INTEGRATED MANAGEMENT OF FUSARIUM WILT AND COLLAR ROT OF CHICKPEA

A THESIS

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Student No: 1701314

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IN

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DEPARTMENT OF PLANT PATHOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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DADICATED TO MY

BELOVED PARENTS

AND HUSBAND

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JUNE 2024 The author

ABSTRACT

Wilt and collar rot of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* and *Sclerotium rolfsii*, respectively is a serious disease responsible for maximum yield loss in Bangladesh. For integrated management of Fusarium wilt and collar rot of chickpea, *Trichoderma* asperellum HSTUT 1, Neem leaf extract and Autostin 50 WDS were selected. In dual culture, *T. asperellum* HSTUT 1 inhibited the mycelial growth of *F. oxysporum* and *S. rolfsii* by 63.33 and 56.66 %, respectively. Well diffusion assay showed that neem leaf extract and Autostin 50 WDG also inhibited the mycelial growth of *F. oxysporum* by 38.88, 65.22, 73.00 & 86.66% and *S. rolfsii* by 35.55, 60.77, 66.33 & 74.88%, respectively over control. In the field conditions, combined application of *T. asperellum* HSTUT 1, neem extract and Autostin 50 WDG resulted higher reduction of wilt incidence by 9.66, 1.21 & 0.00 % and collar rot incidence by 9.14, 2.33 & 0.00% at 30, 45 and 60 DAS, respectively in comparison to other treatments. Combined application of *Trichoderma,* neem leaf extract and Autostin 50 WDG also exhibited the highest germination (84.55 & 90.22%), highest shoot length (18.24 & 16.10 cm), highest root length (5.92 & 7.30 cm), highest shoot weight (0.88 & 0.83g), highest root weight (0.32 & 0.32g), highest pod number per plant (26, 49.33), highest nodule number per plant (46.33 & 31.00) and highest yield per plant (4.16 & 7.89g), respectively in Fusarium wilt and collar rot pathogen infected plant. The finding of the present study explored the potentiality of the combination of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50 WDG in the eco-friendly management of Fusarium wilt and collar rot of chickpea in field conditions.

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CHAPTER I

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the important pulse crops in Bangladesh. It provides energy, protein, minerals, vitamins, fiber and potentially health promoting phytochemicals (Wood *et al*. 2007). Chickpea is a preferred to food legumes in some regions because of its multiple uses. Chickpeas are considered to be special due to its high protein content, which makes up over 40% of its weight. Its also provides potential health benefits, which include reducing cardiovascular, diabetic and cancer risks (Siddique *et al*. 2000). Although most chickpeas are produced for human consumption it provides the livestock industry with an alternative protein and energy feedstuff. In addition, chickpea straw is used as a ruminant feed.

Chickpea is frequently infected by different soil-borne diseases, such as damping-off, wilt, collar rot, root rot etc. Among the diseases, wilt and collar rot caused by *Fusarium oxysporum* f. sp. *ciceri* and *Sclerotium rolfsii*, respectively are considered as the major diseases of chickpea which can contribute up to 100% mortality of seedling crop during congenial environmental conditions (Rajput *et al*. 2010). The pathogens are soil-borne in nature, hence, very difficult to control.

Traditionally, farmers employ chemicals to control such kind of devastating diseases, but the unjudicial application of chemicals have grave impact on the environment as well as human health. In addition, the long term and repeated use of chemicals also lead to develop resistance in pathogens against various chemicals (On *et al*. 2015). But chemical fungicides are farmers' first choice to control such kinds of diseases (EI-Mougy *et al*. 2004). While there are several fungicides on the market that can effectively control infections, they are expensive and can lead to various toxicological and environmental problems (Malkhan *et al*. 2012). Fungicides and other synthetic chemicals cause hormone imbalances in foodborne organisms, cancer, and poor human health in addition to making the disease more resistant (Kumar *et al*. 2007). Due to their harmful effects on non-target organisms and the unfavourable changes they cause to the ecosystem, it is therefore discouraged to use chemicals broadly persistently, and without planning (Arcury and Quandt, 2003; Deising *et al*. 2008). Therefore, it's an immense need to develop an eco-friendly and sustainable way of controlling plant diseases alternative to chemical control. Integration of naturally available plant disease management tools including beneficial fungi such as *Trichoderma* sp. along with medicinal plants such as neem, has gained momentum alternative to chemicals for their cost-effectiveness, durability, and environmental safety nature (Kavita and Dalbeer 2015). *Trichoderma* inhibits or suppresses the growth of the targeted plant pathogens by employing various strategies like competition, antibiosis, inducing host-plant resistance, secretion of chitinolytic enzymes, mycoparasitism etc. (Braun *et al*. 2018). Additionally, it has been discovered that *Trichoderma* spp. improve the vigour index, plant height, fresh weight, and percentage of seedling emergence in a variety of vegetable crops (Begum *et al*. 1999: Chowdhury, *et al*. 2000; Hossain and Shamsuzzaman, 2003; Hossain and Naznin, 2005). Medicinal plants contain phenols, flavonoids, saponins, polysaccharide terpenes, etc. might be responsible for triggering plant growth and yield with reduced plant diseases. The application *Trichoderma* either alone or in combination with phytoextracts as integrated management also reported to reduce disease incidence with higher yield in different crop plants. So far, a limited works has been carried out to investigate the use of formulated *Trichoderma* in combination with neem leaf extracts in controlling wilt and collar rot of chickpea, especially in the northern region of Bangladesh. Therefore, the aim of the study was carried out to develop an integrated management program for the management of Fusarium wilt and collar rot of chickpea.

Objectives

1. To isolate and morphologically characterize of wilt and collar rot pathogen of chickpea
2. To evaluate the *in vitro* efficacy of *Trichoderma asperellum* HSTUT 1, neem leaf extract, and Autostin 50 WDG against *Fusarium oxysporum* and *Sclerotium rolfsii*
3. To develop an integrated approach of managing wilt and collar rot of chickpea using *T. asperellum* HSTUT 1, neem leaf extract, and Autostin 50 WDG in net house conditions

CHAPTER II
REVIEW OF LITERATURES

Chickpea (*Cicer arietinum* L.) is an important pulse crop grown and consumed all over the world, especially in the Afro-Asian countries. It is a good source of carbohydrates and protein, and protein quality is considered to be better than other pulse crops. But the crop is suffering from many diseases like wilt, foot and collar rot, damping off, downy mildew, powdery mildew and many others. Among all the diseases, wilt and collar rot caused by *Fusarium oxysporum, Sclerotium rolfsii* are the major one. These diseases are most destructive and cause serious yield loss of chickpea. Many efforts are done to manage the destructive disease in home and abroad. Some research works reviewed on fungal, botanical and chemical treatment of wilt and collar rot of chickpea.

2.1. Pathogen and symptomatology of Fusarium wilt disease

Miller *et al*. (2011) stated that the general symptoms of *Fusarium* spp. wilt disease include poor and stunted plant growth, yellowing and withering of the leaves, and reddish staining of the xylem vessels.

According to, Agrios (2005) the earliest signs of Fusarium wilt are a minor vein clearing on the outer edges of the younger leaflets. The elder leaves then exhibit epinasty because of the petioles drooping. The most typical symptoms in older plants, however, are vein clearing and leaf epinasty followed by plant stunting, lower leaf yellowing, sporadic adventitious root formation, wilting of leaves and young stems, defoliation, marginal necrosis of the remaining leaves, and ultimately plant death.

Hutmacher *et al*. (2003) and Elliot (2009) observed cotyledons and leaves wilt and fall off immature plants, leaving bare stems when they are in the seedling stage. Fusarium wilt is difficult to identify early because early signs may be readily mistaken with other seedling illnesses such as bacterial, *verticillium* wilt, insect injury, dehydration, stem cankers, crown or root rot, and nutrient shortage.

According to, Zitter (1998) the principal sign used to diagnose Fusarium wilt is interior darkening of the vascular bundles. *Fusarium* also frequently causes the lesion to produce sticky red ooze. wilt, however based on different studies of the symptoms, this shouldn't be confused with slime that develops from bacterial wilt. Rarely, a sudden collapse of certain plants has been linked to Fusarium wilt.

Jiskani *et al*. (2021) stated that *Fusarium* attacks crops causing cholrosis, necrosis, withering, wilting, and defoliation of plant sections, which ultimately leads to the loss of the entire plant.

Garkoti *et al*. (2013) reported that Both in the early (seedling) and mature stages, the disease manifests itself in the field as patches. The abrupt drooping, drying of the leaves, and death of the seedling are the indications of seedling wilt, while the symptoms of adult wilt manifest from late flowering until pod-filling stage and are characterised by the abrupt loss of the afflicted plant's upper leaflets, the closure of leaflets without early shedding, and dull green foliage that is followed by the withering of the entire plant or specific branches.

Oyarzun (1989) experimented *Pythium* spp. were isolated using standard media such as potato dextrose agar and water agar, indicating indications of foot and root rot in leguminous crops.

Ignjatov *et al*. (2012) observed that the vascular system typically shows no external discoloration, but the infection typically manifests as chlorosis, leaf wilting, browning, and vascular necrosis in the stem's cross-section. However, the roots appear healthy, and there is less nodulation and proliferation.

Stoilova and Chadarov (2006) started that Fusarium wilt is a patchy disease that primarily affects crops in the seedling or adult reproductive stages.

Tosi and Cappelli (2001) observed that *F. oxysporum* f. sp. *lentils* cause wilting, internal vascular discolouration of the lower stem, significant decrease of the root system, and stunting when grown in the field.

De Cal *et al*. (2000) reported that advanced stage of *Fusarium oxysporum* infection is characterised by browning of the vascular system. The fungus causes severe wilting of plants by obstructing xylem vascular bundles and obstructing water transport.

According to Madhavi *et al*. (2006), Usually a single leaflet or twig's golden yellow colour, or a single stem's lower leaves drooping and wilting somewhat, obstructing the flow of water in gardens.

According to Madhabi *et al*. (2006), the first signs of Fusarium wilt are typically a single leaflet or twig's golden yellow colour or a little drooping of the lower leaves on a single stem in fields and gardens.

2.2. Pathogen and Symptomology of Collar Rot disease

Rolfs (1892) first discovered *Sclerotium rolfsii* 70% of the crop was lost due to the tomato blight causative bacterium, Sacc. from Florida, USA. It is the most harmful fungus that grows in soil and is polyphagous, omnivore, and widespread. In 1911, Saccardo identified the fungus as *Sclerotium* sp.

Aycock (1966) found that A soil-borne pathogen called *Sclerotium rolfsii* is frequently found in tropical and subtropical regions of the world, where it causes rot in the roots and feet of numerous crops, including mung bean.

Ahmed (1980) reported that *S. rolfsii* is a facultative parasitic fungus that can produce sclerotia to sustain generational continuity in unfavourable conditions.

Fakir (1983) described that Pulses are susceptible to disease development due to various phytopathogenic soil-borne and seed-borne fungi. These pathogens target plants from the seedling to maturity stages, with the seedling stage being the most devastating.

Ahmed (1985) found that Foot rot, which is attributed to *Sclerotium rolfsii,* is regarded as a significant and devastating disease affecting pulses in almost every country that produces legumes.

Agrios (2005) described that Seedling damping-off, stem canker, crown blight, root rot, crown rot, bulb, tuber, and fruit rots are all caused by the pathogens of sclerotia diseases. Many different types of plants, including most vegetables, flowers, legumes, grains, forage plants, and weeds, are commonly afflicted by sclerotia diseases.

Siddique *et al*. (2018) reported that A necrotrophic, soil-borne fungal plant pathogen known as *Sclerotium rolfsii*. That can be extremely harmful to a variety of fruits, vegetables, and pulse crops, including mung bean, lentil, and watermelon, melon, pepper, tomato and eggplant. When the environment is dry, there should be at least a hint of white mycelium on the stem's surface to indicate an infection.

Meah and Khan (2003) and Talukder (1974) described that In Bangladesh, *Sclerotium rolfsii* is a common source of disease in a variety of crops. Common signs include a lesion near the soil that is drenched in water and has a lot of mycelial development.

Nagamma and Nagaraja (2015) stated that more than 500 plant species of agricultural and horticultural crops are susceptible to root rot, stem rot, collar rot, and foot rot diseases due to the soil-borne plant pathogen *Sclerotium rolfsii*. the globe. When collar rot infections develop, yellowing and wilting of the leaves are typically the initial signs of *Sclerotium rolfsii.*

Khalequzzaman (2016) reported that Legume foot and root rot is brought on by the soil-borne pathogenic fungus *Sclerotium rolfsii.* The fungus is more harmful during the seedling stage and can damage the crop at all stages from the seedling to the flowering stage.

Dwivedi and Prasad (2016) described that *Sclerotium rolfsii* hyphae were dispersed inside as well as outside of the infected stem near to the soil's surface, developing upward on the surface of the diseased plant and covered in a cottony white mass of mycelium. When the fungus was young, it produced a large number of uniformly sized, small, round white sclerotia, which when developed turned dark brown. Sclerotia, which resemble mustard seeds in structure and have a tan to brown colour, develop by mycelial coagulation.

2.3. *In vitro* and field efficacy of *Trichoderma* against *Fusarium oxysporum* responsible for wilt Disease

Hussein *et al*. (2022) found in an experiment that the growth of *Fusarium solani* AJA2 (62.3%), *Trichoderma harzianum* antagonistic actions followed, with *F. oxysporum* AJA (55.2%), *F. incarnatum* AJA (53.2%), and *F. solani* AJA1 (50.8%) in the lead. *T. harzianum* had the highest inhibition rate (85%) against *F. incarnatum*. *T. harzianum's* volatile chemicals inhibited *Fusarium* species in a significant percentage.

EI. Sharkawy and Abdelrazik (2022) observed that, under *in vitro* settings, *Trichoderma harzianum* resulted in the largest reduction in the mycelial development of *Fusarium solani* (75%) the causative agent of squash root rot followed by *T. album* and *T. koningii*.

Yassin *et al*. (2021) found that, *Trichoderma viride* showed antagonistic action with mycelial inhibition rates of 80.17% and 70.46% against *Fusarium proliferatum* and *F. verticilliodes,* and 68.38% and 60.64% against *T. harzianum.* The culture filtrates of *T. virde* and *T. harzianum* stains shown antifungal activity against *F. verticilloides* stain, exhibiting suppressive rates of 56.7% and 32.2%, respectively. Meanwhile, the mycelial inhibition rates against the *F. proliferatum* strain were 23.50% and 44.09%, respectively.

Andoji and S, S.A (2021) observed that, For the antagonistic study against *Fusariun soloni* inciting root rot of chickpea (*Cicer arietimum L.),* *Trichoderma virens, T. atroviride, T. viride, T. harzianum, T. koningioosis, T. stibohypoxyli, and T. pseudokongii* species were used. It was observed that all *Trichoderma* species showed greater antagonistic activity than others in the case of a sensitive isolate of test fungus. The resistance was containing the antagonism to some degree.

Mukhopadhyay and Kumar (2020) reported that, The *Trichoderma* species use a number of methods to inhibit the development and spread of dangerous infections include parasitism, competition, and antibiosis.

Faruk (2019) investigated that impact of tricho-compost as a soil amendment on the control of *Sclerotium rolfsii*-caused barley seedling disease at the Plant Pathology Division of the Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh in the years 2013–14, 2014–15, and 2015–16. Due to the use of tricho-compost, the yield of barley improved over control in the years 2013–14, 2014–15, and 2015–16, respectively, at 41.09, 38.55, and 44.03%.

Das *et al*. (2019) conducted an experiment using poultry refuge combined with bio-agent, specifically *Trichoderma harzianum*. Post-emergence mortality of plants caused by damping off, foot rot, collar rot and wilt disease of the lentil variety in BARI-Masur-6 was significantly decreased. The *T. harzianum* strengthened compost treated plot also had the longest roots and shoots observed.

Akter *et.al.* (2018) conducted that, in an *in vitro* pot study, lentil plants treated with Tricho-compost exhibited a much lower incidence of wilt compared with those treated with *T. harzianum* spore solution and other combinations. The highest incidence of wilt disease (63%) was seen when conventional compost and *Fusarium* spore suspension were combined. The largest population of *T. harzianum* is found in the combination of causal compost and Tricho-compost treatment. The population density of both test fungi in the casual compost is high.

Sinha *et.al*. (2018) conducted an experiment and observed that *Fusarium oxysporum* f. sp. *capsica*, which causes wilt disease in chilli, was significantly reduced by 1% talc-based formulation of *T. harzianum* (87.5%) and by 1% talc-based formulation of *T. viride* (83,93%). It has also been shown that the *T. Harzianum* formulation containing 1% talc produces maximal germination in addition to increased plant height, root length, and yield.

Moosa *et al*. (2017) found that, *Trichoderma* isolates and cow manure were evaluated individually and *in vivo*, and it was shown that the combination of *T. viride, T. harzianum*, and cow manure caused the greatest *in vivo* inhibition.

Hasan *et al*. (2016) observed that, *Meloidogyne incognita*-caused root-knot in Indian spinach was evaluated against five treatments: BAU-Biofungicide (*Trichoderma* spp.) as seed coating and side dressing, nematicide (Carbofuran 10G), and allamanda leaf extract as side dressing. A control group was also included in the experiment. The study showed that using all of the treatments over the control resulted in a considerable decrease in the number of galls and egg masses in the roots.

Faruk and Rahman (2016) reported that Tricho-compost showed more effective in reducing seedling mortality, increasing plant growth, and increasing lentil grain output in both field studies and pot cultures infected with *S. rolfsii* and *F. oxysporum*.

Faruk *et al. (*2014) carried out that, the control plot exhibited the highest wilt incidence (43.33%) at 65 DAS, whereas the *Trichoderma harzianum* utilised plot showed the lowest incidence (10%) in terms of suppressing wilt incidence and promoting plant growth and fruit yield.

Kashem *et al.* (2011) evaluated to reduce *Fusarium oxysporum*-induced lentil foot and root rot, 14 isolates of *Trichoderma* spp. According to the study, the isolate of *T. harzianum* exhibited the lowest incidence of foot and root rot (6.9%), the highest germination rate (82.08%), the maximum plant stand (93.12%), and the highest seed output (3726.67 kg/ha), suggesting that it might be utilised to manage the disease in lentils grown in Bangladesh

Harman *et al*. (2004) conducted research on *Trichoderm*a is a pathogenic symbiont that suppresses disease by inducing resistance in the host plant, hyper parasitism, antibiosis, and competition for nutrients and space.

Tjamos *et al*. (1992) observed that, most *Trichoderma* spp. have been used as the most significant biocontrol agent and have the ability to resist plant pathogenic fungus.

Papavizas (1965) conducted an investigation studied a number of strains of *Trichoderma* and *Gilocladium* as possible biocontrol agents, and found that these species can effectively act as biocontrol agents against a variety of soil-borne plant pathogenic fungus, including *Sclerotium*, *Pythium*, *Fusarium*, and *Rhizoctonia.*

2.4 *In vitro* efficacy of *Trichoderma* against *Sclerotium rolfsii*

Lewis and Papavizas (1991) reported that Soybean stem blight induced by *Sclerotium rolfsii* is inhibited by *Trichoderma harzianum*.

Haran *et al*. (1996) revealed that *Trichoderma* sp. is an efficient combatant of *Sclerotium rolfsii*-caused southern stem blight of soybeans.

Biswas and Sen (2000) reported that *Trichoderma harzianum* prevents *Sclerotium rolfsii*-caused groundnut stem rot.

Hermosa *et al*. (2000) revealed that *Trichoderma* species completely inhibits *Sclerotium rolfsii*, which causes the soybean disease known as southern stem blight.

Abd-Allah (2005) revealed that in greenhouse conditions, *Bacillus subtilis* reduces *Sclerotium rolfsii* by 92% in peanuts.

Radawan *et al*. (2006) reported that *Trichoderma harzianum* and *Trichoderma hamatum* showed the most efficacy in inhibiting *Sclerotium rolfsii* mycelial growth, with a 79% reduction in growth.

Karthikeyan *et al*. (2006) reported that in stem-rot of groundnuts, *Trichoderma viride*, *T. harzianum*, and *Pseudomonas fluorescens* were bioagents that inhibited the growth of *Sclerotium rolfsii* (Sacc.). The *T. viride* obstructed the pathogen's mycelial growth by 69.40%, whereas *P. fluorescens* inhibited 64.40% of it.

Eziashi *et al*. (2007) described that *Rhizobium* and *T. Harzianum* cooperated together to greatly inhibit the growth of *S. rolfsii*, the organism that caused stem rot disease, as well as to encourage the growth of groundnut plants and boost seed production.

Ghildiyal and Pandey (2008) studied that *Trichoderma harzianum* produces substances such as gliotoxin, glioviridin, dermin, and trichodermin, which act as antibiotics that have antagonistic activity against *Sclerotium rolfsii*.

Anand and Reddy (2009) evaluated that According to reports, *Trichoderma* sp. shows antagonistic activity *in vivo* against *Fusarium ciceri* and *S. rolfsii.*

Bosah *et al*. (2010) revealed that the growth of *S. rolfsii* was significantly reduced *in vitro* by *Trichoderma*, *Penicillium*, and *Aspergillus* species.

Bhuiyan *et al*. (2012) revealed that the negative effects of the *Sclerotium*-caused stem rot disease are inhibited by the arbuscular mycorrhizal fungus. *Trichoderma harzianum* had the greatest mycelial growth inhibition (83.06%) of *Sclerotium rolfsii*.

Rekha (2012) reported that *Trichoderma* sp. inhibited *S. rolfsii's* mycelial development and sclerotial mass formation.

Samsuzzaman *et al*. (2012) reported that Since *Trichoderma harzianum* increases tomato plant height and yield while decreasing tomato plant mortality when inoculated with *S. rolfsii* in the soil, bio fungicides are a safer alternative to chemical control when it comes to inhibiting *S. rolfsii* growth, which causes tomato collar rot disease.

Podmaja *et al*. (2013) revealed that it has been observed that *Trichoderma* species work as a biocontrol agent against *S. rolfsii.*

Patro and Madhuri (2013) reported that when *S. rolfsii* is grown *in vitro, T. harzianum* prevents its mycelial growth, which results in finger millet foot rot.

Valle *et al*. (2013) described that *Trichoderma asperellum* increased the activities of glucanase, chitinase, and peroxidase in onion bulbs, roots, and leaves. It also prevented *Sclerotium rolfsii* from developing.

Muthukumar and Venkatesh (2014) According to the evaluation, under *in vitro* conditions, *Trichoderm*a and Pseudomonas had the maximum inhibitory activity (68.28% and 74.25%, respectively) against *Sclerotium rolfsii.*

Basumatary *et al*. (2015) recorded *Sclerotium rolfsii's* growth is retarded *in vitro* by six fungal species: *Penicillium sp., Aspergillus niger, Curvularia sp., Trichoderma harzianum, Trichoderma viride,* and *Fusarium* species. This is carried out using a dual culture technique.

Swathi *et al*. (2015) reported that *In vitro* conditions showed that *T. harzianum* and *T. virens* were more active against *S. rolfsii*, exhibiting 100% inhibition.

Aanuoluwa *et al*. (2015) studied that at a suitable temperature of 37 0C and pH 6.0, *Trichoderma* viride has antagonistic activity against *Sclerotium rolfsii*.

Singh *et al*. (2016) studied that the growth of *S. rolfsii* was inhibited by *Trichoderma harzianum* and *T. atroviride*, although the mutant parent bioagent was more effective than the parent bio-agent in treating chickpea collar rot disease, which was caused by *S. rolfsii. T. harzianum* and *T. atroviride* mutant strains were able to effectively block 82.9% of *S. rolfsii's* uncontrollably rapid development.

Khalequzzaman (2016) evaluated that Legumint foot and root rot induced by *Sclerotium rolfsii* is effectively treated by *Trichoderma harzianum* and *Trichoderma viridie*. 35% of diseases are reduced compared to control and 21% in that order.

2.5. *In vitro* and field efficacy of Neem leaf extract against *Fusarium oxysporum*

Butt *et al*. (2016) reported that Under *in vitro* environments, leaf extracts from two significant native plants, *Alstonia scholaris* and *Azadirecta indica*, were more effective against *S. rolfsii* at various concentrations (1%, 2%, 3%, 4%, and 5%).

Hussain *et al*. (2015) reported the fungitoxic characteristics of six distinct plant extracts: Of the thesis plants, *Aspergillus*, *Fusarium*, and *Rhizoctonia* spp. were most effectively inhibited by *Azadirachta indica*, *Ocimum basilicum*, and *Crotalaria juncea*.

Sharf *et al*. (2011) observed that plant extracts from *Azadirecta indica* and *Cannabis sativa* restrict growth by 46% and 36%, respectively. The growth of 15.5 percent of *Alternaria* sp., which causes *Alternaria* leaf spot on *vicia faba*, was reduced by *Aegle marmelos* (25.7 percent) and *Achyranthus aspera*.

Bhardwaj and Laura (2008) found that *Aegle marmelos, Acacia arabicae, Camellia sinensis, Callistemon lanceolatus, Calotropis procera*, *Brassica campestris*, and Aloe vera aqueous extracts have an inhibitory impact on *Fusarium moniliforme*, which causes paddy foot rot.

Ogechi *et al*. (2006) observed that *Fusarium oxysporum* f. sp. *lycopersici*'s mycelial growth was reduced by crude extracts of *Azadirachta indica* (neem) leaf, seed, and *Allium sativum* (garlic) at doses ranging from 5% to 30%. There were no changes in growth inhibition between the several concentrations employed in garlic. Dry *A. indica* seed extract produced 100% suppression of mycelial development.

Singh et. Al. (1980) observed that when compared to aqueous extracts, the oil of neem (*Azadirecta indica*) shown the greatest inhibitory action against the pathogens that cause rot and wilt, specifically *Fusarium oxysporum* f. sp. *ciceri*, *Rhizoctonia solani, Sclerotium rolfsii*.

Latif *et al*. (2006) recorded that the percentage of fungal incidence that was reduced in mustard seeds using a plant extract ranged from 48.4 to 57.0%, with neem leaf extract reducing seed-borne fungus. The fungus that was separated from mustard seeds. A variety of fungus, including *Alternaria, Aspergillus, Chaetomium, Curvularia, Fusarium, Penicillium*, and *Rhizopus* species, were isolated from the mustard seeds.

Guleria and Kumar (2006) studied the induced resistance combined with an enhanced capacity to trigger defence reactions in response to pathogen invasion or elicitor therapy. It was proposed that neem leaf extract may have protected sesame plants from *A. sesame* by enhancing their natural defence mechanism.

Latif *et al*. (2006) observed that the germination ability of neem leaf extracts ranged from 65 to 70 percent. Additionally, he saw that neem leaf extract effectively eliminated seed borne fungus, resulting in a 48.4-57.0% decrease in occurrence.

Hasan *et al*. (2005) found that Neem alcohol extracts totally prevent *Fusarium* sp. from existing. The largest percentages of *Fusarium sp*. (24.33%), *Aspergilus sp.* (17.07%), and *Rhizopus sp. (*17.67%) were found on treated wheat seed.

Rahman *et al.* (1999) observed that Wheat-borne diseases caused by *Alternaria tenuis, Alternaria alternata, Bipolaris sorokiiana, Curvularia iunata*, and *Fusarium spp*. were effectively prevented by neem *(Azadirachta indica*) extracts.

Srivastava *et al*. (2013) assessed that the fungicidal activities of aqueous leaf extracts of *Calotropis procera, Azadirachta indica, Lantata camara*, and

Ocimum basilicum were demonstrated in an *in vitro* test against *Curvularia tuberculate* and *Alternaria isolate* acquired from pear fruits and *Alternaria isolate* "b" from pomegranate fruits that were infected. A study conducted *in vitro* showed that 64–85% of the rot was controlled.

Panda *et al*. (1996) tested the effectiveness of neem leaf extracts *(Azadirachta indica*) in treating Phomopsis-infected seeds of aubergine, tomato, capsicum, cabbage, carrot, and onion, as well as those infected by *Fusarium oxysporum, Peronospora destractor, Alternaria brassicae,* and *Alternaria radicina.*

2.6. *In vitro* and field efficacy of neem leaf extract against *Sclerotium rolfsii*

Butt *et al*. (2016) reported that Under *in vitro* settings, leaf extracts from two significant native plants, *Alstonia scholaris* and *Azadirecta indica*, were more effective against *S. rolfsii* at various concentrations (1%, 2%, 3%, 4%, and 5%).

Amin *et al*. (2013) selected different plants ginger rhizome, neem leaf, tobacco leaf, turmeric rhizome, and urine from cows. Only at greater concentrations did all plant extracts prevent *Sclerotium rolfsii* from growing, although the rhizome of Turmeric slightly slowed down the growth.

Siddique *et al*. (2018) evaluated the effectiveness of plant extracts against *Sclerotium rolfsii*-caused eggplant foot and root rot disease. Neem extract's effects on disease severity and incidence reduction compared to control on eggplant were found to be 60% and 58.64%, respectively. Similarly, on aubergine, the effects of alamanda extract on disease incidence and severity decrease over control were found to be 60% and 44.39%, respectively.

2.7. Chemical control of wilt and Collar rot diseases of chickpea

Parvin *et al*. (2016) conducted an investigation to find out how fungicides affect *S. rolfsii's* radial mycelial growth *in vitro*. Every fungicide that was tested considerably lowered the fungus's radial mycelial growth. There was Bavistin, that potential in lowering the fungus's growth in the lab, as demonstrated by Ridomil Gold.

Puri (2016) described that Methane sodium, methyl bromide, chloropicrin, and methane sodium are examples of soil fumigants that prevent *S. rolfsii* mycelium growth.

Khan and Javaid (2015) reported that the radial growth of *S. rolfsii* is considerably inhibited by the four fungicides tegula (tebuconazole), Thiophanate methyl, ridomil gold (metalaxyl + mancozeb), and mancozeb when used *in vitro*. In addition, two fungicides that significantly inhibit the growth of *S. rolfsii in vivo* and cause collar rot disease in chickpeas are thiophanate methyl and mancozeb.

Manu *et al*. (2012) reported that Hexaconazole, Propiconazole, and Difenoconazole fungicides; combi product; avatar (4% + zineb 68%); nativo (tebuconazole 50% + trifloxystrobin 25%); and vitavax powder (thiram *Sclerothema rolfsii* is inhibited in field conditions by 37.5%+carboxin 37.5%) and the bioagent *Trichoderma harzianum.*

According to Arunasri *et al*. (2011) Four fungicides were used to control the significant flower plant Crossandra, which was afflicted by the *Sclerotium rolfsii* collar rot disease: propiconazole, thiophanate-methyl 70% WP, captan 50% WP, and thiram at 75% SD and 25% EC at five distinct concentrations. Propiconazole, thiram, and captan, three fungicides, significantly inhibited *S. rolfsii's* mycelial growth.

Toorray *et al*. (2007) evaluated Seven fungicides were tested *in vitro* against *S. rolfsii* at varying concentrations. *S. rolfsii's* growth was completely inhibited when exposed to Captan, Thiram, Mancozeb, and Hinosan (edifenphos). and Antracol, but low concentrations of chlorothalonil demonstrated partial inhibition.

Yaqub and Shahzad (2006) reported that six fungicides were found to be effective against *Sclerotium rolfsii*: benomyl, carbendazim, thiovit, sancozeb, and dithane M-45. However *Sclerotium rolfsii* growth was significantly inhibited by high concentrations of dithane M-45 and mancozeb.

CHAPTER III
MATERIALS AND METHODS

3.1 Collection of diseased plant parts

The diseased plant parts ware collected from naturally infected Chickpea (*Cicer arietinum* L.) plants grown in the experimental plot.

3.2 Preparation of potato dextrose agar (PDA)

PDA media was prepared by adding 19.5g PDA powder on 500 mL of distilled water and stirred vigorously. After that, the mixture was autoclaved at 121 °C for 15 min and allowed to cool at room temperature (Tijjani *et al*. 2014)

3.3 Isolation and identification of *F. oxysporum* f. sp. *ciceri* and *Sclerotium rolfsii*

The infected tissue of the collar region of the plant was collected and repeatedly washed in fresh water and surface was sterilized with 10% Clorox for 1 minute followed by three times washing in distilled water. Then the pieces of infected tissue were placed on PDA acidified with one drop of 5% lactic acid and inoculated at 22±2 °C for 7 days. After incubation, white mycelia and sclerotia were formed. The pathogen was purified and multiplied subsequently through hyphal tip culture on PDA, for preparation of inoculum.

3.4 Preparation of pure culture of *F. oxysporum* f. sp. *ciceri* and *Sclerotium rolfsii*

Pure cultures of the fungal pathogen were prepared following hyphal tip culture method (Tutte, 1969). A cork borer was used to cut a disc of fungal colony and it was placed in the middle of a plain PDA containing petri plate and incubated for 2 days. After that, the culture plate was observed under a compound microscope and subsequently transferred to fresh PDA plates. Petri dishes containing pure culture of *Fusarium oxysporum* and *Sclerotium rolfsii.*

3.5 Inoculum preparation of *F. oxysporum* f. sp. *ciceri and Sclerotium rolfsii*

Ten 250 mL conical flasks were used for each culture of *Sclerotium rolfsii* and *Fusarium oxysporum*. For a full day, the wheat grains were submerged in water and poured into one half of each conical flask, which was then autoclave-sterilized. Five (5) mm discs of the pathogens' active mycelia that were cultivated on PDA were used to inoculate the flasks' contents. Every flask was incubated at room temperature with regular daylight that was dispersed. Following a 15-day incubation period, each wheat grain was taken out of the flasks and dried in the shade. A refrigerator was used to retain the dried inoculum.

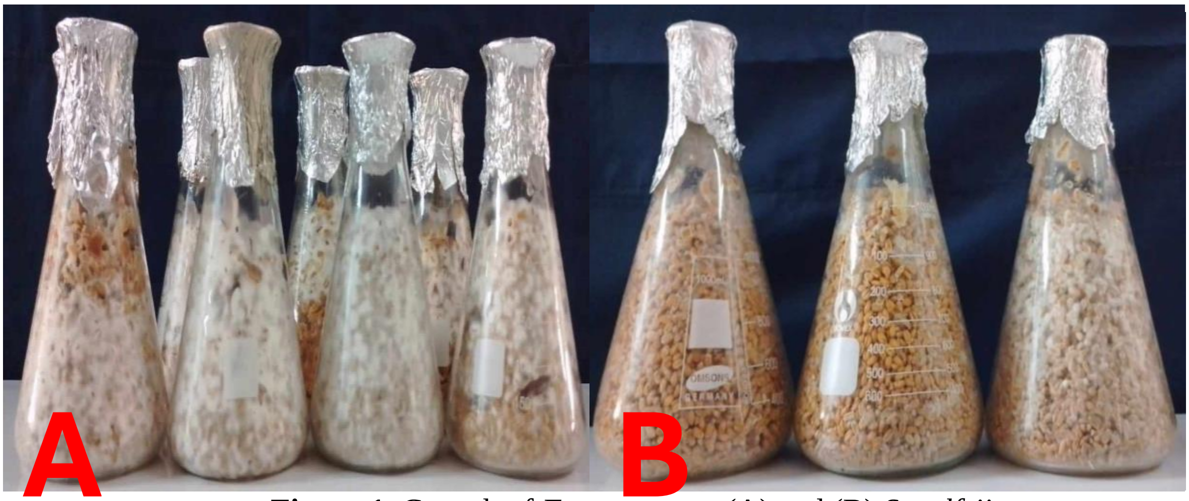

Figure 1. Growth of *F. oxysporum* (A) and (B) *S. rolfsii*

3.6 Collection, multiplication and formulation of *Trichoderma*

Trichoderma asperellum HSTUT 1 were collected from the stock culture of the Plant Pathology Laboratory of HSTU, Dinajpur and multiplied as per our need. Experiments were conducted to find out the antagonistic effect of *Trichoderma* HSTU 1 against *Fusarium oxysporum* and *sclerotium rolfsii* controlling wilt and collar rot of chickpea. Brans of check pea (*Cicer arietinum*) were prepared by running tap water for four hours. After soaking 200g of substrates were poured into 500ml conical flasks and autoclaved at 121°C with 15 psi for 15 minutes. Each conical flasks were inoculated with five mycelial discs of 7 days old culture of *T. asperellum* HSTU 1, incubated at room temperature (25±3 °C) for 21 days. After incubation, conical flasks were opened and colonized materials were air dried and packaged for soil application (Islam *et al*.2007)

Figure 2. Formulated *T. asperellum* HSTUT 1

3.7 Collection and preparation of neem leaf extract

Fresh leaves were collected from HSTU campus and extracts prepared using distilled water (1:1, w/v basis). Briefly, 250 g thoroughly washed fresh leaves were shade dried, surface-sterilised for a min in 0.1% NaOCl solution and washed thrice with double distilled water (DDW). Leaves were then grind in 250 mL of DDW using mortar and pestle and ground material was filtered through double layer of muslin cloth and filtrate was centrifuged at 5000 rpmfor

15 min. It was further placed on Whatman filter paper no. 1 and the collected plant extract was assumed to be 100% standard solution. To avoid contamination, heating of the collected extract was done in a water bath at 40 °C for 5 min.

3.8 Preparation of Autostin 50 WDG

Fungicide formulation of required concentration was prepared by mixing the desired amount of Autostin 50 WDG 2g/L in DDW prior to the experimental work.

3.9 *In vitro* evaluation of *Trichoderma* HSTUT 1, neem leaf extract and Autostin 50 WDG against *F. oxysporum* f. sp. *ciceri and Sclerotium rolfsii*

Dual culture technique was in case of *T. asperellum* whereas poisoned food technique followed in case of neem leaf extract and Autostin 50 WDG to record the inhibition percentages of the radial growth of *F. oxysporum* & *S. rolfsii* were calculated following the formula as suggested by Sunder *et al*. (1995).

Growth inhibition (%) = X-Y/X×100

Where,

X = Mycelial growth of pathogen in control condition

Y = Mycelial growth of pathogen in presence of *T. asperellum* HSTUT 1 , neem Leaf extract & Autostin 50 WDG.

3.10 Experimental location

The experiment was carried out at the research field under the Department of Plant Pathology, HSTU, Dinajpur.

3.11 Experimental period

The experiment was conducted during the period of December 2023 to March 2024.

3.12 soil type

The experimental soil was sandy loam texture soil. The pH value of the soil is above 5.5 measured with pH meter.

3.13 Experimental details

3.13.1 Planting material

Chickpea variety BARI Chola 5 was used as the test crop in this experiment. The seeds were collected from the Agronomy Division of Bangladesh Agricultural Research Institute, Joydebpur, Gazipur.

3.13.2 Treatments of the experiments

The experiment comprised of the following 6 treatments including control condition for wilt and collar rot diseases caused by *Fusarium oxysporum* and *Sclerotium rolfsii*.

Treatment combinations in case of management of Fusarium wilt of chickpea

T_0: control (only *F. oxysporum*)

T_1: *F. oxysporum* + formulated *T. asperellum* HSTUT 1

T_2: *F. oxysporum* + neem leaf extract

T_3: *F. oxysporum* + formulated *T. asperellum* HSTUT 1 + neem leaf extract

T_4: *F. oxysporum* + Autostin 50 WDG

T_5: *F. oxysporum* + formulated *T. asperellum* HSTUT 1 + neem leaf extract + Autostin 50 WDG

Treatment combinations in case of management of collar rot of chickpea

T_0: control (only *S. rolfsii*)

T_1: *S. rolfsii* + formulated *T. asperellum* HSTUT 1

T_2: *S. rolfsii* + neem leaf extract

T_3: *S. rolfsii* + formulated *T. asperellum* HSTUT 1 + neem leaf extract

T_4: *S. rolfsii* + Autostin 50 WDG

T_5: *S. rolfsii* + formulated *T. asperellum* HSTUT 1 + neem leaf extract + Autostin 50 WDG

3.14 Net house experiment

3.14.1 Soil preparation

Pot soil was prepared by mixing sandy loam soil with sand and well decomposed cow dung at the ratio of 2:2:1 and the soil was sterilized with 40% formaldehyde solution at the rate of 5 ml formaldehyde solution diluted with 20 mL of water. The formaldehyde treated soil was covered with a polythene sheet for 72 hours and then exposed for 48 hours for aeration to remove the residues of formaldehyde. The sterilized soil was used to fill the sterilized plastic pot and apparently disease-free healthy chickpea seeds were sown in the pot.

3.14.2 Application of fertilizer and manure

Well decomposed cow-dung was applied at the time of final soil preparation. The sources of N, P, K was Urea, TSP, MOP. During final soil preparation, full dosage of fertilizers was applied.

3.14.3 Sowing of seeds in the pots

Pots were filled with sterilized soil. Wheat grain inoculum of each isolate of *F. oxysporum* f. sp. ciceri and *Sclerotium rolfsii* was thoroughly mixed with the soil of each pot at the rate of 20 g/kg soil. Control pots were prepared using wheat grain inoculum soil only. Fifteen (15) seeds of BARI Chola-5 were sown in each pot. The seed germination was recorded 15 days after sowing. Observations on number of plants wilted in each pot were recorded at 30, 45 and 60 days after sowing.

Figure 3. Experimental plot in the farm of HSTU

3.15 Intercultural operation

Weeding, thinning and watering were done as when necessary.

3.16 Data collection

Plant growth features and disease data were collected four times. Started from 15 DAS at 5 days interval and continued up to harvesting. The pots of chickpea were visited consciously to measure the data of different agronomic parameter and to inspect and record the data of wilt and collar rot disease. Dead plants were removed from the pots after recording about the infected plants. The data were collected on the basis of following parameters:

3.16.1 Seed germination (%)

Seed germination (%) was counted at 15 days after seeds sowing. Percent of germination was calculated by following formula.

$$\text{Seed germination (\%)} = \frac{\text{Number of germinated seedling}}{\text{Total number of seeds sown}} \times 100$$

3.16.2 Disease incidence (%)

Number of wilt and collar rot incidence or infected plants/pot was counted and recorded by the visual observation at different stages of plant growth. The data was recorded at 30, 45 and 60 days after sowing of seeds. Disease incidence was recorded as the number of plants infected by wilt and collar rot disease out of the total number of plants. Percent of plant disease was calculated by following formula.

Figure 4. Typical symptom of Fusarium wilt and collar rot of chickpea

3.16.3 Shoot length (cm)

Shoot length was measured with the help of a meter scale. Shoot length was recorded from randomly selected 3 plants from each pot. Shoot lengths were recorded at15 DAS.

3.16.4 Root length (cm)

Root length was measured with the help of a meter scale. Root length was recorded from randomly selected 3 plants from each pot. Root lengths were recorded at 15 DAS.

3.16.5 Shoot weight (g)

The shoot weight (g) was recorded at 15 DAS by using of an electrical balance and the mean was computed using an excel sheet

3.16.6 Root weight (g)

The root weight (g) was recorded at 15 DAS by using of an electrical balance and the mean was computed using an excel sheet

3.16.7 No. of pods per plant

Plants were selected as before, and pod numbers were recorded at 125 DAS.

3.16.8 No. of root nodules per plant

Three (3) plants were randomly selected from every treatment of each pot to record the number of root nodules. Total root nodules from the selected plants were recorded at 125 DAS.

3.16.9 Harvesting of crops

When 80% to 90% plant was on its' peak of maturity, harvesting was done. Maturity was determined considering some indicators. Pod colour, pod filling, water content of plants etc. At the time of harvesting, pods were picked by hand and packed into different packs with proper tags based on plot and treatment.

3.16.10 Yield per plant (g)

After harvesting, firstly weight of the pods and then seeds were taken immediately. Total yield was got at 130 DAS. Total yield was measured in gram for final data preparation.

3.17. Data analysis

Data obtained from different parameters were statistically analysed to find out the level of effectiveness of different treatments to control wilt and collar rot of chickpea. The analysis of variance was performed by using Statistix 10. 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

RESULTS

The *in vitro* efficacy of different treatments against the fungal pathogen causing wilt and collar rot of chickpea was carried out to observe their antifungal efficacy. The selected treatments were also applied in the field level to evaluate their efficacy for the eco-friendly management of wilt and collar rot disease of chickpea. However, the efficacy of different treatments was evaluated separately as well as combined application for the management of wilt and collar rot disease caused by *F. oxysporum* and *S. rolfsii*.

4.1. Identification of *F. oxysporum* & *S. rolfsii*

The colony formed with white aerial mycelia which later produce dark violate pigment on media that is a well-known morphological characters of *F. oxysporum*. Micro conidia produced on micro conidiophores found elliptical shaped and single or no septation. On the other hand, the growth rate of *S. rolfsii* were recorded after two days. *S. rolfsii* was very fast growing. Firstly, mycelium was silky white in color after than turned to dull white radial spreading given fan like appearance after three days. Hyaline, thin mycelium observed after microscopic examination of the fungal culture. After seven days, at the edges of the petridish turned to mustard seed like sclerotia which were deep brown or brownish black in color.

4.2. *In vitro* study

4.2.1 *In vitro* efficacy of different treatments against *F. oxysporum*

Different treatments used in this study were showed significant inhibitory effect in *in vitro* against the fungal pathogen causing wilt disease in chickpea. Highest reduction (86.66 %) of *F. oxysporum* was occurred by 100 ppm Autostin 50 WDG followed by 50 ppm Autostin 50 WDG (73%), 10 ppm

Autostin 50 WDG (65.22%), *Trichoderma* (63.33%), neem leaf extract (38.88%) over control.

Table 1. *In vitro* efficacy of different treatments against *F. oxysporum*

Treatments	**Mycelial growth**	**% inhibition of mycelial growth over control**
Control	9.00 a ± 0	-
Trichoderma	3.30 c ± 0.1	63.33
Neem leaf extract	5.50 b ± 0.3	38.88
Autostin 10 ppm	3.13 c ± 0.15	65.22
Autostin 50 ppm	2.43 d ± 0.05	73
Autostin 100 ppm	1.20 e ± 0.2	86.66
LSD ($p \leq 0.05$)	0.45	

Means ± Standard deviation followed by the same letter did not differ at 5% level of probability

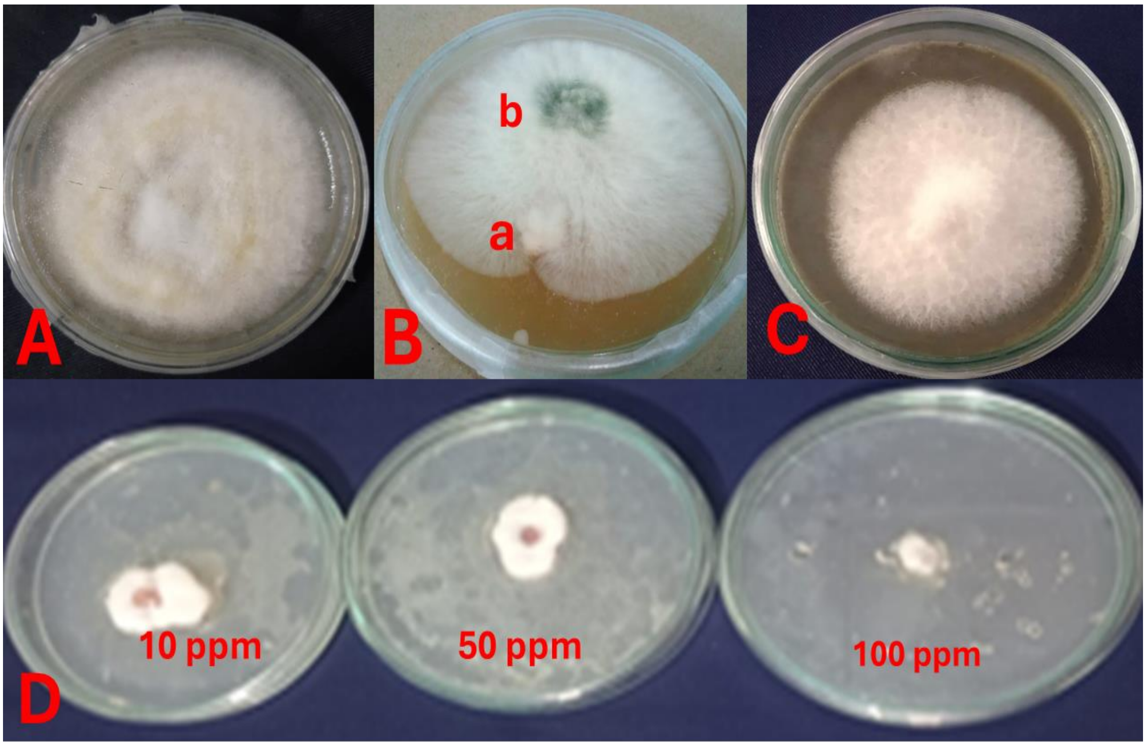

Figure 5. *In vitro* efficacy of different treatments against *F. oxysporum* against wilt of chickpea (A) Control (B); a) *F. oxysporum*, b) *T. asperellum* HSTUT 1, (C); Neem leaf extract PDA media, (D); Autostin 50 WDG at (10,50,100) ppm

4.2.2 *In vitro* efficacy of different treatments against *S. rolfsii*

Different treatments used in this study were showed significant inhibitory effect in *in vitro* against the fungal pathogen causing wilt disease in chickpea. Highest reduction of *S. rolfsii* occurred by 100 ppm Autostin 50 WDG (74.88%) followed by 50 ppm of Autostin 50 WDG (66.33%), 10 ppm of Autostin 50 WDG (60.77%), *Trichoderma* (56.66%), neem leaf extract (35.55%) over control.

Table 2. *In vitro* efficacy of different treatments against *S. rolfsii* causing collar rot disease of chickpea

Treatments	Mycelial growth	% inhibition of mycelial growth over control
Control	9.00 a ± 0	-
Trichoderma	3.90 c ± 0.1	56.66
Neem leaf extract	5.80 b ± 0.2	35.55
Autostin 10 ppm	3.53 c ± 0.25	60.77
Autostin 50ppm	3.03 d ± 0.15	66.33
Autostin 100ppm	2.26 e ± 0.05	74.88
LSD ($p \leq 0.05$)	0.41	

Means ± Standard deviation followed by the same letter did not differ at 5% level of probability

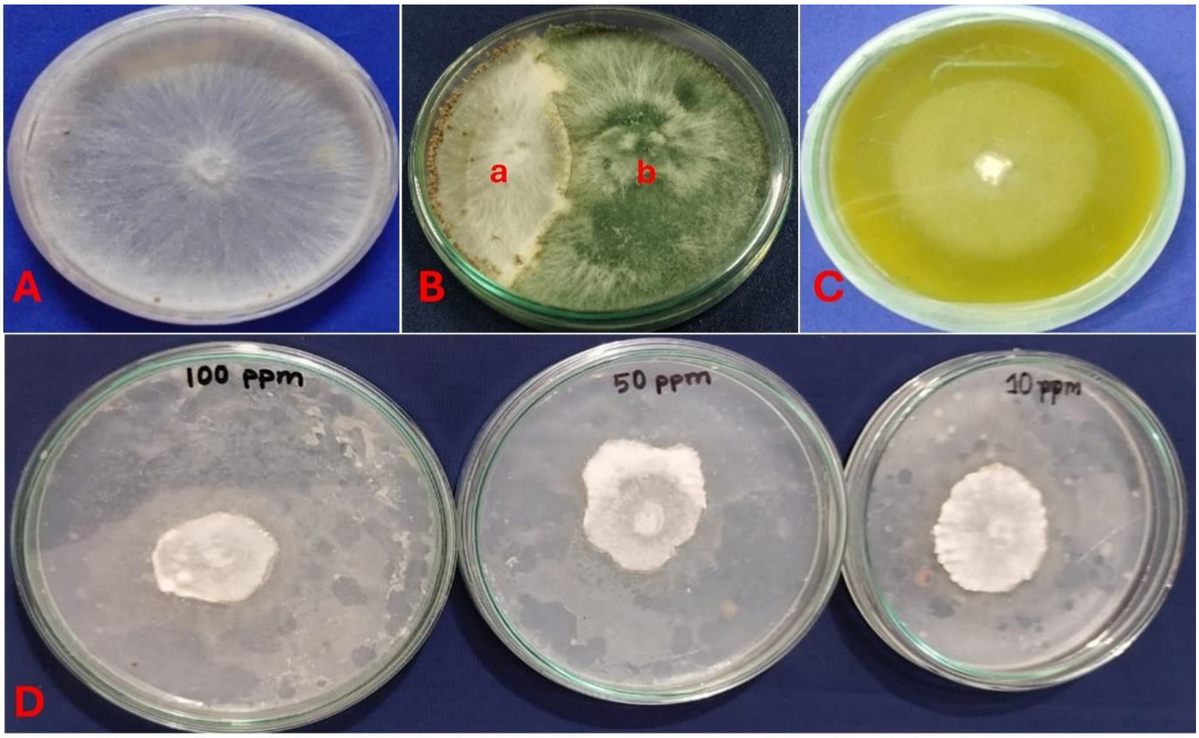

Figure 6. *In vitro* efficacy of different treatments against *S. rolfsii* against collar rot of chickpea (A)Control (B); a) *S. rolfsii*, b) *T. asperellum* HSTUT 1, (C); Neem leaf extract PDA media, (D); Autostin 50 WDG at (10,50,100) ppm

4.3 Field experiment

4.3.1. Efficacy of different treatments on germination (%) of chickpea against Fusarium wilt and collar rot diseases of chickpea

Combined application of *T. asperellum* HSTUT1, neem leaf extract and Autostin 50 WDG showed the highest germination (84.55%) and statistical similar germination with the single application of Autostin 50 WDG (84.44%); followed by single application of *T. asperellum* HSTUT1 (77.76%), single application of neem leaf extract (75.53%) and combined application of *T. asperellum* HSTUT 1 with neem leaf extract (73.44%). However, application *F. oxysporum* f. sp. *ciceri* only (control) showed the lowest germination (66.66%).

Combined application of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50 WDG showed the highest germination (90.22%); single application of Autostin 50 WDG (85.11%); combination of *T. asperellum* HSTUT 1 with neem leaf extract (82.66%), single application of neem leaf extract (80.33%)

and single application of *T. asperellum* HSTUT 1 showed statistical similar germination percentage (79.99%). However, application *Sclerotium rolfsii* only (control) showed the lowest germination (73.88%).

Table 3. Effect of different treatments on germination percentage of chickpea against Fusarium wilt and collar rot diseases of chickpea

Treatments	Seed germination (%)			
	Fusarium wilt	**Increase over control (%)**	**Collar rot**	**Increase over control (%)**
Control	66.66c ±3.66	-	73.88 c±1.83	-
Trichoderma formulation	77.76 ab ±3.85	16.75	79.99 b±2.66	8.27
Neem leaf extract	75.53abc± 4.68	13.40	80.33b±2.18	8.73
Trichoderma formulation + Neem leaf extract	73.44 bc± 4.67	10.27	82.66b±1.76	11.88
Autostin	84.44a ± 3.84	26.78	85.11ab±1.6	15.20
Trichoderma formulation+ Neem leaf extract+Autostin	84.55 a±2.16	26.95	90.22 a±1.16	22.11
LSD($p \leq 0.05$)	10.71		5.13	

Means ± Standard deviation followed by the same letter did not differ at 5% level of probability

4.3.2 Efficacy of different treatments against wilt and collar rot incidence (%) of chickpea caused by *Fusarium oxysporum* and *Sclerotium rolfsii* at 30 DAS

At 30 DAS, combined application of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50 WDG showed the lowest disease incidence (9.66%), followed by the application of Autostin 50 WDG (10.37%) and statistical similar disease incidence with the single application of *T. asperellum* HSTUT 1(11.05 %); application of neem leaf extract (12.31%) and combined application of *T. asperellum* HSTUT 1 & neem leaf extract (12.96%). However, application *F. oxysporum* f. sp. *ciceri* only (control) showed the highest disease incidence (14.40%).

50WDG showed the lowest disease incidence (9.14%), followed by the application of Autostin 50WDG (12.66%); single application of neem leaf extract (17.26%) and statistical similar disease incidence with the combined application *T. asperellum* HSTUT 1 & application of neem leaf extract (18.11%); single application of *T. asperellum* HSTUT 1 (25.74%). However, application of *Sclerotium rolfsii* only (control) showed the highest disease incidence (28.04%).

Table 4. Effect of different treatments against wilt and collar rot incidence (%) of chickpea caused by *Fusarium oxysporum* and *Sclerotium rolfsii* at 30 DAS

Treatments	Disease incidence (%)			
	Fusarium wilt	Reduction over control (%)	Collar rot	Reduction over control (%)
Control	14.40 a ± 1.11	-	28.04a± 5.57	-
Trichoderma formulation	11.05 bc± 1.03	23.26	25.74 a±1.09	8.20
Neem leaf extract	12.31 abc±1.12	14.51	17.26 b±2.51	38.44
Trichoderma formulation+ Neem leaf extract	12.96 ab±1.16	10	18.11 b±2.16	35.71
Autostin	10.37 bc±0.64	23.26	12.66bc±1.52	54.85
Trichoderma formulation+ Neem leaf extract+Autostin	9.66 c± 0.57	32.91	9.14 c± 0.83	67.40
LSD ($p \leq 0.05$)	2.66		7.62	

Means ± Standard deviation followed by the same letter did not differ at 5% level of probability

4.3.3 Efficacy of different treatment against wilt and collar rot incidence (%) of Chickpea caused by *Fusarium oxysporum* and *Sclerotium rolfsii* at 45 DAS

At 45 DAS, combined application of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50WDG showed the lowest disease incidence (1.21%); followed by the single application of Autostin 50WDG (3.18%); single application of neem leaf extract (4.12%); combined application of *T. asperellum* HSTUT 1 & neem leaf extract (4.07 %) are statistical similar; single application of *T. asperellum* HSTUT 1 (5.55%). However, application *F. oxysporum* f. sp. *ciceri* only (control) showed the highest disease incidence (17.77%).

Combined application of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50WDG showed the lowest disease incidence (2.33%); followed by the single application of Autostin 50 WDG (2.86%) statistical similar disease incidence with the combined application of *T. asperellum* HSTUT 1 & neem leaf extract (3.25%); single application of *T. asperellum* HSTUT 1 (4.38%); single application of neem leaf extract (6.29%). However, application of *Sclerotium rolfsii* only (control) showed the highest disease incidence (20.03%)

Table 5. Effect of different treatment against wilt and collar rot incidence (%) of chickpea caused by *Fusarium oxysporum* and *Sclerotium rolfsii* at 45 DAS

Treatments	Disease incidence (%)			
	Fusarium wilt	**Reduction over control (%)**	**Collar rot**	**Reduction over control (%)**
Control	17.77 a ± 1.32		20.03 a ±1.45	-
Trichoderma formulation	5.55 b ± 0.97	68.76	4.38 bc ±0.77	78.13
Neem leaf extract	4.12 bc ± 1.65	76.81	6.29 b ± 0. 84	68.59
Trichoderma formulation+ Neem leaf extract	4.07 bc ± 0.81	77.09	3.25 c ± 1.89	83.77
Autostin	3.18 bc ± 0.67	82.10	2.86 c ± 0.32	85.72
Trichoderma formulation+ Neem leafextract+Autostin	1.21 c ± 1.08	93.19	2.33 c ± 0.80	88.36
LSD ($p \leq 0.05$)	3.11		2.44	

Means ± Standard deviation followed by the same letter did not differ at 5% level of probability

4.3.4 Efficacy of different treatment against wilt and collar rot incidence (%) of chickpea caused by *Fusarium oxysporum* and *Sclerotium rolfsii* at 60 DAS

At 60 DAS, combined application of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50 WDG showed the lowest disease incidence (0.00%); followed by the single application of Autostin 50 WDG (2.33%); single application of neem leaf extract (3.16%) and single application of *T. asperellum* HSTUT 1 (3.70%) are statistically similar; combined application of *T.*

asperellum HSTUT 1 (4.5%). However, application *F. oxysporum* f. sp. *ciceri* only (control) showed the highest disease incidence (6.66%).

At 60 DAS, combined application of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50WDG showed the lowest disease incidence (0.00%); followed by the application of single *T. asperellum* HSTUT 1(2.37%); application of single neem leaf extract (3.33%) and statistical Similar disease incidence with the single application of Autostin 50 WDG (4.76); combined Application of *T. asperellum* HSTU T neem leaf extract (5.48%). However, application of *Sclerotium rolfsii* only (control) showed the highest disease incidence (11.77%)

Table.6 Efficacy of different treatment against wilt and collar rot incidence (%) of chickpea caused by *Fusarium oxysporum* and *Sclerotium rolfsii* at 60 DAS

Treatments	Disease incidence (%)			
	Fusarium wilt	Reduction over control (%)	Collar rot	Reduction over control (%)
Control	6.66 a ± 0.53	-	11.77 a ± 1.34	-
Trichoderma formulation	3.70 b ± 1.16	44.44	2.37 cd ± 0.63	79.86
Neem leaf extract	3.16 b ± 0.97	48.04	3.33 bc ± 0.67	71.70
Trichoderma formulation+ Neem leaf extract	4.5 ab ± 1	32.43	5.48 b ± 1.34	53.44
Autostin	2.33 b ± 0.80	65.01	4.76 bc ± 0.34	59.55
Trichoderma formulation+ Neem leaf extract+Autostin	0.00 d± 0	100	0.00 d ± 0	100
LSD ($p \leq 0.05$)	2.30		2.39	

Means ± Standard deviation followed by the same letter did not differ at 5% level of probability

4.3.5 Efficacy of different treatments on shoot length (cm) of chickpea at 15 Days after Sowing

Combined application of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50WDG showed the highest shoot length (18.24cm); followed by the application of Autostin 50 WDG (16.54 cm) and statistical similar shoot length with the single use of *T. asperellum* HSTUT 1 (15.78 cm) & single use of neem leaf extract (15.78 cm) respectively; combined *application of T. asperellum* HSTUT 1 with neem leaf extract (13.23 cm). However, application of *F. oxysporum* f. sp. *ciceri* only (control) showed the lowest shoot length (12.93 cm).

Combined application of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50 WDG showed the highest statistical similar shoot length (16.10 cm) with the single application of Autostin 50 WDG (15.76 cm); followed by the single application of *T. asperellum* HSTUT 1 (15.34 cm) and combined application of *T. asperellum* HSTUT 1& neem leaf extract (15.36 cm) respectively; single use of neem leaf extract (14.41cm). However, *S. rolfsii* only (control) showed the lowest shoot length (12.02 cm).

Table.7. Effect of different treatments on shoot length (cm) of chickpea at 15 Days after Sowing

Treatments	**Shoot length (cm)**			
	Fusarium wilt	**Increase over control (%)**	**Collar rot**	**Increase over control (%)**
Control	12.95 c ± 0.24	-	12.02 c ± 0.52	-
Trichoderma formulation	15.78 b ± 0. 08	21.85	15.34 ab ±0.14	27.62
Neem leaf extract	15.82 b ± 0.98	22.16	14.41 b ± 0.09	19.88
Trichoderma formulation+ Neem leaf extract	13.23 c ± 0.60	2.16	15.36 ab ±0.26	27.78
Autostin	16.54 b ± 0.65	27.72	15.76 a ± 0.26	31.11
*Trichoderma f*ormulation+ Neem leaf extract+Autostin	18.24 a ± 0.58	40.84	16.10 a ± 0.49	33.94
LSD ($p \leq 0.05$)	1. 64		1.02	

Means ± Standard deviation followed by the same letter did not differ at 5% level of probability

4.3.6 Efficacy of different treatments on root length (cm) of chickpea at 15 Days after Sowing

Autostin 50 WDG showed the highest statistical similar root length (6.03 cm) with the combined application of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50 WDG (5.92 cm); single use of neem leaf extract (5.66 cm) and single use of *T. asperellum* HSTUT 1 (5.20 cm); combination of *T. asperellum* HSTUT1 & neem leaf extract (4.66cm). However, *F. oxysporum* f. sp. *ciceri* only (control) showed the lowest root length (3.91 cm).

The combined application of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50 WDG showed the highest statistical similar root length (7.30 cm) with the application of Autostin 50 WDG (7.22 cm) & single use of *T. asperellum* HSTUT 1 (7.12 cm); single application of neem leaf extract (6.67 cm) and combination of *T. asperellum* HSTUT 1 with neem leaf extract (5.97 cm). However, *S. rolfsii* only (control) showed the lowest root length (12.02 cm).

Table 8. Effect of different treatments on root length (cm) of chickpea at 15 Days after Sowing

Treatments	Root length (cm)			
	Fusarium wilt	**Increase over control (%)**	**Collar rot**	**Increase over control (%)**
Control	3.91 c± 0.19	-	4.30 c ± 0.3	-
Trichoderma formulation	5.20 b ± 0.30	32.99	7.12 a ± 0.12	65.58
Neem leaf extract	5.66 ab ± 0.35	44.75	6.67 ab ±0.19	55.11
Trichoderma formulation+ Neem leaf extract	4.39 c ± 0.29	12.27	5.97 b ± 0.52	38.83
Autostin	6.03 a ± 0.07	54.21	7.22 a ± 0.23	67.90
Trichoderma formulation+ Neem leafextract +Autostin	5.92 a ± 0.26	51.40	7.30 a ± 0.55	69.76
LSD ($p \leq 0.05$)	0.71		0.98	

Means ± Standard deviation followed by the same letter did not differ at 5% level of probability

4.3.7 Effect of different treatments on shoot weight (g) of chickpea at 15 Days after Sowing

Combined application of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50WDG showed the highest statistical similar shoot weight (0.88g) with the single application of Autostin 50 WDG (0.87g); single application of neem leaf extract (0.74g); single application of Autostin 50 WDG (0.70g) and combination of *T. asperellum* HSTUT 1 & neem leaf extract (0.62g). However, *F. oxysporum* f. sp. *ciceri* only (control) showed the lowest shoot weight (0.56g).

Combined application of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50 WDG showed the highest shoot weight (0.83g); single application of Autostin 50 WDG statistical similar shoot weight (0.76g) with the single use of *T. asperellum* HSTUT 1 (0.72g); single application of neem leaf extract (0.69 g) and combination of *T. asperellum* HSTUT 1 with neem leaf extract (0.62g). However, *S. rolfsii* only (control) showed the lowest shoot weight (0.61g).

Table 9. Effect of different treatments on shoot weight (g) of chickpea at 15 Days after Sowing

Treatments	Shoot weight (g)			
	Fusarium wilt	**Increase over control (%)**	**Collar rot**	**Increase over control (%)**
Control	0.56 c ± 0.07	-	0.61 d ± 0.04	-
Trichoderma formulation	0.87 a ± 0.02	55.35	0.72 b ± 0.03	18.03
Neem leaf extract	0.74 ab ± 0.06	32.14	0.69 bc ±0.01	13.11
Trichoderma formulation+ Neem leaf extract	0.62 bc ± 0.08	10.71	0.62 cd ±0.02	1.63
Autostin	0.70 bc ± 0.06	25	0.76b ± 0.03	24.59
Trichoderma formulation+ Neem leaf extract+Autostin	0.88 a ± 0.03	57.14	0.83 a ± 0.01	36.06
LSD ($p \leq 0.05$)	0.16		0.07	

Means ± Standard deviation followed by the same letter did not differ at 5% level of probability

4.3.8 Efficacy of different treatments on root weight (g) of chickpea at 15 Days after Sowing

Combination of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50 WDG showed the highest root weight (0.32g); single application of Autostin 50WDG (0.28g); followed by single application of neem leaf extract (0.26g) and combination of *T. asperellum* HSTUT 1with neem leaf extract (0.25g); single application of *T. asperellum* HSTUT 1(0.23g). However, *F. oxysporum* f. sp. *ciceri* only (control) showed the lowest root weight (0.18g).

Combination of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50 WDG showed the highest root weight (0.32g); followed by single application of Autostin 50 WDG (0.30g); single application of *T. asperellum* HSTUT 1(0.27 g) and single use of neem leaf extract (0.2667g) are statistical equal. However, *S. rolfsii* only (control) showed the lowest shoot weight (0.61g).

Table 10. Effect of different treatments on root weight (g) of chickpea at 15 Days after Sowing

Treatments	**Root weight (g)**			
	Fusarium wilt	**Increase over control (%)**	**Collar rot**	**Increase over control (%)**
Control	0.18 d ± 0.01	-	0.17 d ± 0.01	-
Trichoderma formulation	0.23 c ± 0.01	27.77	0.27 bc ± 0.01	58.82
Neem leaf extract	0.26 bc ± 0.01	44.44	0.266 bc ±0.01	56.88
Trichoderma formulation+ Neem leaf extract	0.25 bc ± 0.01	38.88	0.263 c ± 0.01	54.88
Autostin	0.28 b ± 0.00	55.55	0.30 ab ± 0.01	76.47
Trichoderma formulation+ Neem leaf extract+Autostin	0.32 a ± 0.01	77.77	0.32 a ± 0.01	88.23
LSD ($p \leq 0.05$)	0.0342		0.0372	

Means ± Standard deviation followed by the same letter did not differ at 5% level of probability

4.3.9 Efficacy of different treatments on number of pods per plant of chickpea at the time of harvesting at 130 days after sowing

Combination of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50 WDG showed the highest number of pods/plants (26.00); single application of Autostin 50 WDG (20.00) and combination of *T. asperellum* HSTUT 1 & neem leaf extract (15.33); single application of *T. asperellum* HSTUT1 (11.33) showed statistical similar numbers of pods/ plant with the single application of neem leaf extract (11.00). However, *F. oxysporum* f. sp. *ciceri* only (control) showed the lowest pods number per plants (7.00).

Combined application of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50WDG showed the highest number of pods/plants (49.33); single application of Autostin 50 WDG (31.00) showed statistical similar numbers of pods/ plant with the combined application of *T. asperellum* HSTUT 1 with neem leaf extract (30.33); single application of *T. asperellum* HSTUT 1 (24.00) and single application of neem leaf extract (17.00). However, *S. rolfsii* only (control) showed the lowest number of pods per plant (14.33).

Table 11. Efficacy of different treatments on number of pods per plant of chickpea at the time of harvesting at 130 days after sowing

Treatments	Number of pods			
	Fusarium wilt	Increase over control (%)	Collar rot	Increase over control (%)
Control	7.00 e ± 1	-	14.33 d ± 2.51	-
Trichoderma formulation	11.33 d ± 1.54	61.85	24.66 bc ±4.72	72.08
Neem leaf extract	11.00 d ± 1	57	17.00 cd ±4.35	18.63
Trichoderma formulation + Neem leaf extract	15.33 c ± 0.33	119	30.33 b ± 3.21	111.65
Autostin	20.33 b ± 0.67	190.42	31.00 b ± 2.64	116.32
Trichoderma formulation + Neem leafextract+Autostin	26.00 a ± 1	271	49.33 ± 3.51	244.24
LSD ($p \leq 0.05$)	2.47		9.84	

Means ± Standard deviation followed by the same letter did not differ at 5% level of probability

4.3.10 Efficacy of different treatments on number of nodules per plant of chickpea at the time of harvesting at 130 days after sowing

Combination of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50 WDG showed the highest number of nodules/plant (31.00); single application of Autostin 50 WDG (24.00); single application of single application of neem leaf extract (14.66), single application of *T. asperellum* HSTUT 1 (14.33) and combined application of *T. asperellum* HSTUT 1 & neem leaf extract showed statistical similar numbers of nodules (12.33). However, *F. oxysporum* f. sp. *ciceri* only (control) showed the lowest nodules number per plant (8.40).

Combination of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50 WDG showed the highest number of nodules/plant (46.33); single application of Autostin 50 WDG (42.00); single application of neem leaf extract (29.33); combined application of *T. asperellum* HSTUT 1& neem leaf extract (23.61) and single application of *T. asperellum* HSTUT 1 showed statistical similar numbers of nodules (21.66). However, *Sclerotium rolfsii* only (control) showed the lowest nodules number per plant (12.00).

Table 12. Efficacy of different treatments on number of nodules per plant of chickpea at the time of harvesting at 130 days after sowing

Treatments	**Number of nodules**			
	Fusarium wilt	**Increase over control (%)**	**Collar rot**	**Increase over control (%)**
Control	8.40 d ± 0.65	-	12.00 e ± 1	-
Trichoderma formulation	14.33 c ± 0.57	109.81	21.66 d ± 1.52	109.81
Neem leaf extract	14.66 c ± 1.52	74.52	29.33 c ± 1.32	74.52
Trichoderma formulation+ Neem leaf extract	12.33 c ± 1.52	51.54	23.61 d ± 1.76	51.54
Autostin	24.00 b ± 1	185.71	42.00 b ± 2	185.71
Trichoderma formulation + Neem leaf extract+Autostin	31.00 a± 2	269	46.33 a ± 1.52	269
LSD ($p \leq 0.05$)	3.61		4.27	

Means ± Standard deviation followed by the same letter did not differ at 5% level of probability

4.3.11 Efficacy of different treatments of yield per plant of chickpea at the time of harvesting at 130 days after sowing

Combination of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50 WDG showed the highest yield/plant (4.16g); single application of Autostin 50 WDG (3.25g); combined application of *T. asperellum* HSTUT 1 with neem leaf extract (2.42g); single application of *T. asperellum* HSTUT 1(1.81g) and single application of neem leaf extract showed statistical similar result of yield/plant (1.76g). However, *F. oxysporum* f. sp. *ciceri* only (control) showed the lowest yield per plant (1.12g).

Combination of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50 WDG showed the highest yields/plant (7.89g); single application of Autostin 50 WDG (4.96g); combined application of *T. asperellum* HSTUT 1 with neem leaf extract (4.85g); single application of *T. asperellum* HSTUT 1 (3.94g) and single treatment of neem leaf extract (2.72 g). However, *Sclerotium rolfsii* only (control) showed the lowest yield per plant (2.29 g).

Table 13. Effect of different treatments of yield per plant of chickpea at the time of harvesting at 130 days after sowing

Treatments	**Yield per plant (g)**			
	Fusarium wilt	**Increase over control (%)**	**Collar rot**	**Increase over control (%)**
Control	1.12 e ± 0.06	-	2.29 d ± 0.40	-
Trichoderma formulation	1.81 d ± 0.10	61.60	3.94 bc ± 0.75	72.05
Neem leaf extract	1.76 d ± 0.16	57.14	2.72 cd ± 0.69	18.77
Trichoderma formulation+ Neem leaf extract	2.42 c ± 0.02	116.07	4.85 b ±0.51	111.79
Autostin	3.25 b ± 0.05	190.17	4.96 b ± 0.42	116.59
Trichoderma formulation+ Neem leaf extract + Autostin	4.16 a ± 0.04	271.42	7.89 a ± 0.56	244.54
LSD ($p \leq 0.05$)	0.23		1.57	

Means ± Standard deviation followed by the same letter did not differ at 5% level of probability

4.3.12 Relation between wilt incidence (%) and yield (g) per plant

In the regression, wilt disease incidence (%) of chickpea plant considered as independent and yield (g) per plant as dependent variable. A strong negative relation was found between wilt incidence (%) of chickpea plant and yield (g) per plant. Due to wilt disease, (3.1421%) loss in yield per plant was observed.

The regression equation also showed that for every one unit increase in wilt incidence, yield regression of (0.7982%) will be occurred (Fig. 7).

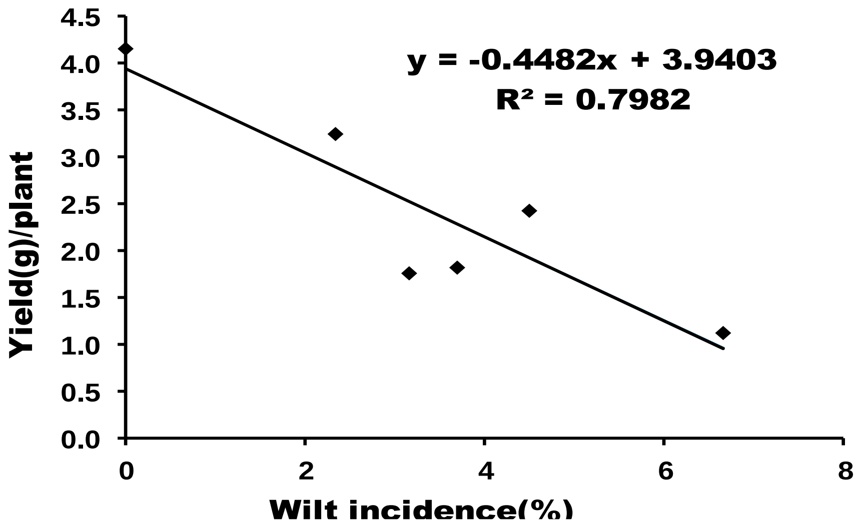

Figure 7. Correlation of co-efficient between wilt disease incidence (%) and yield (g) per plant

4.3.13 Relation between collar rot incidence (%) and yield (g) per plant

In the regression, collar rot disease incidence (%) of chickpea plant considered as independent and yield (g) per plant as dependent variable. A strong negative relation was found between collar rot incidence (%) of chickpea plant and yield (g) per plant. Due to collar rot disease, (5.5623%) loss in yield per plant was observed. The regression equation also showed that for every one unit increase in collar rot incidence, yield regression of (0.4703%) will be occurred (Fig.8).

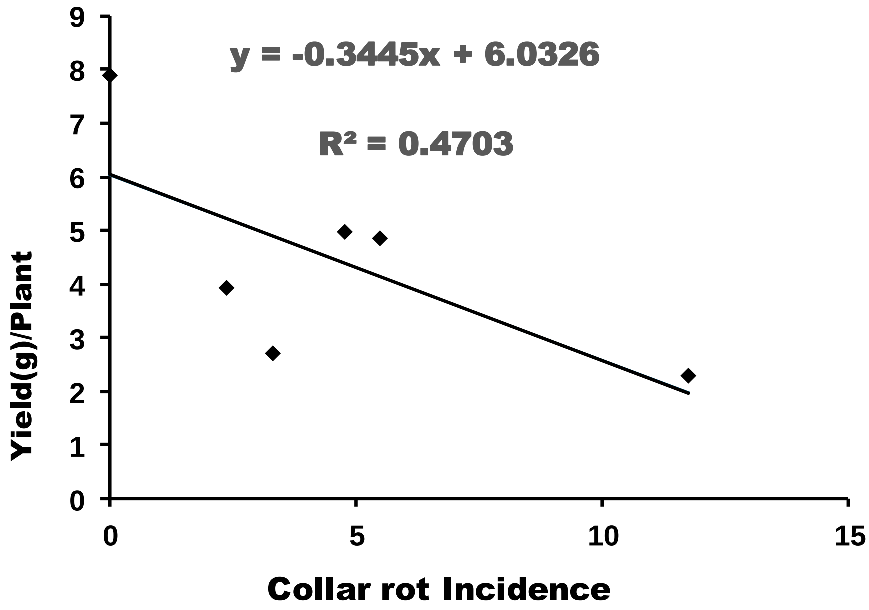

Figure 8. Correlation of co-efficient between collar rot disease incidence (%) and yield (g) per plant

CHAPTER V

DISCUSSIONS

Fusarium oxysporum and *S. rolfsii* are soil borne pathogens responsible for wilt and collar rot of disease in various crops. Wilt and collar rot of chickpea potentially very destructive disease and the pathogen can attack during various growing stages of plant from seedling to maturity. The efficacy of different treatments including formulated *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50 WDG were used single or in combination to control the diseases. The consortia made up of *B. cereus* HSTUB 17, *T. harzianum*, and *C. gigantea* also results reduced bacterial wilt of brinjal in Bangladesh (Qulsum, *et al* 2023). The formulated *T. harzianum*, either alone itself or in combination with its spore suspension, decreased the damping off and foot rot of chilli. In addition, *T. harzianum* application improved other agronomic parameters that are directly related to chilli yield, including seed germination (Islam *et al* 2021). *Trichoderma* species introduce cell wall-degrading enzymes such as proteases and chitinases together with other secondary metabolites that function as elicitors of plant defence mechanisms (Deng *et al*. 2019; Jamil 2021). *Trichoderma* spp. colonise plant roots as endophytic symbionts and release chemical stimulants that are good for plants were also reported with ten bio agents including control. Macerated extract of *Fusarium solani* + *Trichoderma harzianum* had the best result in controlling root rot of lentil with the highest seed germination (100%), number of branch/5 plants (15.56). The antifungal and antibacterial effects of *A. indica* extract are also attributed to quercetin and β-sitosterol. Increased phytohormone biosynthesis may be the cause of the wilt-infected chickpea plants' increased growth and yield when they were treated with plant extract (Harman *et al*. 2012; Kashem *et al*. 2011). Alkaloids, glycosides, flavonoids, and saponins are among the phytochemicals found in *A. indica's* leaves, fruits, and bark. Nimbin, Nimbandiol, imbolide, Nimbinene, and a few others are properties with antioxidant, antibacterial, and anti-pesticide qualities include nimbiol, 6-desacetylnimbinene, acorbic acid, 7-desacetyl-7-benzoylgedunin, n-7-desacectyl-7-benzoylazadiradione, and 17-hydroxyazadiradione azadirachtin and gedunin were also found if seeds are treated with all the tested fungicides/botanicals, it reduced disease severity and increases pod number and crop yield of lentil in comparison to untreated control ones (Elzaawely *et al*. 2017., Sadre *et al*. 1983; Shahiduzzaman 2015). Alkaloids, glycosides, flavonoids, and saponins are among the phytochemicals found in *A. indica's* leaves, fruits, and bark. Nimbin, Nimbandiol, Nimbolide, Nimbinene, and a few others (Dubey *et al*. 2020). The combined application of Vitavax and *T. harzianum* reduced the incidence of wilt and enhanced plant development and tomato yield. Jamil and Kumar 2022 observed the incidence of *Solanum melongena* Fusarium *wilt* was reduced by the topical application of *T. harzianum* and Bavistin, which also improved crop yield, chlorophyll and phenolic contents, and seed emergence and plant growth. Choudhary and Ashraf (2019) reported the combined application of *T. harzianum* and *A. indica* cake significantly reduced plant disease mortality and the pathogen's population in the soil while also improving the plants' physiological parameters, growth, yield, and seed emergence rate. This resulted in a reduction in dry root rot of mung bean. Jamil *et al*. (2021) supported implementing an integrated management approach which involves the use of Bavistin, *T. harzianum*, and *A. indica* leaf extract in various combinations significantly improved these characteristics. It suppressed the wilt fungus growth in the soil.

CHAPTER VI

SUMMARY AND CONCLUSION

Chickpea is a vital legume crop of the world, grown in more than fifty countries. Wilt and collar rot are the most destructive diseases of chickpea. Chemical fungicides may control these diseases to some extent, but it has residual effects on human health and environment as well. So, in the recent study, it was experimented to manage the wilt and collar rot diseases of chickpea in an eco- friendly manner. To study the effect of different treatments on conducting of wilt and collar rot diseases of chickpea by using *Trichoderma,* neem leaf extract and Autostin 50 WDG. Firstly, *in vitro* experiment; botanical and chemical efficacy of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50 WDG against *Fusarium oxysporum* and *Sclerotium rolfsii* were studied. The result indicated that *Trichoderma asperellum* (63.33%), neem leaf extract (38.88%) and Autostin 50 WDG at (10,50,100) ppm (65.22%, 73% & 86.66%) significantly inhibited the mycelial growth of *F. oxysporum*. The result indicated that *Trichoderma asperellum* (56.66%), neem leaf extract (35.55%), and Autostin 50 WDG at (10,50,100) ppm (60.77%, 66.33% & 74.88%) significantly inhibited the mycelial growth of *S. rolfsii* respectively. After inoculation of *F. oxysporum* and *S. rolfsii* combined application of *T. asperellum* HSTUT 1 with neem extract and Autostin 50 WDG resulted higher reduction of wilt incidence by 32%, 93.19% & 100% and collar rot incidence by 67.40%, 88.36% & 100% respectively. The combined application of *Trichoderma asperellum* HSTUT 1, neem leaf extract and Autostin 50 WDG also exhibited the highest result of all agronomical data of chickpeas.

The findings of the present study, it may be concluded that combined application of *T. asperellum* HSTUT 1, neem leaf extract and Autostin 50 WDG showed maximum result of germination percentage and other agronomical parameter comparing with single treatment. And which ultimately offer eco-friendly, cost-effective management of Fusarium wilt and collar rot of chickpea. However, before final recommendation to the farmers, on farm research in different locations need to contact.

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