.65 mg/g FW); *Trichoderma* HSTUT-6 grown on potato-dextrose broth and yeast molasses broth resulted (0.45 & 0.99 mg/g FW, respectively); The pathogenic fungi *F. oxyxporum* showed the lowest chlorophyll content (1.67 mg/g FW) (Fig. 11).

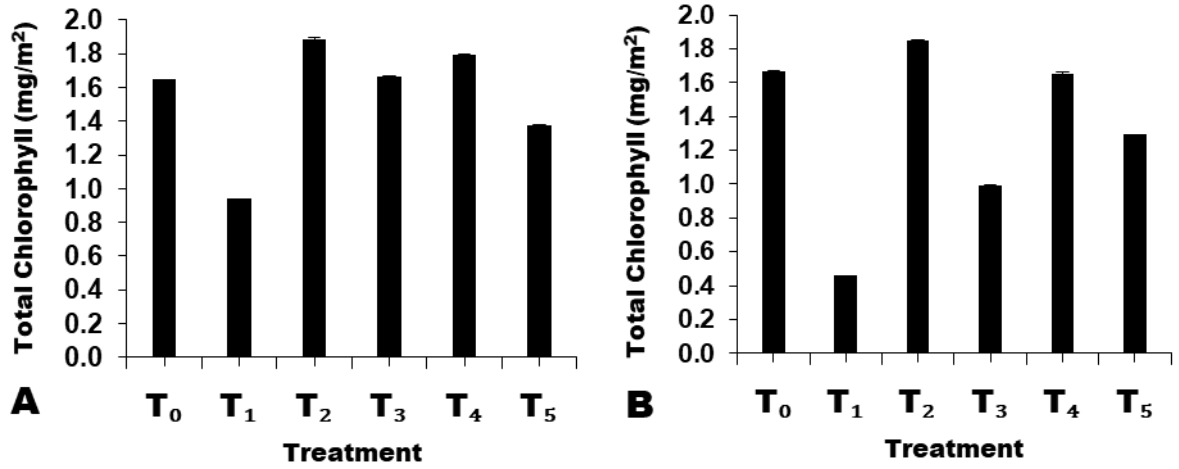

Figure 11. Effect of HSTUT-6 and HSTUT-8 on total chlorophyll of leaves of A) Naaz Green, B) Diana (*Means followed by different letter(s) in the column are significantly different according to Duccans's multiple range test at p=0.05. (T0= Control; T1= *Trichoderma* HSTUT-6 potato dextrose formulation T2 = *Trichoderma* HSTUT-8 potato dextrose formulation; T3 = *Trichoderma* HSTUT-6 yeast molasses formulation; T4 = *Trichoderma* HSTUT-8 yeast molasses formulation; T5 =Turbo 50WP).

4.18 Effect of developed liquid formulation of *Trichoderma* spp. on total phenols of the fruits of Naaz Green and Diana Bottle Gourd

In Naaz Green, *Trichoderma* HSTUT-8 grown on potato-dextrose liquid formulation showed the highest total phenol content (0.583 mg/100g FW) followed by *Trichoderma* HSTUT-8 grown on yeast-molasses formulation (0.523 mg/100g FW). These results were followed by the effects of *Trichoderma* HSTUT-6 yeast-molasses-based formulation, and *Trichoderma* HSTUT-6 potato-dextrose-based formulation (0.306 & 0.152 mg/100g FW, respectively); The pathogenic fungi *F. oxyxporum* showed the lowest result of phenol content (0.142 mg/100g FW) (Fig. 12).

In Diana, *Trichoderma* HSTUT-8 grown on potato-dextrose liquid formulation showed the highest total phenol content (0.553 mg/100g FW)

followed by *Trichoderma* HSTUT-8 grown on yeast-molasses formulation (0.531 mg/100g FW). These results were followed by the effects of *Trichoderma* HSTUT-6 potato-dextrose-based formulation, *Trichoderma* HSTUT-6 yeast molasses formulation (0.486, 0.209 mg/100g FW, respectively); The pathogenic fungi *F. oxyxporum* showed the result of phenol content (0.471 mg/100g FW) (Fig. 12).

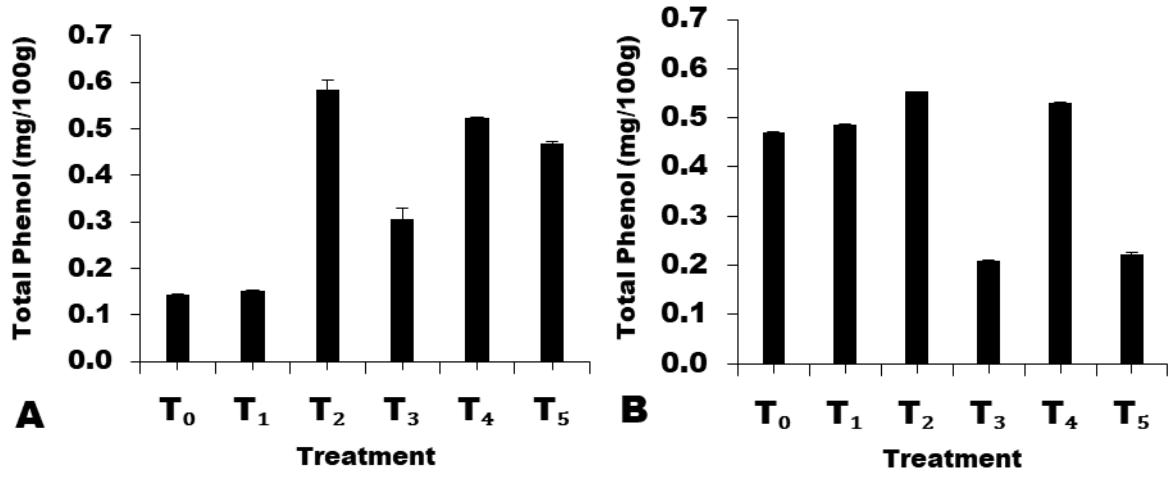

Figure 12. Effect of *Trichoderma* HSTUT-6 and *Trichoderma* HSTUT-8 on total Phenol of fruits of A) Naaz Green, B) Diana (*Means followed by different letter(s) in the column are significantly different according to Duccans's multiple range test at p=0.05. (T0= Control; T1= *Trichoderma* HSTUT-6 potato dextrose formulation T2 = *Trichoderma* HSTUT-8 potato dextrose formulation; T3 = *Trichoderma* HSTUT-6 yeast molasses formulation; T4 =HSTUT-8 yeast molasses formulation; T5 =Turbo 50WP).

4.19 Effect of developed liquid formulation of *Trichoderma* spp. on Total Soluble Solids (TSS) in fruits of both Naaz Green and Diana Bottle Gourd

In Naaz Green Bottle Gourd, *Trichoderma* HSTUT-8 potato dextrose formulation showed the highest total soluble solid (TSS %) (6.23 %) followed by *Trichoderma* HSTUT-8 yeast molasses formulation which showed the second highest result (5.5 %). These results were followed by *Trichoderma* HSTUT-6 yeast molasses formulation, Turbo 50WP, and *Trichoderma* HSTUT-6 potato dextrose formulation (5.46 %, 4.86 %, & 4.6 % respectively). The

pathogenic fungi *Fusarium* showed the lowest result of TSS (3.23 %) (Figure 13).

In Diana Bottle Gourd, *Trichoderma* HSTUT-8 potato dextrose formulation showed the highest TSS % value (5.7 %) followed by *Trichoderma* HSTUT-8 yeast molasses formulation and *Trichoderma* HSTUT-6 yeast molasses formulation which showed the second and third highest results (5.23 % & 5.13 %, respectively). The pathogenic fungi *Fusarium* showed 3.1 %. Surprisingly, the *Trichoderma* HSTUT-6 potato dextrose formulation showed the lowest result (2.7 %) (Fig. 13).

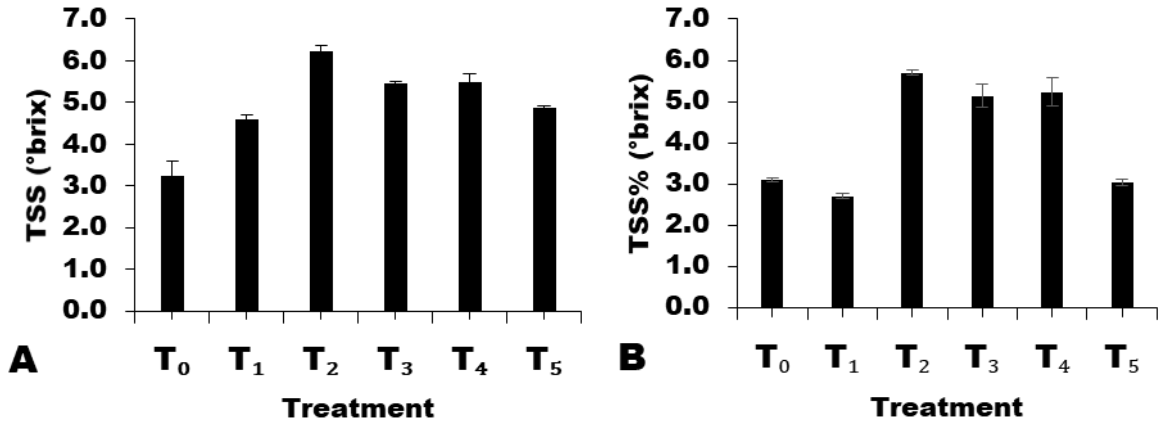

Figure 13. Effect of *Trichoderma* HSTUT-6 and *Trichoderma* HSTUT-8 on total soluble solids in fruits of A) Naaz Green, B) Diana (*Means followed by different letter(s) in the column are significantly different according to Duccans's multiple range test at p=0.05. (T0= Control; T1= *Trichoderma* HSTUT-6 potato dextrose formulation T2 = *Trichoderma* HSTUT-8 potato dextrose formulation; T3 = *Trichoderma* HSTUT-6 yeast molasses formulation; T4 = *Trichoderma* HSTUT-8 yeast molasses formulation; T5 =Turbo 50WP).

4.20 Effect of developed liquid formulation of *Trichoderma* spp. on firmness (Kg/m²) in Bottle Gourd fruits

In the Naaz Green Bottle Gourd, the pathogenic fungi *Fusarium* which showed the highest result (2.9 Kg/m²). These results were followed by *Trichoderma* HSTUT-6 molasses-yeast formulation, *Trichoderma* HSTUT-6 potato-dextrose formulation, and *Trichoderma* HSTUT-8 molasses-yeast formulation (2.4 Kg/m², 2.26 Kg/m², & 2.17 Kg/m² respectively). The

Trichoderma HSTUT-8 potato dextrose formulation showed the lowest result of firmness (2.09 Kg/m²) (Fig. 14).

In Diana Bottle Gourd, the plant inoculated with pathogenic fungi *Fusarium* showed the highest firmness (2.67 Kg/m²). These results were followed by *Trichoderma* HSTUT-6 molasses-yeast formulation, *Trichoderma* HSTUT-8 molasses-yeast formulation, and *Trichoderma* HSTUT-6 potato-dextrose formulation (2.58 Kg/m², 2.31 Kg/m², & 2.14 Kg/m² respectively). The *Trichoderma* HSTUT-8 potato dextrose formulation showed the lowest result (2.14 Kg/m²) (Fig. 14).

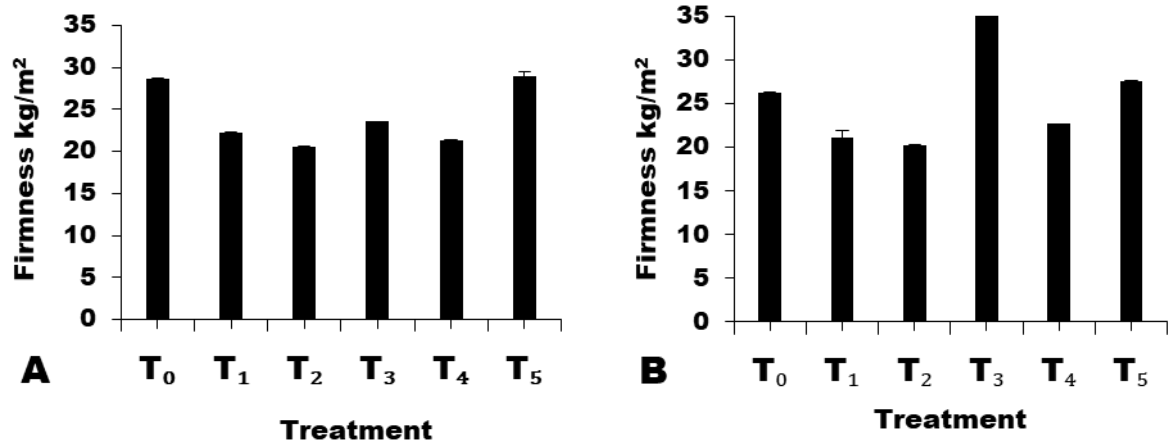

Figure 14. Effect of *Trichoderma* HSTUT-6 and *Trichoderma* HSTUT-8 on the firmness (Kg/m^2) in Bottle Gourd fruits of A) Naaz Green, B) Diana (*Means followed by different letter(s) in the column are significantly different according to Duccans's multiple range test at p=0.05. (T0= Control; T1= *Trichoderma* HSTUT-6 potato dextrose formulation T2 = *Trichoderma* HSTUT-8 potato dextrose formulation; T3 = *Trichoderma* HSTUT-6 yeast molasses formulation; T4 = *Trichoderma* HSTUT-8 yeast molasses formulation; T5 =Turbo 50WP).

4.21 Effect of developed liquid formulation of *Trichoderma* spp. on pH in Bottle Gourd Fruits

In the Naaz Green Bottle Gourd, The *Trichoderma* HSTUT-8 potato dextrose formulation showed the highest pH level of (6.8) and the *Trichoderma* HSTUT-8 yeast-molasses formulation showed the second highest pH level of (6.36). The results were followed by *Trichoderma* HSTUT-6 potato-dextrose liquid formulation and *Trichoderma* HSTUT-6 yeast-molasses liquid

formulation (6.25 & 5.96, respectively). The plant treated with pathogenic fungi *Fusarium* showed the lowest pH (5.16) (Fig. 15).

In Diana Bottle Gourd, The *Trichoderma* HSTUT-8 potato dextrose formulation showed the highest pH level of (6.64) and the *Trichoderma* HSTUT-8 yeast-molasses formulation showed the second highest pH level of (6). The results were followed by *Trichoderma* HSTUT-6 potato-dextrose liquid formulation and *Trichoderma* HSTUT-6 yeast-molasses liquid formulation (5.55 & 5.43, respectively). Turbo 50WP resulted in (5.5) pH and the pathogenic fungi *Fusarium* showed the lowest result of pH (5.67) (Fig. 15).

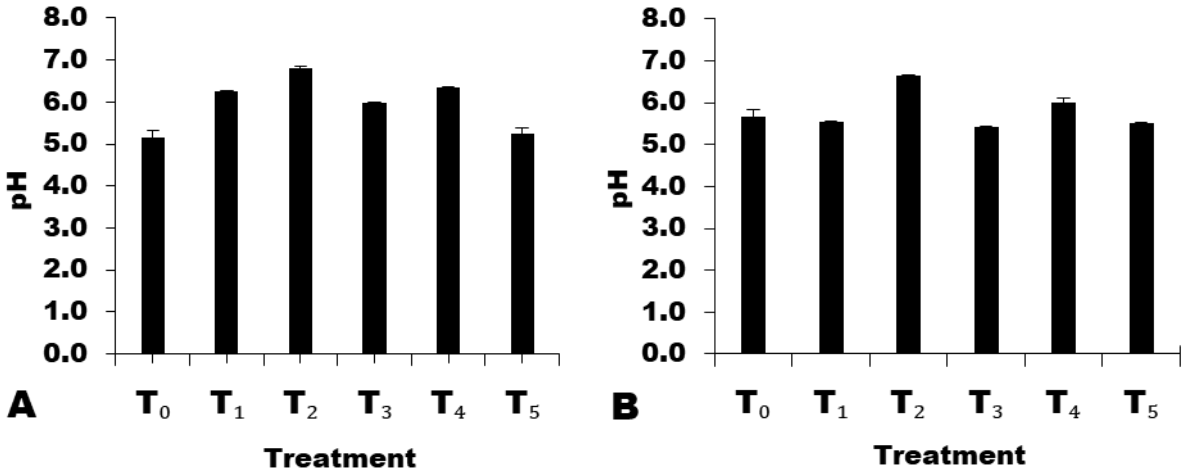

Figure 15. Effect of *Trichoderma* HSTUT-6 and *Trichoderma* HSTUT-8 on the pH of Bottle Gourd fruits of A) Naaz Green, B) Diana (*Means followed by different letter(s) in the column are significantly different according to Duccans's multiple range test at p=0.05. (T0= Control; T1= *Trichoderma* HSTUT-6 potato dextrose formulation T2 = *Trichoderma* HSTUT-8 potato dextrose formulation; T3 = *Trichoderma* HSTUT-6 yeast molasses formulation; T4 = *Trichoderma* HSTUT-8 yeast molasses formulation; T5 =Turbo 50WP).

4.22 Effect of developed liquid formulation of *Trichoderma* spp. on Moisture Content in Bottle Gourd Fruits

In the Naaz Green Bottle Gourd, The *Trichoderma* HSTUT-8 potato dextrose formulation showed the highest moisture level of (97.2 %) and the *Trichoderma* HSTUT-8 yeast-molasses liquid formulation showed the second highest moisture level of (95.26 %). These results were followed by *Trichoderma* HSTUT-6 yeast-molasses liquid formulation and *Trichoderma*

HSTUT-6 potato-dextrose formulation (94.43 % & 93.5 %, respectively). The plant treated with pathogenic fungi *Fusarium* showed the lowest moisture content (90.65 %) (Fig. 16).

In the Diana Bottle Gourd, The *Trichoderma* HSTUT-8 potato dextrose formulation showed the highest moisture level of (97.63 %) and the *Trichoderma* HSTUT-8 yeast-molasses formulation showed the second-highest moisture level of (96.68 %). The results were followed by *Trichoderma* HSTUT-6 potato-dextrose liquid formulation and *Trichoderma* HSTUT-6 yeast-molasses liquid formulation (96.16 % & 95.96 %, respectively). The plant infected with the pathogenic fungi *Fusarium* showed the lowest moisture percentage (90.65 %) (Fig. 16).

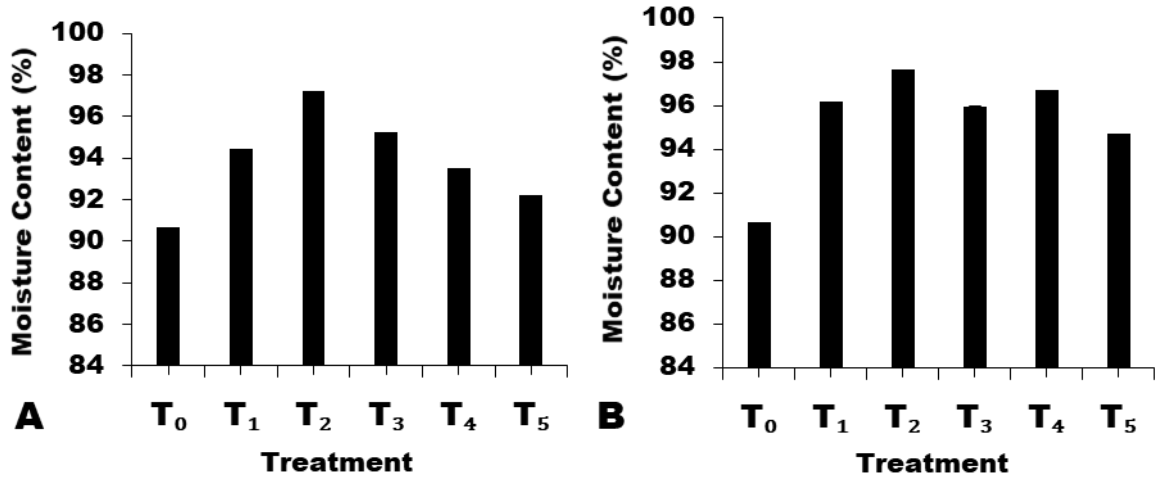

Figure 16. Effect of *Trichoderma* HSTUT-6 and *Trichoderma* HSTUT-8 on the Moisture content (%) in Bottle Gourd fruits of A) Naaz Green, B) Diana (*Means followed by different letter(s) in the column are significantly different according to Duccans's multiple range test at p=0.05. (T0= Control; T1= *Trichoderma* HSTUT-6 potato dextrose formulation T2 = *Trichoderma* HSTUT-8 potato dextrose formulation; T3 = *Trichoderma* HSTUT-6 yeast molasses formulation; T4 = *Trichoderma* HSTUT-8 yeast molasses formulation; T5 =Turbo 50WP).

CHAPTER V

Discussion

The Leaf spot disease caused by *Fusarium* is a severe problem for Bottle Gourd cultivators in Bangladesh. The pathogen responsible for the disease is soil-borne, making chemical control methods expensive and challenging. Moreover, chemical controls pollute the environment and pose health risks to humans. People around the world are concerned about the deleterious effects of chemical pesticides in controlling different pests and diseases (Mazzola, 1998; Mark *et al.*, 2006). One such strategy to avoid such consequences is using beneficial microorganisms like *Trichoderma,* which can help control soil-borne fungal pathogens.

Fusarium was isolated from spotted Bottle Gourd leaves and identified based on morphology. Pathogenicity tests confirmed its potential to attack Bottle Gourd leaves. There have been reports of a few diseases affecting Bottle Gourd, including powdery mildew, downy mildew, fruit rot, anthracnose, root rot, root-knot, insect pests, and viral diseases. (Maholay, 1989). Additionally, some fungi have been found in Bottle Gourd seeds, those are *Altenaria alternata, Aspergillus flavus, A. niger, Botryodiplodia theobromae, Chaetomium sp., Curvularia lunata, Drechslera tetramera, Fusarium equiseti, F. moniliforme, F. solani, Macrophomina phaseolina, Myrothecium roridum, Rizoctonia solani, Sclerotium rolfsii* and *Trichoderma* spp., (Maholay & Sohi, 1976; Richardson, 1979; Maholay 1989; Shakir & Mirza, 1992).

Two *Trichoderma* isolates were collected from the rhizospheric soil of plants of which both the strains showed inhibitory effects against *Fusarium*. *Trichoderma* HSTUT-8 showed the highest inhibitory effect (76.67 %) followed by *Trichoderma* HSTUT-6 (72.23 %) against *Fusarium* in vitro. Among all the developed liquid formulated *Trichoderma,* potato-dextrose based *Trichoderma* HSTUT 6 showed the highest reduction over control against the pathogen up to

90.19 % in Naaz Green and 88.89 % in Diana Bottle Gourd plants. *Trichoderma* may have produced diffusible inhibitory substances that suppressed *F. oxysporum* growth, resulting in the inhibition zone. *Trichoderma* have been shown to produce antibiotics based on the presence and size of their zone of inhibition (Jackson *et al.,* 1991; Crawford *et al.,* 1993). *Trichoderma* produce cell-free metabolites that can reduce *F. oxysporum* colony size. Although antibiotic substances from *Trichoderma* were not extracted and determined in this study, some antibiotics such as tubercidin, candicidin, phosphlactomycin, phenasin, and 4- diacetylphloroglucinol, which have been produced by some antagonists, like *Pseudomonas flourescens*, *Streptomyces* spp., and *Trichoderma* spp., have been reported by researchers (Hwang *et al.,* 1994; Shanahan *et al.,* 1992). *Trichoderma* contains species that are well-documented biocontrol agents for various crop pathogens, including *Fusarium* (Degenkolb *et al.,* 2015; Reino *et al.,* 2008; Vinale *et al.,* 2008). *Trichoderma* species may use various modes of antagonism, including toxin production (e.g., antifungals, chitinases), mycoparasitism (physical disruption of pathogen hyphal growth, coiling, penetration, dissolution of cytoplasm), inducing a host defense response, or monopolizing rhizosphere nutrients and space (Howell, 2003). Recent studies on the role of *Trichoderma* in biological control have primarily used strains such as *T. harzianum* (Yedidia *et al.,* 2001; Cheng *et al.,* 2010; Chen *et al.,* 2012), *T. viride* (Zhuang *et al.,* 2005; Cheng *et al.,* 2010; Bi 2016), *T. longibrachiatum* (Li *et al.,* 2010; Bi 2016; Zhang S W *et al.,* 2016), *T. reesei* (Luo *et al.,* 2016). Very few studies have focused on *T. asperellum* and *T. koningense* (Qi & Zhao 2013).

In the present study, the plant height was also increased in *Trichoderma*-treated plants. The increase in plant growth might be associated with the secretion of auxins, gibberellins, and cytokinins (Hwang *et al.,* 1994). Cheng *et al.* (2010) observed that *T. viride* T23 and *T. harzianum* T22 prevent cucumber *Fusarium* wilt, with an efficacy of 66.04 %; while Zhuang *et al.* (2005)

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 cucumber *Fusarium* wilt, with efficacies of 75.74 and 70.76 %, respectively. 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 *T. harzianum* T203 in the soil increased the cucumber root area by 95 %, the plant dry weight by 80 %, the root length by 75 %, the plant height by 45 %, and the leaf area by 80 %. Zhang S W *et al.* (2016) demonstrated that *T. longibrachiatum* spore suspension significantly promoted the growth of Meloidogyne incognita-inoculated cucumbers. In addition, several strains of *Trichoderma* also promoted plant growth and biomass accumulation in other plants, such as cabbage (Xing *et al.* 2017) and *Sedum plumbizincicola* (Luo *et al.* 2016). The plant chlorophyll content and the leaf nitric nitrogen content are important indicators for evaluating photosynthesis and nitrogen assimilation and utilization.

In this study, the plants that were treated with the upgraded liquid formulations of *Trichoderma* bear fruits with higher length, weight, and diameter than those treated with chemical treatments. The increase in fruit health may be associated with photorespiratory amino acids, harzianic acid (HA), 6-pentyl-α-pyrone (6PP), secondary metabolites, antioxidants, polyphenols, chitinase, glucanase, and ammonium (Pascale *et al.*, 2017). *T. longibrachiatum* significantly promotes the growth and yield of tomato and contents of lycopene and some amino acids increased in the plum tomato fruits after the application of *T. harzianum* by increasing nitrogen metabolism (Carillo *et al.*, 2020).

Plant chlorophyll and leaf nitric nitrogen content are key indicators of photosynthesis and nitrogen utilization. According to Liu *et al.* (2013), *T. viride*

broth inhibits mango anthracnose pathogens and boosts antioxidant enzyme activity in mango. Liu *et al*. (2014) found that *T. harzianum* T23 increased the production of phytoalexin and lignin in eggplants, as well as the activity of PAL, PPO, POD, and SOD, leading to increased resistance to *Fusarium* wilt. Consistent with previous studies In vivo results revealed that combining *Trichoderma* HSTUT-6 and *Trichoderma* HSTUT-8 significantly increases photosynthetic pigment and total phenols in Bottle Gourd plant leaves. Also, it improved fruit quality, including firmness, pH, TSS and moisture content.

CHAPTER VI

SUMMARY AND CONCLUSION

The leaf spot disease of Bottle Gourd is a newly emerging disease in Bangladesh which is negatively impacting the yield of the Bottle Gourd. As the chemical method of disease control is harmful to the environment and does not improve the fruit quality, this study aimed to determine the *in-vitro* efficacy of isolated *Trichoderma* strains against *F. oxysporum* and evaluate the practical application in the field of *Trichoderma* spp. in enhancing systemic resistance and improving overall health and yield in Bottle Gourd crops facing *Fusarium*-related biotic stresses. The study involved collecting leaves showing signs of leaf spot disease from Bottle Gourd plants and identifying *Fusarium*. The isolated fungi were identified by examining their morphological and cultural characteristics. The potential antagonistic effects of collected *Trichoderma* HSTUT-6 and HSTUT-8 on *Fusarium* were investigated using the dual-culture technique.

The fungi *Trichoderma* HSTUT-6 and *Trichoderma* HSTUT-8 were grown on liquid formulations and applied to enhance systemic resistance in Bottle Gourd against Fusarium leaf disease. Total 6 treatments were applied: Control (*Fusarium oxysporum* only), *Trichoderma* HSTU-6 grown on Potato dextrose broth, *Trichoderma* HSTU-6 grown on Molasses yeast solution, *Trichoderma* HSTUT-8 grown on Potato dextrose broth, *Trichoderma* HSTUT-8 grown on Molasses yeast solution, and Turbo 50 WP (50 % Carbendazim). *Trichoderma* HSTUT-8 grown on potato-dextrose liquid formulation showed a maximum inhibition percentage followed by *Trichoderma* HSTUT-8 yeast-molasses liquid formulation. Molecular characterization of the *Trichoderma* isolate (*Trichoderma* HSTUT-6 and *Trichoderma* HSTUT-8) showed antifungal efficacy against *Fusarium*.

In Naaz Green and Diana Bottle Gourd, *Trichoderma* HSTUT-8 potato-dextrose liquid formulation resulted in the height plant height (51.33 cm & 49 cm, respectively); Same formulated Trichoderma also resulted in higher TSS value (6.23 % & 5.7 %, respectively).

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