Compatibility of *Trichoderma harzianum* with Selected Botanicals and Their Efficacy against *Fusarium oxysporum f.sp.lentis*

A Thesis

By

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Examination Roll: MS- 170812

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DEDICATED TO

MY BELOVED PARENTS

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ABSTRACT

Two experiments were conducted in the Plant Protection Laboratory of Agrotechnology Discipline, Khulna University, Khulna. At first, the compatibility of *Trichoderma harzianum* with four different botanicals (neem, tulsi, arhar, durba leaf extracts) maintains five concentrations (1%, 5%, 10%, 15% and 20%) was evaluated. The effectiveness of *Trichoderma harzianum* suspension along with these four bio-agents was evaluated against a pathogenic fungi *Fusarium oxysporum f.sp. lentis* the causal agent of *Fusarium* wilt of lentil. The experiment was conducted in Completely Randomized Design (CRD) with five replications. *Trichoderma harzianum* revealed compatibility at 1% to 10% concentration of neem and tulsi leaf extracts and all the concentrations of arhar and durba leaf extracts. On the other hand, 20% concentration of both neem and tulsi leaf extracts demonstrated incompatibility with *Trichoderma harzianum*. To suppress the growth of Neem and tulshi leaf extracts were effective at 20% concentration. *Trichoderma* suspension was effective against *Fusarium oxysporum f. sp. lentis* at 15% and 20% concentration in comparison to other botanical extracts. Moreover, regression equation between different concentrations of all the treatments and mycelial growth inhibition percentage revealed positive functional relationship except in case of durba leaf extract. In addition, colony characteristics of the tested pathogens were varied from control plate at different concentrations. Among all the treatments, *Trichoderma* suspension inhibited the radial mycelial growth of *Fusarium oxysporum f. sp. lentis* significantly when they applied in combination with other botanicals. On the contrary, *Trichoderma harzianum* revealed incompatibility with higher concentration (15% to 20%) of neem and tulsi leaf extracts. So, *Trichoderma* may be successfully used in combination with arhar, durba leaf extracts and lower concentration of neem and tulsi leaf extracts to control *Fusarium oxysporum f.sp. lentis.*

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INTRODUCTION

Fusarium oxysporum f. sp. lentis causes *Fusarium* wilt of lentil which is the second most important diseases resulting about 44% yield loss in Bangladesh (Anonymous, 1986) and it is considered as destructive disease of pulses in almost all legume-growing countries of the world (Anonymous, 1986; Dey et al. 1993, Ahmed, 1985). The incidence of this disease has been increasing, causing substantial yield losses of lentil and it may cause 100% seedling mortality in field condition under monoculture and favorable weather conditions (Begum, 2003). Singh and Kamal (2012) reported 10-90% yield loss in lentil in sub-tropical region due to this destructive disease. The disease may cause complete crop failure under favorable conditions and can be the major limiting factor for lentil cultivation in certain areas (Chaudhary and Amarjit, 2002).

Several procedures have been attempted for managing this destructive disease in the field and greenhouse condition. Ioannou (1999) used soil solarization technique to reduce the population density of *Fusarium* species in soil, and provided effective control measures. Bioactive compounds extracted from different species of fungi were reported to inhibit the growth of many plant pathogenic fungi, including *Fusarium* that causes wilt of lentil (Kanokmedhakul et al., 2006 and 2003; Thongsri and Soytong, 2004; Srinon et al., 2004, Suwannapong and Soytong, 2002).

Despite of the many achievements in modern agriculture, chemical control still holds a strong performance in combating certain destructive plant diseases (Hoque et al. 2014). Fungicide applications to soil, kills important beneficial fungi and also weakens the natural antagonistic

activity (Lenteren and Woets, 1988). In spite of well known side effects of chemicals on environment, these are continuously used to control soil borne plant pathogens. To reduce the use of pesticides, biological control method has been considered as more natural and environmentally acceptable approach (Bagwan, 2010). Several species of *Trichoderma* are well documented mycoparasites and have been used successfully against certain soil borne pathogenic fungi. Significant growth inhibition by *Trichoderma* has been reported for Armillaria mellea (Tapwal et al., 2004), *Dematophora necatrix* (Tapwal et al., 2005), *Phytophthora cinnamomi* (Singh et al., 2010), *Fusarium oxysporum* and *Rhizoctonia solani* (Dar et al., 2011) and *Sclerotium rolfsi* (Jegathambigai et al., 2010). Many other workers (Salehpour et al., 2005; Abdollahzadeh et al, 2006; Mir et al., 2011; Osman et al., 2011) utilized *Trichoderma* species as a potential biological control agent.

Besides *Trichoderma,* plant extracts are recently advocated by several researches as a potential control method of plant diseases (Belabid et al., 2010). Many plant products have been reported to have antimicrobial activities against plant pathogenic root fungi (Bashar and Baharat, 1992; Bowers and Locke, 2000). In an IDM package, incorporation of natural products provides a viable solution to the environmental problems caused by synthetic pesticides. Identification of these compounds and their further testing may be an effective approach to minimize the use of hazardous chemicals (Duke, 1990).

To develop an effective disease management program, the compatibility of potential bio-agents with botanicals is essential. For better results, farmers sometimes apply *Trichoderma* in soil with other botanicals for biological control of the soil borne diseases without knowing the interaction effects. As there may be some inhibitory effects of biological agent with

botanicals so for the use of these botanicals in an integrated disease management program, the botanicals must be compatible with each other.

With this background information, the present investigation was under taken with the following objectives:

- To evaluate the compatibility of *Trichoderma harzianum* with some selected botanicals
- To find out the efficacy of some selected botanicals against *Fusarium oxysporum f. sp. lentis*

CHAPTER II

REVIEW OF LITERATURE

2.1 Compatibility of *Trichoderma harzianum* with other botanicals

Maheshwari (2014) reported that *Trichoderma harzianum* is an exceptionally good model of bio-control agent as it is ubiquitous, easy to isolate and culture multiply rapidly on many substrates. There are several mechanisms involved in *Trichoderma* antagonism namely antibiosis whereby the antagonistic fungus shows production of volatile metabolites including ethylene and acetone as well as diffusible antibiotics and environmentally safe and economically viable strategy for control of various plant diseases has led to an increased plant based products in agriculture. The results of neem leaves, garlic and onion bulb extracts significantly reduced the growth of isolates of *Trichoderma* species. It was also noted that an increase in the concentration (5% to 15%) resulted in subsequent decrease in growth of the isolates. The inhibition per cent at lower concentration i.e., 5 per cent of neem, garlic and onion extracts was ranging from 20.89 to 27.99, 9.51 to 17.11 and 7.95 to 12.5, respectively. Whereas, at higher concentration i.e., 15 per cent, it was 44.53 to 55.09, 34.59 to 39.86 and 31.56 to 39.55, respectively. The average percent inhibition in mycelial growth of isolates species wise indicated that all the seven species were more or less similar regarding sensitivity towards tested plant extracts.

Wedajo (2015) evaluated the compatibily of fungicides viz., curzate and sancozeb were used at different concentrations that is., 100, 200, 400, 600, 800 and 1000 ppm active ingredient to with *Trichoderma* species viz., *Trichoderma harzianum* (AUT1) and *Trichoderma viride* (AUT2) in favour of tolerance to fungicides. By increasing the fungicide concentrations to 400 ppm (sancozeb) and 600 ppm (curzate), the *Trichoderma* species tolerate the fungicides 50% and slightly incompatible at higher concentrations of 800 and 1000 ppm, and completely inhibited beyond 1000 ppm compared to the control for both fungicides. The highest 97.8% was recorded at 100 ppm for curzate fungicides when combined with AUT2 and 96.7% of compatibility was recorded at concentration of 100 ppm when AUT1 is combined with the same fungicides. But, in the case of sancozeb the highest compatibility (97.8%) was recorded when combined AUT2, and 95.5% with AUT1 at 100 ppm. Therefore, the present compatibility study assist in the selection of botanicals, which can be used with reduced amount of preferred fungicides for the control of plant pathogenic fungi.

Khirallah et al. (2016) found that the compatibility between strains of *Trichoderma harzianum* (Tcomp, TH1 and TH3) and *T. viride* (TV1) and different active ingredients used as fungicides against seeds borne and foliar diseases of some cultures were tested under *in vitro* conditions. The activities of the formulations, pyraclostrobin + boscalid, cyprodinil + fludioxanil, fenhexamid and mepanipyrim were null to low on the mycelial growth of the strains tested.

However, thiram showed good compatibility at 500 and 666.6 ppm ranging from 45.5 to 84.9%. Similary, boscalid + Pyraclostrobin at 125.5 ppm and cyprodinil + fludioxanil at 93.7 ppm were compatible respectively with TH3 and TV1 (50.6-56.1%) and TH1-TH3 (90.4-79.3%). After 24 hours of incubation, the *Trichoderma* conidia could germinate in the presence of different concentrations of mepanipyrim and fenhexamid showing compatibility respectively ranging from 45.0 to 98.0% and 37.5 to 97.1%. The Tcomp strain showed good ability to germinate in presence of low doses of thiram (500 ppm), chlorothalonil (375 ppm), boscalid + pyraclostrobin (125.2 ppm) and cyprodinil + fludioxonil (93.7) with respective compatibility percentages of 54.3, 54.2, 75.5 and 57.2%.

Bagwan (2010) conducted compatibility under in vitro condition to find out safer fungicides, pesticides, different cakes and botanicals against *Trichoderma.* For this different fungicide, pesticides, cakes and botanicals were tested against *Trichoderma harzianum* (Th 09) and *Trichoderma viride* (Tv 11). Results indicate that among the fungicides tested, thiram (0.2%), copper oxychloride (0.2%) and mancozeb (0.2%) were found comparatively safer against *Trichoderma harzianum* and *Trichoderma viride* as compared to other fungicides. *Trichoderma* was most sensitive to captan, tebuconazole, vitavax, propiconazole and chlorothalonil. But *Trichoderma* was tolerant to all the pesticides and weedicides tested. None of the pesticide and weedicide inhibited the growth of *Trichoderma.* Among the botanicals tested, 10% fresh leaf extract of karanj leaves (*Pongamea pinnata*) and cumin leaves inhibited 32.19% 27.15% growth of *Trichoderma*, respectively as compared to control.

Another intresting thing observed that, neem oil (5%), neem leaves extract (10%), wild sorghum leaves extract (10%), neem cake, castor cake and mustard cake extract (10%) enhanced the growth of *Trichoderma*. This finding indicates that seed treatment or furrow applications of *Trichoderma* would be compatible with thiram, copper oxychloride, mancozeb, pesticides, weedicides, neem oil, neem leaves extract, wild sorghum leaves extract, neem cake, castor cake and mustard cake extracts for the integrated management of soil borne diseases of groundnut.

2.2 Effect of different botanicals on growth of *Fusarium oxysporum*

Arya and Kushwaha (2018) evaluated an investigation on the evaluation of chemicals to see their effect on the fungal pathogen *Fusarium oxysporum f.sp. lentis*, causing wilt disease of lentil crop. Fungal pathogen was evaluated in the laboratory by employing the Poisson Food Technology along with seed treatment to the lentil seeds was given for evaluation of the chemicals against the pathogen under in vivo conditions. Percent inhibition in the radial growth of mycelia of pathogen in the petri dish was used for evaluating the potential of chemicals in laboratory to manage pathogen growth and reduction in the disease incidence was recorded to see the effect of chemicals under field conditions.

The compatibility percentages were low to moderate the first week, respectively 35.9, 10.0, 28.7 and 34.4% for TH3 and 59.1, 32.8, 61.9 and 84.1% for Tcomp at the recommended doses 501, 375, 750 and 400 ppm. They become high during the second week respectively 88.1, 75.4 and 100%. Seven days after incubation, the compatibility of Tcomp and TH3 was moderate (51.4%) at 200 ppm of pyrimethanil and become important after 16 days, respectively 65.0 and 78.7%. 32 days after incubation, this fungicide showed a moderate activity at the recommended dose (800 ppm), the compatibility percentages varied between 33.3 and 68.5%. After the second week, the chlorothalonil at 500 ppm showed good compatibility with Tcomp and TV1 (51.9 and 48.2%) and all the strains tested except TH1 become moderately compatible (43.5 – 72.2%) with the different concentrations after 24 days of incubation. However, thiram was able to completely inhibit mycelial growth of the *Trichoderma* stains during 4 weeks. No compatibility was observed between them. After the second week, the chlorothalonil at 500 ppm showed good compatibility with Tcomp and TV1 (51.9 and 48.2%) and all the strains tested except TH1 become moderately compatible (43.5 – 72.2%) with the different concentrations after 24 days of incubation. However, thiram was able to completely inhibit mycelial growth of the *Trichoderma* stains during 4 weeks. No compatibility was observed between them. After one month, the *Trichoderma* strains produced conidia in the presence of low concentrations of chlorothalonil and in the recommended dose of fenhexamid, showing compatibility respectively ranging from 50.5 to 89.7% and 51.5 to 97.1%. The conidia production of the tested strains was inhibited at the recommended doses of the other fungicides tested.

he eight chemicals namely Azoxystrobin, Propiconazole, Provax, Captaf, Carbendazim, Thiram, Raxil and Vitavax Power, were used against the pathogen. Among all the chemicals evaluated, Raxil was found most effective giving 100 percent mycelium inhibition in laboratory at 500ppm followed by 88.75 percent, Captaf (75%), Vitavax power (71%), Azoxystrobin (69.5%), Carbendazim (56.66%), Thiram (53.75%), Provax (37.5%) and control where no inhibiton of mycelium was observed. The seed treatment with the chemicals showed minimum disease incidence in case of Raxil treatment i.e.0.97 percent in the year 2016 and 0.87 percent in the year 2017, and the maximum disease incidence was recorded in case of seed control (9.23%). The present study concluded that there are several new chemicals those have the potential to manage the disease even in a lower concentrations.

Belabid et al. (2010) found that *Fusarium* lentil wilt caused by *Fusarium oxysporum* Schechet. emend. Snyder & Hansen *f. sp. lentis* Vasudeva and Srinivasan (Fol) as an important disease and considered as a limiting factor to lentil production in Algeria. The experiment aimed at evaluating the antifungal activity of powders and essential oils formulation of some local medicinal plants (*Anacyclus valentinus* L., *Artemisia herba.*, *Eucalyptus sp, Inula viscosa* L., *Laurus nobilis* L., *Mentha pepirita* L., *Rosmarinus officinalis* L., *Salvia officinalis* L., *Tetraclinis articulata* (Vahl) Masters and Thymus vulgaris L.) on Fol population in the soil.

Results obtained showed that the treatments 10 and 5% with the powders of *I. viscosa* and *M. pepirita* and the essential oils formulation in all treatments have significantly reduced the soil population densities of Fol and the disease incidence on lentil. The observed reduction in the pathogen population and increased healthy plant indicates that these extracts could have important roles in biologically based management strategies for control of *Fusarium* wilt disease.

Garkoti et al. (2013) evaluated the antifungal activity of some plant extracts *in vitro* and in vivo condition. Field experiments were conducted at N. E. Borlaug Crop Research Centre of G. B. Pant University of Agriculture and Technology, Pantnagar. Field trials were carried out consecutively during Rabi 2010-11 and 2011-12 crop seasons in Randomized Block Design (RBD) with three replications, using 'Pant L-639' a released cultivar to wilt. The plot size was 3.0×1.5m2 with row spacing of 30cm. On the basis of in vitro studies, effective plant extracts were used for seed treatment. Effect of selected plant extracts on disease incidence, 1000 grain weight and yield of lentil was recorded. All the botanicals were observed effective. However, garlic extract seed treatment followed by ginger, showed lowest disease incidence, highest grain yield as well as maximum 1000-grains weight in comparison to check plot. The reduction in the pathogen population/ growth, decreased disease incidence and increased grain yield indicates that these extracts could have important roles in biologically based management strategies for control of *Fusarium* wilt disease under organic mode of lentil cultivation.

Dahal and Shrestha (2018) conducted a study in IAAS, Lamjung Campus to test the efficacy of fungicides *in-vitro* by poisoned food technique in PDA medium against *Fusarium oxysporum f. sp. lentis*. The fungicides tested were Carbendazim (50% WP), Chlorothalonil (75% WP) and Dithane M-45 (75% WP) at three different concentrations (100 ppm, 150 ppm and 200 ppm). The treatments were arranged in complete randomized design and replicated five times. The measurement of diameter of the fungal mycelium was taken 8 times at 48 hours interval until the fungus nearly covered the plate in control treatment and inhibition percent of the chemicals were calculated. All the fungicides inhibited the fungal growth significantly, among which carbendazim was highly effective in all the concentrations reducing 100% of mycelial growth followed by chlorothalonil. Dithane M-45 showed least inhibition i.e. 26.62% in 200 ppm (day 13). The chemicals exhibited increased tendency of inhibition with increased concentration.

Chandra et al. (2018) reported that use of synthetic fungicides has led to the emergence of several problems like environment pollution, residual effect in grains and killing of non-target organism(s). Hence, for minimizing the losses caused by *Fusarium* wilt, four plant extracts such as Garlic, Neem, Eucalyptus, Tulsi and three antagonistic botanicals viz. *Trichoderma viride, Trichoderma harzianum* and *Pseudomonas fluorescence* were evaluated. The disease incidence was not much reducing by Neem (40.50 %) and Tulsi (34.45 %) at 10 per cent concentration of 60 days after sowing. The same trends were also found in per cent disease reduction.

However, per cent disease reduction in between Eucalyptus (25.50 %) and Garlic (28.00 %), Tulsi (34.45 %) was at par to each other. The Maximum per cent disease reduction was found in Eucalyptus (25.50 %) and Garlic (28.00 %), Lowering the disease incidence and higher per cent disease reduction was also found in *Trichoderma viride* (19.64 %) as compared to *Trichoderma harzianum* (35.50 %) and *Pseudomonas fluorescence* (28.43 %) at 10 per cent concentration of 60 days after sowing.

Abdisa (2018) found *Fusarium* wilt of lentil (*F. oxysporum f.sp. lentis*) caused huge lentil yield losses in central highlands of Ethiopia. In his study, extensive wilt survey of eight major lentil-growing districts, viz. Adaa, Aleltu, Lume, Gimbichu, Minjar-Shenkora, Siyadebrena Wayu, Moretina-jiru and Ensaro in central highlands of Ethiopia was conducted during the 2017cropping season. The study of the objectives were to: 1) assess the distribution of lentil *Fusarium* wilt in the central highlands of Ethiopia; 2) determine the extent of seed infection due to lentil *Fusarium* wilt pathogen on seed lots collected from different sources in the central highlands of Ethiopia; and 3) evaluate the effect of lentil varieties and seedbed types as components of integrated management option. Data of the survey revealed 100% mean wilt prevalence and 32.8% mean wilt incidence were observed. The highest (75%) and the lowest (2%) wilt incidence were recorded in Moretina-jiru and Lume, respectively.

Morphological and cultural assessment of 192 isolates of wilt causal agents showed highest frequencies of *Fusarium oxysporum f.sp. lentis* (100%) followed by *Rhizoctonia spp.* (23.4%) and *Sclerotium rolfsii* (3.0%). factorial experiment involving lentil variety and seedbed type, each at four levels, was carried out in a split-plot design with three replications. The four lentil varieties were ILL-590 (susceptible check), Alemaya, Derash and Denbi, and four seedbed types were flat bed, open raised bed, tie-raised bed and farmer's practice. Among the seedbed types, raised seedbed exhibited relatively lower disease incidence than others. Interaction of the varieties and seedbed types was significant in wilt reduction. The highest wilt incidence (ca. 82.0%) was recorded on ILL-590, susceptible lentil line, planted on flat bed; whereas, the lowest (ca. 8.8%) *Fusarium* wilt incidence was noted on cv. Derash planted on raised bed. A combination of cv. Derash and raised bed gave significantly ($P < 0.05$) higher grain yield (3827.0 kg/ha) than all other treatment combinations at Chefe Donsa. Unlike Chefe Donsa, raised bed contributed significantly ($P < 0.05$) higher grain yield of not only cv. Derash but also cv. Alemaya than all other treatment combinations at Debre Zeit. Significantly ($P < 0.05$) lower grain yields (in the order of 68.0 kg ha-1) were obtained from integration of the susceptible genotype (ILL-590) with flat bed than all other treatment integration irrespective of the trial locations. The highest (1018.0% unit/days) in AUDPC values were obtained by flat seedbed type and ILL-590, while the lowest (342.4% unit/days) in AUDPC values were obtained by raised seedbed type and Derash variety at Debre Zeit.

Wilt incidence and AUDPC values were significant and negatively correlated with yield parameters. Lentil seed infection due to *Fusarium oxysporum f.sp. lentis* ranged from 0 to 22.2%. It was concluded that using resistant variety with raised seedbed significantly reduced *Fusarium* wilt incidence and gave reasonably high yields. The future research work should focus on screening several lentil genotypes for source of resistance.

Ramaiah and Garampalli (2008) described *Fusarium oxysporum f. sp lycopersici* as an important disease that causes wilt disease in tomato crop world over. Management through chemical fungicides cause serious environmental problems and are toxic to non-target organisms as well. Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides. In an approach towards the development of eco-friendly management, in vitro antifungal assay was conducted against *Fusarium oxysporum f. sp. lycopersici* (FOL), using plant extracts of fifteen plants. Out of fifteen plants, three plants proved to be potential in inhibiting the growth of the FOL viz. *Solanum indicum* (78.33%), *Azadirachta indica* (75.00%), *Oxalis latifolia* (70.33%). Antifungal potency was compared with three chemical fungicides namely via (Mancozeb with 82.66%, Copper oxychloride 79.33% and Copper sulphate 82.33%) in different concentration.

The poison food technique was employed for the evaluation of antifungal activity of the extracts at four different concentrations (10%, 20%, 40% and 60%) on mycelial growth of FOL. This study indicates that the botanical extracts could be a good alternative in developing a potent plant based fungicides which can be used in organic farming for the management of *Fusarium oxysporum f. sp. lycopersici*.

Khursid and Marley (2016) conducted an experiment to assess antifungal potential of the allelopathic grass Cenchrus pennisetiformis Hochst. & Steud. against the fungal plant pathogen *Fusarium oxysporum f. sp. lycopersici* (cause of tomato wilt disease) under chromium stress. Laboratory experiments were performed in 10 mL volume glass test tubes each containing 1.0 mL of malt extract broth with seven concentrations (0, 50, 100, 150, 200, 250, 300 and 350 ppm) of each of Cr(III) and Cr(VI), and two concentrations (5% and 6%) of methanolic leaf, stem or root extract of *C. pennisetiformis*. A metal + weed extract amended medium was inoculated with the pathogen and incubated for 7 days at 25 C. Different concentrations of Cr(III) and Cr(VI) proved equally inhibitory resulting in 10-84% and 18-89% reduction in fungal biomass, respectively. Methanolic leaf, stem and root extracts of the weed reduced fungal biomass by 12-25%, 14-23% and 46-50%, respectively, over negative control.

In combined application of methanolic extracts of different parts of *C. pennisetiformis* and metal solutions, root extract in combination with either Cr (III) or Cr (VI) showed the highest inhibitory potential against the fungus followed by leaf and stem extracts. In combination with methanolic root, leaf and stem extracts, different concentrations of Cr(III) and Cr(VI) significantly reduced fungal biomass by 54-99%, 14-99% and 9-95%, respectively, over negative control. Such studies have not been carried out previously. Results of the present investigation suggest that *F. oxysporum f. sp. lycopersici*, the cause of *Fusarium* wilt disease in tomato, can be managed by application of extracts (or alternatively biomass) of *C. pennisetiformis* in chromium contaminated soils.

kerkeni et al. (2007) marked suppression in growth of *Fusarium oxysporum* by the medicinal leaves extracts of *Anagalis arvensis, Aspergillus sp, Trichoderma viride* strain 2 and *T. viride* strain 1 isolated from an animal manure compost are tested for their in vitro and in vivo antagonistic activity against *Fusarium oxysporum f. sp. radicis-lycopersici*, the causal agent of the *Fusarium* Crown and Root Rot of tomato. Dual culture experiments, observed after incubation at 25°C on PDA during 5 days, showed that all tested fungi significantly inhibited the mycelial growth of *F. oxysporum f. sp. radicis-lycopersici* comparatively to the untreated control. Inhibition varied from 25% for *Trichoderma viride* (strain 1) to 100% for *Aspergillus sp.* Competition for media was the predominant mechanism of action noted on PDA.

In vivo, tomato plants (cv. Riogrande), simultaneously inoculated and treated individually by the compost fungi conidial suspensions (107 spores mL-1), showed reduced severity of the *Fusarium* Crown and Root Rot, when observed 30 days after transplantation, comparatively to the untreated control. The compost fungi *T. viride* (strain 1) was the most effective, it decreased severity of the disease by 48%.

Naz et al. (2006) evaluated the antifungal activity of *Azadirachta indica* against *Fusarium oxysporum.* The present paper reports the antifungal activity of plant diffusates from 5 indigenous medicinal plant species of Potohar region viz., *Adhatoda zeylanica, Azadirachta indica, Capparis decidua, Dodonaea viscosa* and *Salvadora oleoides.* Antifungal activity was tested against 3 pathogens attacking commercial crops viz., *Alternaria solani, Rhizoctonia solani* and *Macrophomina phaseolina.* All selected medicinal plants exhibited considerable distinction in radial mycelial growth of tested pathogens. Overall, *Dodonaea viscosa* appeared significantly the most effective and suppressed the radial mycelial growth of the *Alternaria solani* and *Rhizoctonia solani,* whereas, *Adhatoda zeylanica* exhibited maximum inhibition (77.44%) against *Macrophomina phaseolina.* However, *Salvadora oleoides* exhibited minimum inhibition against all tested pathogens. It was also observed that radial mycelial growth of selected pathogens reduced at an increase of plant diffusates concentration. Among 5 concentrations of plant diffusates, the highest inhibition in radial mycelial growth of all 3 pathogens was observed at 100 and 200g/l respectively, as compared to control, while minimum inhibition was recorded at 10g/l in all plant diffusates.

Dhttura and Calotropi (AKK) were found less effective as compared to other three botanical extracts. All the botanical extracts at their highest doses significantly retarded the growth of fungus as compared to control (82.66 mm).Its concluded that wilt is actually responsible of basal rot in onions.

Sahayaraj et al. (2006) evaluated that extracts of *Allium sativum* bulbs showed a strong effect in inhibiting mycelial growth of *F. oxysporum* f. sp. *Ciceris*. Three plant extracts viz. bulbs of *Allium sativum* L. (Liliaceae), seeds of *Annona squamosa* L. (Annonaceae) and leaves of *Vitex negundo* L. (Verbenaceae) were evaluated against cowpea wilt pathogen, *Fusarium oxysporum f. sp. ciceris* by mycelial dry weight method under laboratory condtions. The mean mycelium dry weights of *F. oxysporum* of methanol and benzene extracts of *A. sativum* obtained from 125 g of crused dry plant material (bulbs) were 0.0113 and 0.0174 mg, respectively. This was followed by methanol and petroleum ether extracts of *A. squamosa* (0.2396 and 0.2381 mg). They effectively controlled mycelial growth of cowpea wilt pathogen, however *V. negundo* extracts did not cause any significant mycelium growth inhibition when compared to other plant extracts tested. Among the three plant extracts, methanol extracts of *A. sativum* bulbs could possibly be used for controlling *F. oxysporum*.

CHAPTER III

MATERIALS AND METHODS

Two *in vitro* experiments namely, "Compatibility of *Trichoderma harzianum* with selected botanicals" and "The Efficacy of bio-agents against *Fusarium oxysporum f.sp. lentis*" were conducted in the plant protection laboratory of Agrotechnology Discipline, Khulna University, Khulna, during February 2018 to October 2018. For these the following activities were done:

3.1 Collection of samples

Pure culture of *Fusarium oxysporum f. sp. lentis* was collected from Plant Pathology Division, Pulse Research Centre, BARI, Ishurdi, Pabna. Commercial Biotech Care *Trichoderma* Suspension (*Trichoderma harzianum*, strain number: IPM-22 CP and accession AB935952) prepared by IPM Lab, Bangladesh Agricultural University (BAU), Mymensingh was collected from there. Fresh leaves of neem, tulsi, durba and arhar were collected from Khulna University campus and its adjacent area.

3.2 Preparation of Potato Dextrose Agar (PDA) Medium

PDA was prepared following the standard procedure (Anonymous, 1968). 200 g peeled, sliced potato was boiled in 1000 ml water. Boiled potato was sieved using cheese cloth. The potato extract was taken in a 100 ml braker and 15 g dextrose was added with the extract. 20 g agar

was added slowly using a magnetic stirrer so that the agar can easily be melted and mixed with the extract. Then the total volume was adjusted to 1000 ml. The prepared PDA was dispensed in 250 ml conical flask, plugged by cotton, covered by aluminium foil and then sterilized in autoclave at 121°C temperature for about 20 minutes.

3.3 Multiplication of Fungi

The fungal isolate was multiplied on PDA medium described by Tuite (1969) for further use. Multiplication procedure was conducted under Laminar Air Flow hood. 20 ml sterilized PDA was poured in each sterilized petri dishes. After solidification of PDA, the plates were inoculated with *Fusarium oxysporum f.sp. lentis* and *Trichoderma harzianum* separately.

3.4 Preservation of Isolates

The isolates of *Fusarium oxysporum f. sp. lentis* and *Trichoderma harzianum* were preserved in test tube slant with PDA. Five to ten days old test tube slants with profuse mycelium and conidia were stored in refrigerator at 4°C.

3.5 Preparation of media of leaf extracts

Leaves of four plants were used against *T. harzianum* in this experiment. Media were prepared by using a newly adapted method following the Poison Food Technique (Grover and Moore, 1962).

Table 1. List of Plants with concentration of leaf extracts used

Name of the Plants	Scientific Name	Family Name	Concentrations (%)
Neem	*Azadirachta indica*	Meliaceae	1%, 5%, 10%, 15%, 20%
Tulsi	*Ocimum tenuiflorum*	Lamiaceae	1%, 5%, 10%, 15%, 20%
Durba	*Cynodon dactylon*	Poaceae	1%, 5%, 10%, 15%, 20%
Arhar	*Cajanus cajan*	Fabaceae	1%, 5%, 10%, 15%, 20%

Healthy leaves of (neem, tulsi, durba and arhar) were collected, cleaned, weighted. 20 g leaves of each plant with 20 ml distilled water (1:1) was taken into a blender and blended, sieved to produce a stock solution. Then 1 ml, 5 ml, 10 ml, 15 ml and 20 ml extracts were mixed separately with 99 ml, 95 ml, 90 ml, 85 ml and 80 ml PDA, respectively in separate 100 ml PDA medium in 250 ml conical flask to prepare 1%, 5%, 10%, 15% and 20% extracts concentrations. After that these were mixed thoroughly by using magnetic stirrer for mixing properly. Then the medium was sterilized in an autoclave at a temperature of 121°C temperature for about 20 minutes.

Experiment 1: Compatibility of *Trichoderma harzianum* with different botanicals

In this experiment, four plant leaf extracts at five concentrations were used as botanicals to evaluate the compatibility of *Trichoderma harzianum.*

3.6 Inoculation and incubation

100 ml sterilized plant extract amended medium was poured in five sterilized petridishes, 20 ml in each. Culture disc of 5 mm *Trichoderma harzianum* was placed on the center of petridish of 90 mm diameter. Five replicated petridishes were used for each treatment. The

inoculated petridishes were kept in the growth chamber at 25±1°C until the mycelia touch the edge of petridish. The growth of control plates (*Trichoderma harzianum* on PDA without leaf extracts) was observed regularly.

3.7 Measurement of radial mycelial growth and determination of per cent inhibition of *Trichoderma harzianum*

After 6 days of incubation, when the radial growth of control plates covered about 80% of the petri plate area, the radial growth of *T. harzianum* was recorded. The radial growth was recorded in mm averaging growth of two dimensions measured at right angle through the centre of the plate.

The per cent inhibition was calculated by using following formula as given by Vincent (1947).

$$I = \frac{C-T}{C} \times 100$$

Where,

I: Percent of inhibition

C: Colony diameter in control petri plate.

T: Colony diameter in the treated petri plate.

Compatibility test was determined according to the method of Bagwan, 2010. Here, less than 30% of growth inhibition was considered as compatible and more than 30% of growth inhibition was considered as non-compatible.

3.8 Observation of Colony Characters

After 80% radial growth of control treatment, mycelial characters such as texture, margin, mycelial growth and color of mycelium were observed visually and recorded. (Akter et al., 2016)

Experiment 2: Efficacy of selected bio-agents against *Fusarium oxysporum f. sp. lentis*

3.9 Preparation of *Trichoderma harzianum* added media

To prepare media containing *Trichoderma* suspension commercial Biotech Care *Trichoderma* Suspension (*Trichoderma harzianum*, strain number: IPM-22 CP and accession AB935952) was used. After sterilization of the PDA medium, *Trichoderma* suspension was mixed with warm PDA by using magnetic stirrer. Five concentrations (1%, 5%, 10%, 15%, 20%) were prepared by adding 1ml, 5 ml, 10 ml, 15 ml and 20 ml suspension to 99 ml, 95 ml, 90 ml, 85 ml and 80 ml PDA medium, respectively to prepare 100 ml media in 250 ml conical flasks. Then, 20 ml media poured in sterilized petridishes.

3.10 Inoculation and incubation

The effect of different bio-agents on mycelial growth of *Fusarium oxysporum f. sp. lentis* was determined following poison food technique. The basic PDA medium was modified by adding leaf extracts of different plant sources and *Trichoderma harzianum* at different concentrations. Five mm culture discs were cut from the edge of 6 days old culture of *Fusarium oxysporum f.sp. lentis* grown on PDA. Each petri dish contained 20 ml media. Five

replicated petri dishes were used for each treatment. The inoculated petri dishes were kept in the growth chamber at 25±1°C until the mycelia of control plates tend to touch the edge of petri dish. The growth of *Fusarium oxysporum f.sp. lentis* at control plates (without *Trichoderma harzianum* and leaf extracts) was observed regularly.

3.11 Measurement of radial mycelial growth and determination of per cent inhibition of *Fusarium oxysporum f. sp. lentis*

The radial growth of mycelium of each plate was recorded as an average of two dimensions measured at right angles to one another through centre of the colony. The percentage inhibition of growth was calculated using the following formula (Naz et al., 2006).

$$\text{Inhibition (\%)} = \frac{X-Y}{Y} \times 100$$

Where,

X = Average growth of *Fusarium oxysporum* f. sp. *lentis* in control Petri dish

Y = Average growth of *Fusarium oxysporum* f. sp. *lentis* in each treated Petri dish

3.12 Observation of Colony Characters

Before touching the edge of Petri dishes by the mycelia in control treatment, mycelial characters such as texture, margin, mycelial growth and color of mycelium were observed visually and recorded. (Nath et al., 2017)

3.13 Data Transformation

Percent data were transformed using square-root transformation and Arcsine transformation.

3.14 Experimental Design and Data Analysis

Completely Randomized Design (CRD) with five replications was followed to conduct the experiment. The data was analyzed statistically using STAR (Statistical Tool for Agricultural Research, version-02, IRRI, Los Banos, Phillipines) and means were compared following Tukeys's Honest Significant Difference (HSD) Test.

CHAPTER IV
RESULTS AND DISCUSSIONS

4.1 Compatibility of *Trichoderma harzianum* with some selected botanicals at different concentrations

For compatibility test of *Trichoderma harzianum* with four extracts viz., Neem, Tulsi, Durba and Arhar. Neem and tulsi leaf extarcts significantly inhibited ($P<0.01$) the radial mycelial growth of *Trichoderma harzianum* but durba and arhar leaf extracts revealed statistically similar inhibition per cent at five concentrations.

Tulsi leaf extracts

The compatibility of *Trichoderma harzianum* with tulsi leaf extracts reduced significantly as the concentration of the media increased. As maximum mycelial growth (80.00 mm) was reported at 1% concentration and there was no mycelial growth inhibition, the fungus showed the most compatibility at this concentration. Similarly, upto 15% concentration the fungus was compatible with tulsi leaf extracts representing less than 30% inhibition but it revealed least compatibility at 20% concentration exhibiting the highest percentage of inhibition (43.12%) (Table 3).

There was a significant positive relation between mycelial growth inhibition (%) and tulsi leaf extract concentration and about 92% variation in mycelial growth inhibition could be explained by different extract concentration (Figure 1).

Neem leaf extracts

The compatibility of *Trichoderma harzianum* with neem leaf extracts reduced significantly as the concentration of the media increased. The radial mycelial growth was maximum (75.50 mm) at 1% concentration which was statistically similar to 5% concentration (74.00 mm) thus the mycelial growth inhibition was minimum at 1% concentration (5.62%) which was statistically similar to 5% concentration (7.50%). Up to 15% concentration, the inhibition percentage was less than 30% as such compatibility was found from 1 to 15% concentration. On the other hand, the highest percentage inhibition (32.50%) was observed at 20% concentration thus representing incompatibility. So it can be concluded that *Trichoderma harzianum* revealed compatibility with neem leaf extracts at 1-15% concentration however, it was less compatible at 20% concentration (Table 3).

Regression equation between concentrations of neem leaf extract and mycelial growth inhibition percentage revealed that, there is a significant, positive relation between mycelial growth inhibition (%) and neem leaf extract concentration and about 91% variation in increased inhibition percentage could be explained by different extract concentration (Figure 1).

Shah et al. (2011) evaluated that *Azadirachta indica* inhibited the mycelial growth of *Trichoderma hazianum* significantly (34.1%). Bagwan, (2010) conducted the compatibility tests under in vitro condition and found neem leaves extract (10%) enhanced the growth of *Trichoderma.* Rajput and Zacharia (2017) also reported that neem leaf extract @ 10% concentration inhibited the mycelial growth (48.71%).

Arhar leaf extracts

In case of arhar leaf extracts, *Trichoderma harzianum* revealed compatibility at 1% - 20% concentrations. Here, the maximum mycelial growth (80 mm) was reported at 1-15% concentration and it was 75.40 mm at 20% concentration. As such no mycelial growth inhibition was reported at 1%-15% concentration while only 5.75% was observed at 20% concentration thus demonstrated compatibility (Table 3).

Regression equation between concentrations of arhar leaf extract and mycelial growth inhibition percentage revealed that there is a significant positive relation between mycelial growth inhibition (%) and arhar leaf extract concentration and about 50% of the variation in increased inhibition percentage could be explained by the increase of concentration (Figure 1).

Durba leaf extracts

No significant variations were observed in radial mycelial growth and mycelial growth inhibition percentage in case of different concentrations of durba leaf extracts. At all levels of concentrations, mycelial growth was 80 mm and no mycelial growth inhibition was reported thus *Trichoderma harzianum* clearly depicted compatibility with durba leaf extracts (Table 3).

Table 2. Mycelial growth and inhibition percentage of *Trichoderma harzianum* at different concentrations

Plant extracts		Mycelial growth Mean (mm)	Inhibition percentage (Transformed data)
Control		80.00 a	0.00 f (0.00)
	1%	80.00 a	0.00 f (0.00)
	5%	75.50 b	5.62 e (0.24)
Tulsi	10%	71.50 c	10.62 d (0.33)
	15%	60.40 d	24.50 c (0.49)
	20%	45.50 f	43.12 a (0.66)
	1%	75.50 b	5.62 e (0.24)
	5%	74.00 b	7.50 e (0.27)
Neem	10%	71.00 c	11.25 d (0.33)
	15%	59.60 d	25.5 c (0.50)
	20%	54.00 e	32.5 b (0.57)
	1%	80.00 a	0.00 f (0.00)
	5%	80.00 a	0.00 f (0.00)
Arhar	10%	80.00 a	0.00 f (0.00)
	15%	80.00 a	0.00 f (0.00)
	20%	75.40 b	5.75 e (0.24)
	1%	80.00 a	0.00 f (0.00)
	5%	80.00 a	0.00 f (0.00)
Durba	10%	80.00 a	0.00 f (0.00)
	15%	80.00 a	0.00 f (0.00)
	20%	80.00 a	0.00 f (0.00)
Level of significance		0.01	
CV%		1.19	8.78

The parenthesis data is transformed (Square root and Arcsine transformation) data

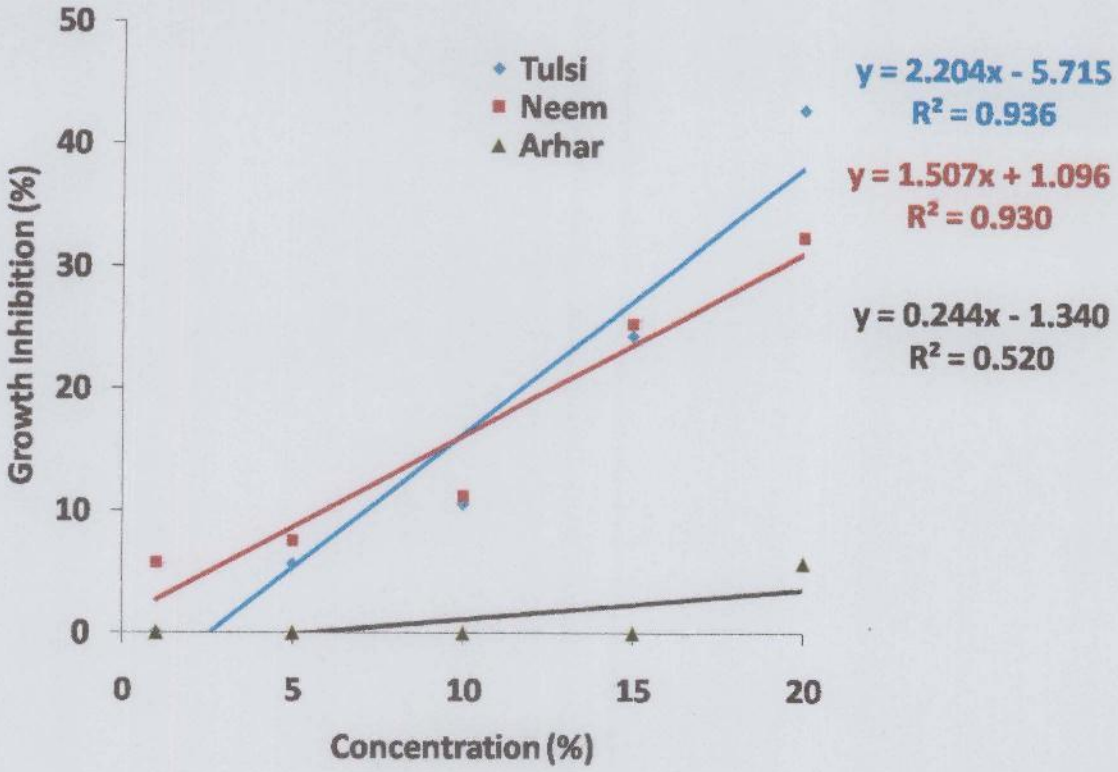

Figure 1. Functional Relationship between concentration and mycelial growth inhibition percentage of *Trichoderma harzianum*

4.2 Colony characteristics of *Trichoderma harzianum* at different concentration of leaf extracts containing media

Colony characteristics of *Trichoderma harzianum* at Tulsi leaf extract

Mycelial growth and colony characteristics of *Trichoderma harzianum* varied from control plate and there was variation among different concentrations of tulsi leaf extracts. Mycelial growth was suppressed at 1%, 15% and 20% concentration and cottony at 5% and 10% concentration. Colony texture was loose at 1%, 5%, 15% and 20% concentration but compact at 10% concentration. Variation also found in marginal characteristics. Regular margin were found at 1% but irregular margin were at 5%, 10%, 15% and 20% concentration. Whitish mycelium was observed in all concentration of tulsi leaf extracts (Plate 1).

Colony characteristics of *Trichoderma harzianum* at Neem leaf extract

No variation was observed in mycelial growth of *Trichoderma harzianum* at different concentrations but there was variation among the colony characteristics. Mycelial growth was cottony at different concentrations of neem leaf extracts. Colony texture was compact at 1% concentration and loose at 5%, 10%, 15% and 20% concentration. Irregular margin was reported from 1% and 10% concentration but regular margin was at 5%, 15% and 20% concentration. Mycelium color was whitish in all concentration of neem leaf extracts (Plate 2).

Colony characteristics of *Trichoderma harzianum* at Arhar leaf extract

There was no variation in mycelial growth of *Trichoderma harzianum* at different concentrations except colony characteristics. Mycelial growth was cottony at different concentrations of arhar leaf extract. Colony texture was compact at 1%, 5%, 10% concentration and loose at 15% and 20% concentration. Variations found in marginal characteristics. Irregular margin was found at 1% and 15% concentration and regular margin was found at 5%, 10% and 20% concentration. Greenish mycelium were found in all concentration of arhar leaf extracts (Plate 3).

Colony characteristics of *Trichoderma harzianum* at Durba leaf extract

There was no variation in mycelial characteristics and colony characteristics of *Trichoderma harzianum* at different concentrations. Cottony mycelial characteristics, regular margin, loose texture were reported at different concentrations of durba leaf extract containing media. Mycelial color was whitish in all concentration of durba leaf extracts (Plate 4).

Table. 3 Colony characteristics of different medicinal plant extracts on *Trichoderma harzianum* at different concentrations

Treatment	Concentration%	Colony Characteristics			
		Mycelial growth	Margin	Texture	Color
Tulsi	Control	Cottony	Regular	Compact	Whitish
	1	Suppressed	Regular	Loose	Whitish
	5	Cottony	Irregular	Loose	Whitish
	10	Cottony	Irregular	Compact	Whitish
	15	Suppressed	Irregular	Loose	Whitish
	20	Suppressed	Irregular	Loose	Whitish
Neem	1	Cottony	Irregular	Compact	Whitish
	5	Cottony	Regular	Loose	Whitish
	10	Cottony	Irregular	Loose	Whitish
	15	Cottony	Regular	Loose	Whitish
	20	Cottony	Irregular	Loose	Whitish
Arhar	1	Cottony	Irregular	Compact	Greenish
	5	Cottony	Regular	Compact	Greenish
	10	Cottony	Regular	Compact	Greenish
	15	Cottony	Irregular	Loose	Greenish
	20	Cottony	Regular	Loose	Greenish
Durba	1	Cottony	Regular	Loose	Whitish
	5	Cottony	Regular	Loose	Whitish
	10	Cottony	Regular	Loose	Whitish
	15	Cottony	Regular	Loose	Whitish
	20	Cottony	Regular	Loose	Whitish

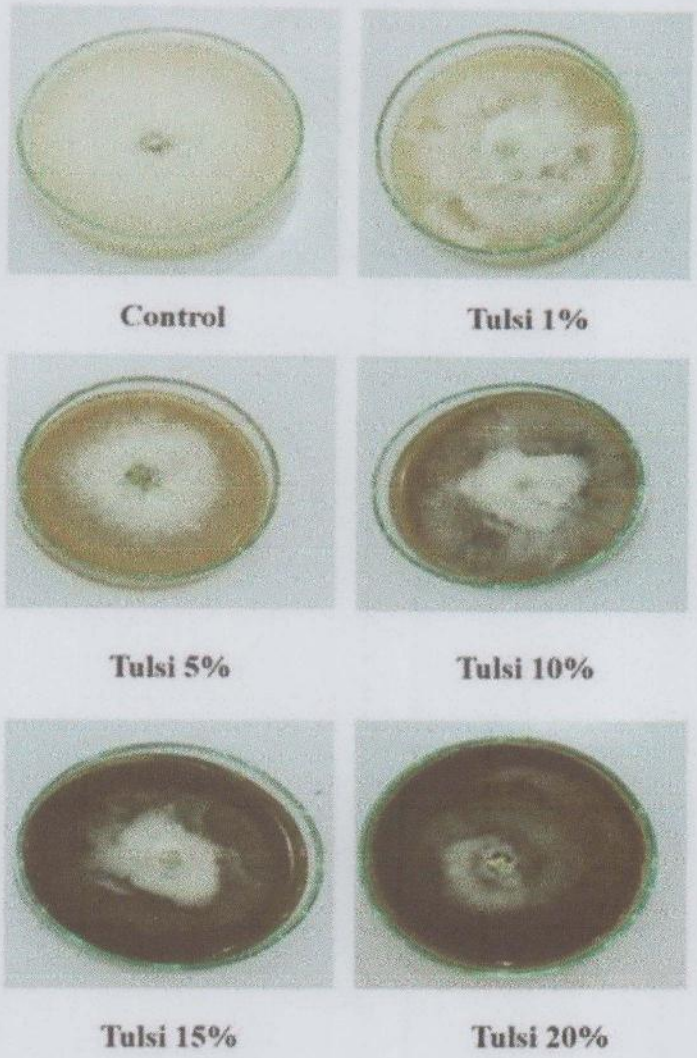

Plate 1. Mycelial growth of *Trichoderma harzianum* at different concentration of tulsi leaf extracts

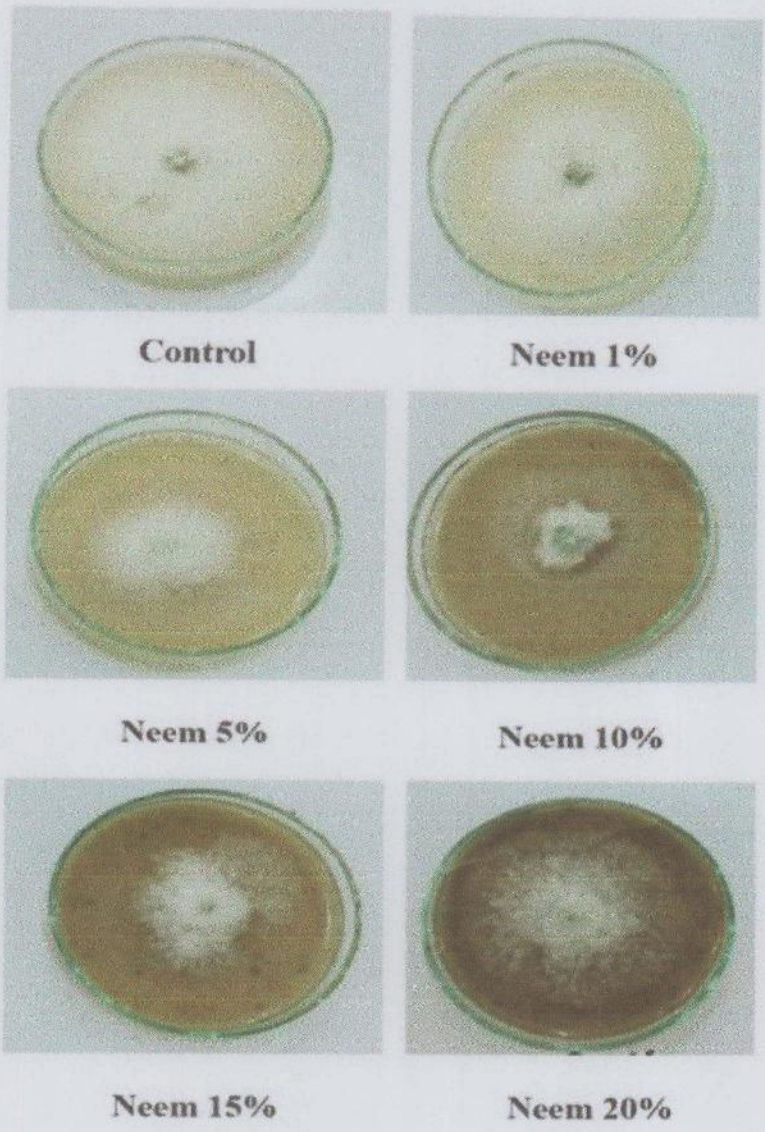

Plate 2. Mycelial growth of *Trichoderma harzianum* at different concentration of neem leaf extracts

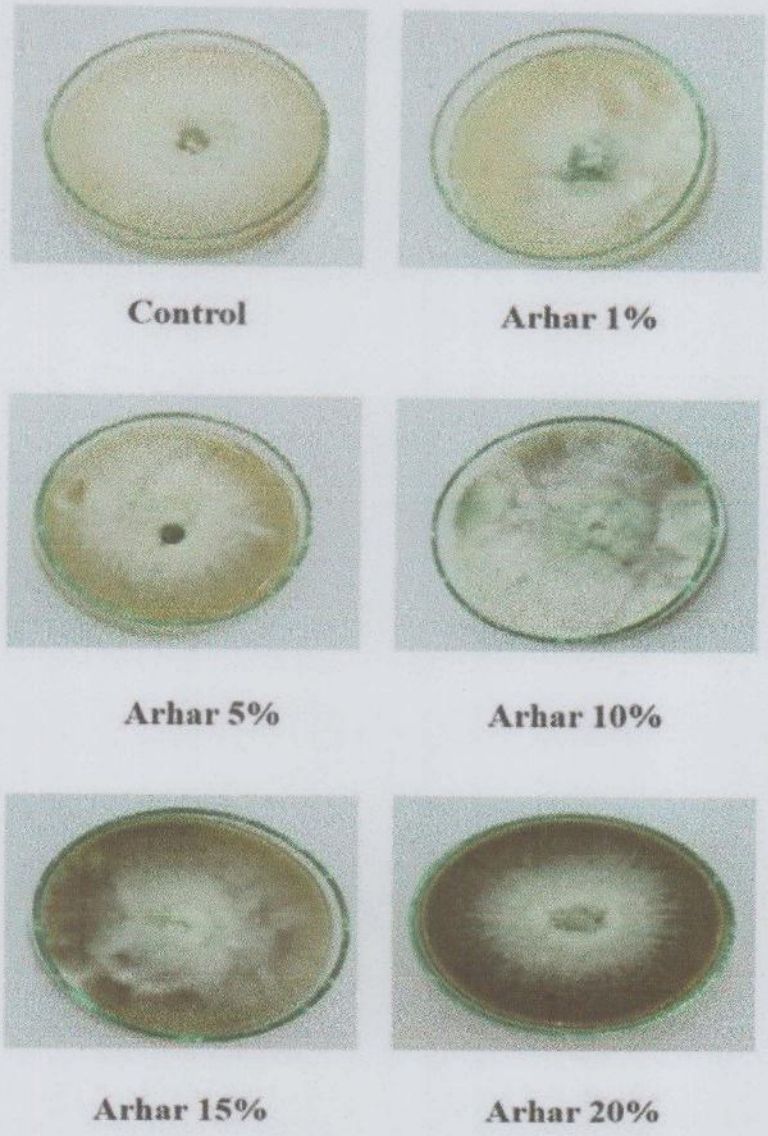

Plate 3. Mycelial growth of *Trichoderma harzianum* at different concentration of arhar leaf extracts

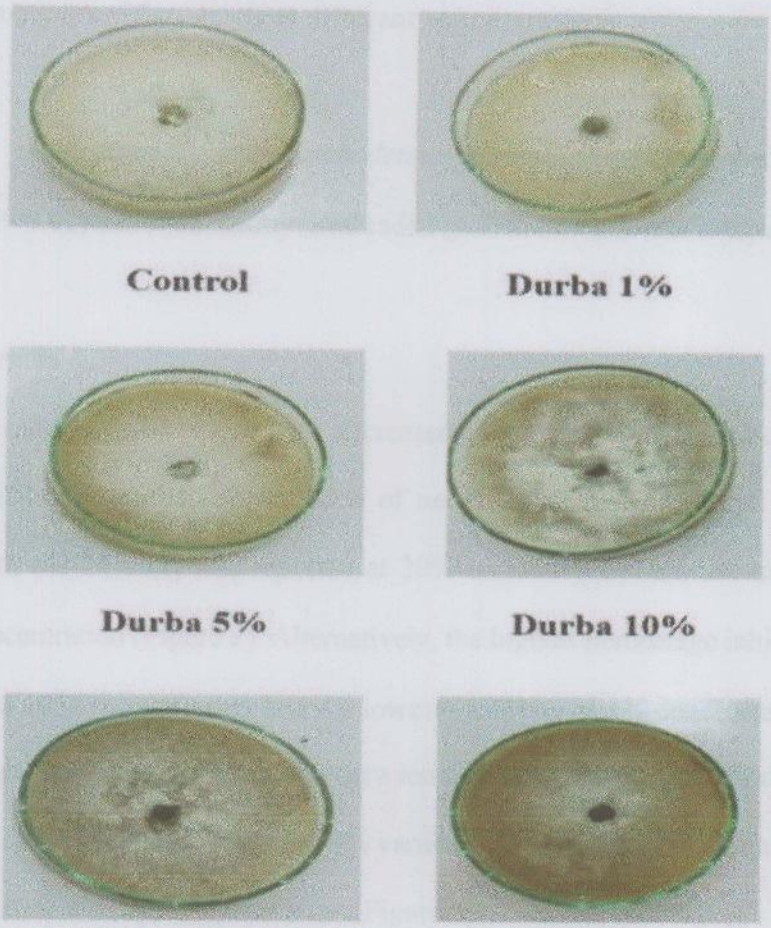

Plate 4. Mycelial growth of *Trichoderma harzianum* at different concentration of durba leaf extracts

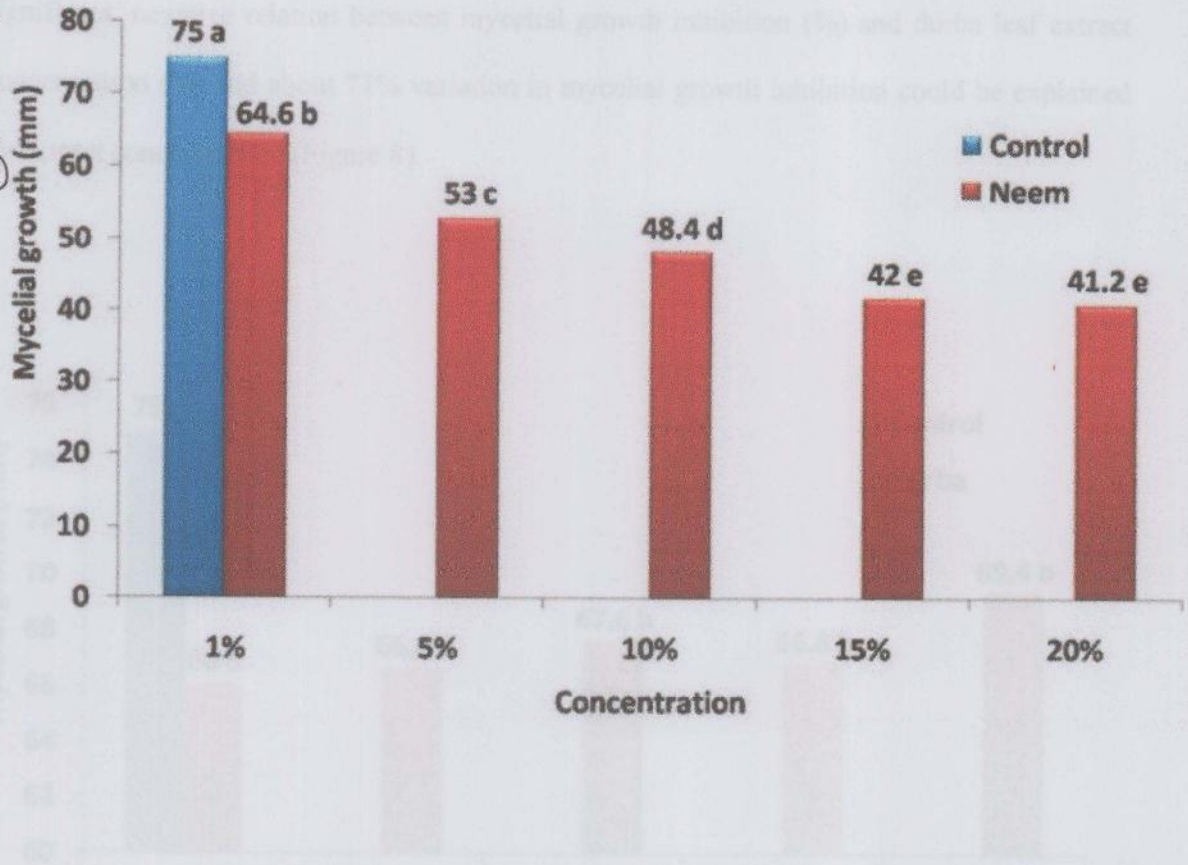

Figure 2. Effect of different concentrations of neem leaf extracts on mycelial growth of *Fusarium oxysporum f.sp. lentis*

Tulsi leaf extracts

Fungal mycelial radial growth in the tulsi leaf extracts containing media reduced significantly and inhibition percentage increased significantly as the concentration increased. The media containing 20% concentration produced minimum (42.60 mm) fungal growth whereas, 1% concentration provided maximum (64.00 mm) mycelial growth which was statistically similar to 10% concentration (62.00 mm) (Figure 4). Alternatively, the highest inhibition percentage (43.16%) was found at 20% concentration and the lowest percentage inhibition (14.66%) was at 1% concentration. There was a significant, positive relation between mycelial growth inhibition (%) and tulsi leaf extract concentration (%) and about 81% variation in mycelial growth inhibition could be explained by extract concentration (Figure 8).

The result was in favor to the findings of earlier workers Joseph et al. (2008) who studied that *Ocimum sanctum* was effective against *Fusarium solani* f. sp. *melongenae* causing brinjal wilt. On the other hand, Mengane and Kamble found extracts of *Ocimum sanctum* (20%) least effective in inhibition of growth (1.91 cm) of *Fusarium Oxysporum f.sp. Cubense*.

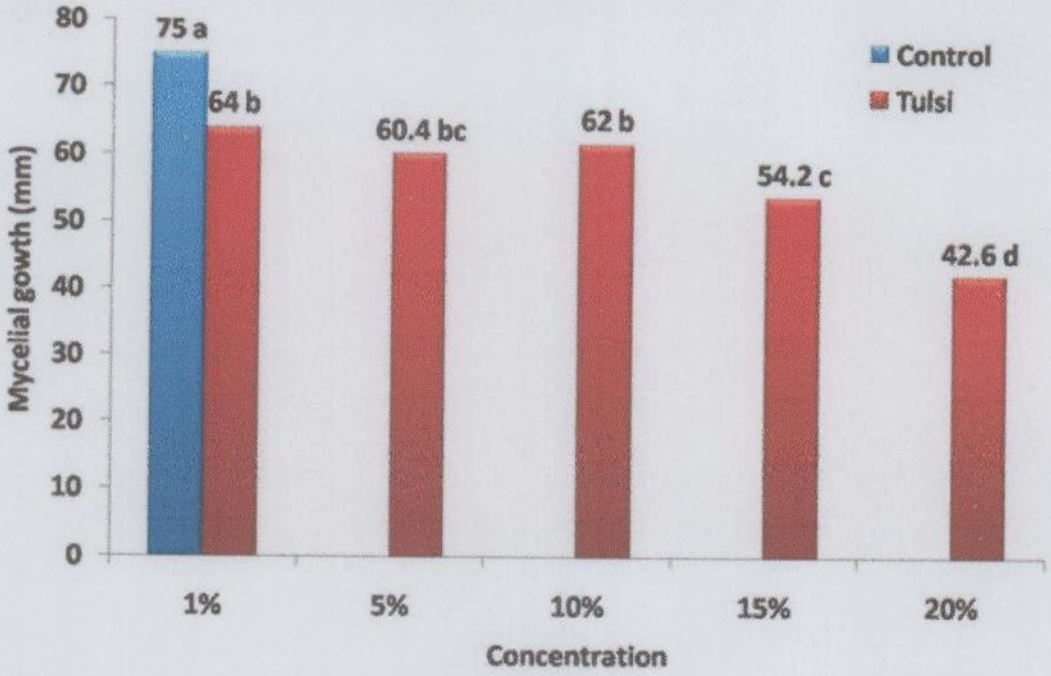

Figure 4. Effect of different concentrations of tulsi leaf extracts on mycelial growth of *Fusarium oxysporum f.sp. lentis*

Arhar leaf extracts

Mycelial growth of fungus and inhibition percentage was more or less statistically similar at 1% to 20% concentration of arhar leaf extracts. As such, the maximum (67.80 mm) radial mycelial growth was observed at 15% concentration and minimum (60.60 mm) at 1% concentration (Figure 5). On the other hand, the highest percentage inhibition (18.62%) was reported at 20% concentration which was statistically similar to 1% and 10% concentration (Table 7). There was a significant, positive relation between mycelial growth inhibition (%)

and arhar leaf extract concentration and about 12% variation in mycelial growth inhibition could be explained by extract concentration (Figure 8).

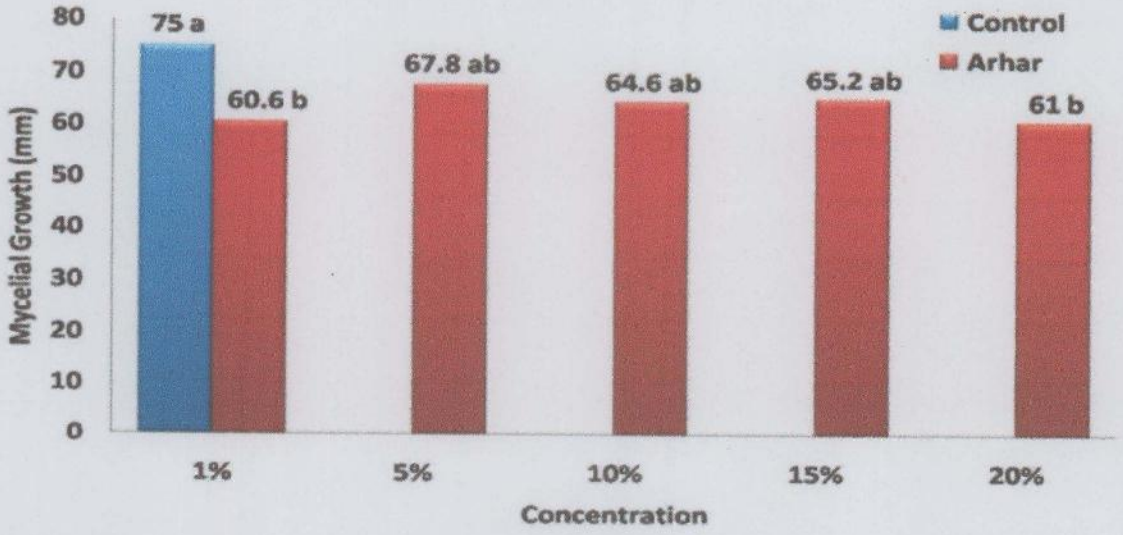

Figure 5. Effect of different concentrations of arhar leaf extracts on mycelial growth of *Fusarium oxysporum f.sp. lentis*

Trichoderma suspension

The radial mycelial growth in *Trichoderma* containing media declined significantly and inhibition percentage increased significantly as the increasing of media concentration. *Trichoderma* suspension rendered statistically similar minimum mycelial growth (15.4 mm and 15.8 mm) at 15% and 20% concentration, respectively. Whereas, mycelial growth was maximum (31.00 mm) at 1% concentration of media (Fig. 6). On the contrary, the highest inhibition percentage (79.44%) was observed at 15% concentration which was statistically similar to 20% concentration (78.92%) and the lowest percentage inhibition (58.66%) was at 1% concentration (Figure 8). There is a significant, positive relation between mycelial growth inhibition (%) and *Trichoderma* suspension and about 92% variation in mycelial growth inhibition could be explained by extract concentration (Fig. 8).

Gwa and Nwankiti (2017) recorded highest percentage growth inhibition of mycelia of the pathogen (77.99%) which was similar to our study. Cherkupally and Amballa (2017) also reported that *T. harzianum* showed maximum extent of inhibition 81.11%.

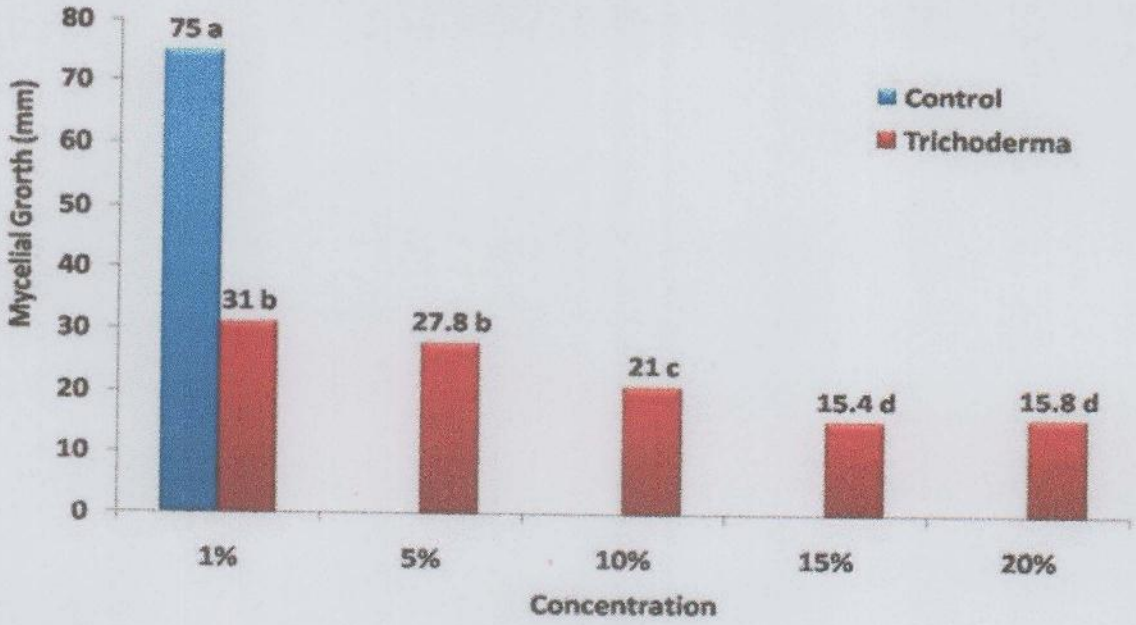

Figure 6. Effect of different concentrations of *Trichoderma* suspension on mycelial growth of *Fusarium oxysporum f.sp. lentis*

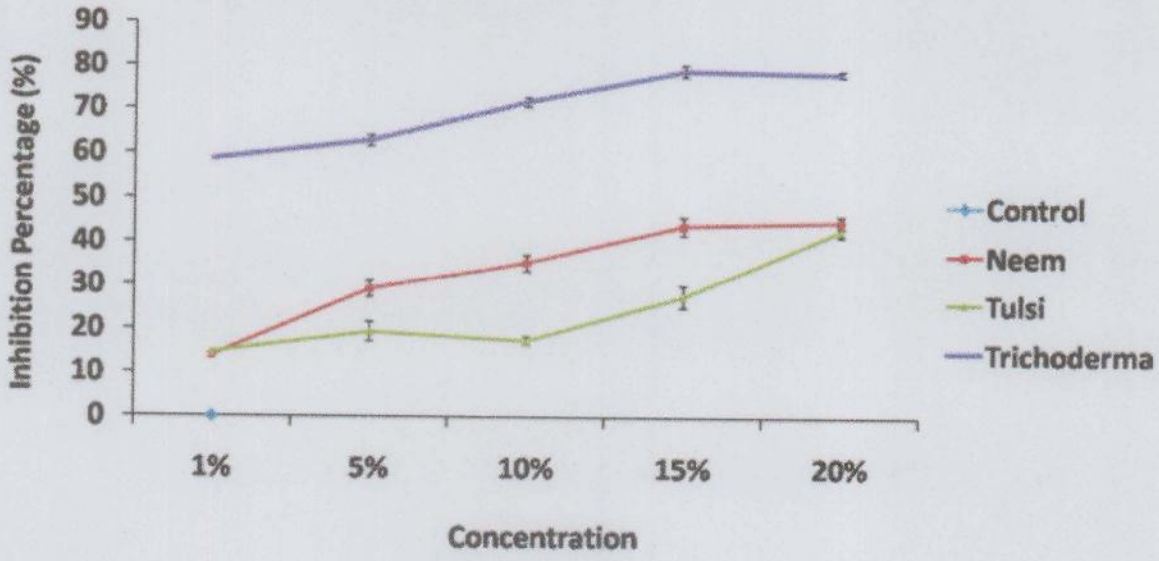

Figure 7. Effect of neem and tulsi leaf extracts and *Trichoderma* suspension at different concentration on growth inhibition of *Fusarium oxysporum f.sp. lentis*

Table 4. Effect of durba and arhar leaf extracts at different concentration on growth inhibition of *Fusarium oxysporum f.sp. lentis*

Plant extracts	Percentage inhibition at different concentration (Transformed data)				
	1%	5%	10%	15%	20%
Control			-		
			(0.00)		
Durba	11.96 a	11.18 a	9.84 a	10.92 a	7.42 a
	(0.12)	(0.11)	(0.10)	(0.11)	(0.07)
Arhar	16.78 a	9.56 ab	13.82 a	13.02 ab	18.62 a
	(0.17)	(0.10)	(0.14)	(0.13)	(0.19)
Level of significance			0.01		

The parenthesis data is transformed (Square root and Arcsine transformation) data

Among all the treatments, the control plate showed maximum mycelial growth while minimum mycelial growth was reported from *Trichoderma* suspension at 15% concentration (15.40 mm) which was statistically similar to 20% concentration (Table 6). Furthermore, *Trichoderma* suspension inhibited the radial mycelial growth of *Fusarium oxysporum* significantly at 15% and 20% concentration in comparison to other botanical extracts. To summarize, the media containing 15% and 20% concentration of *Trichoderma* provided the best performance with 79.44% radial mycelial growth inhibition of the target fungus. Whereas, the control plate represented no mycelial growth inhibition (Table 6).

Table 5. Effect of plant extracts and *Trichoderma* suspension at different concentration on growth inhibition of *Fusarium oxysporum f.sp. lentis*

Plant extracts	Percentage inhibition at different concentration (Transformed data)				
	1%	5%	10%	15%	20%
Control			-		
Neem	13.84 hi	29.30 ef	35.44 de	43.98 d	45.04 d
	(0.14)	(0.30)	(0.36)	(0.46)	(0.47)
Durba	11.96 hi	11.18 hi	9.84 hij	10.92 hi	7.42 ij
	(0.12)	(0.11)	(0.10)	(0.11)	(0.07)
Tulsi	14.66 hi	19.44 fgh	17.32 ghi	27.68 efg	43.16 d
	(0.15)	(0.20)	(0.17)	(0.28)	(0.45)
Arhar	16.78 hi	9.56 hij	13.82 hi	13.02 hi	18.62 gh
	(0.17)	(0.10)	(0.14)	(0.13)	(0.19)
Trichoderma suspension	58.66 c	62.88 c	71.96 b	79.44 a	78.92 ab
	(0.63)	(0.68)	(0.80)	(0.92)	(0.91)
Level of significance			0.01		
CV%			12.90		

The parenthesis data is transformed (Square root and Arcsine transformation) data

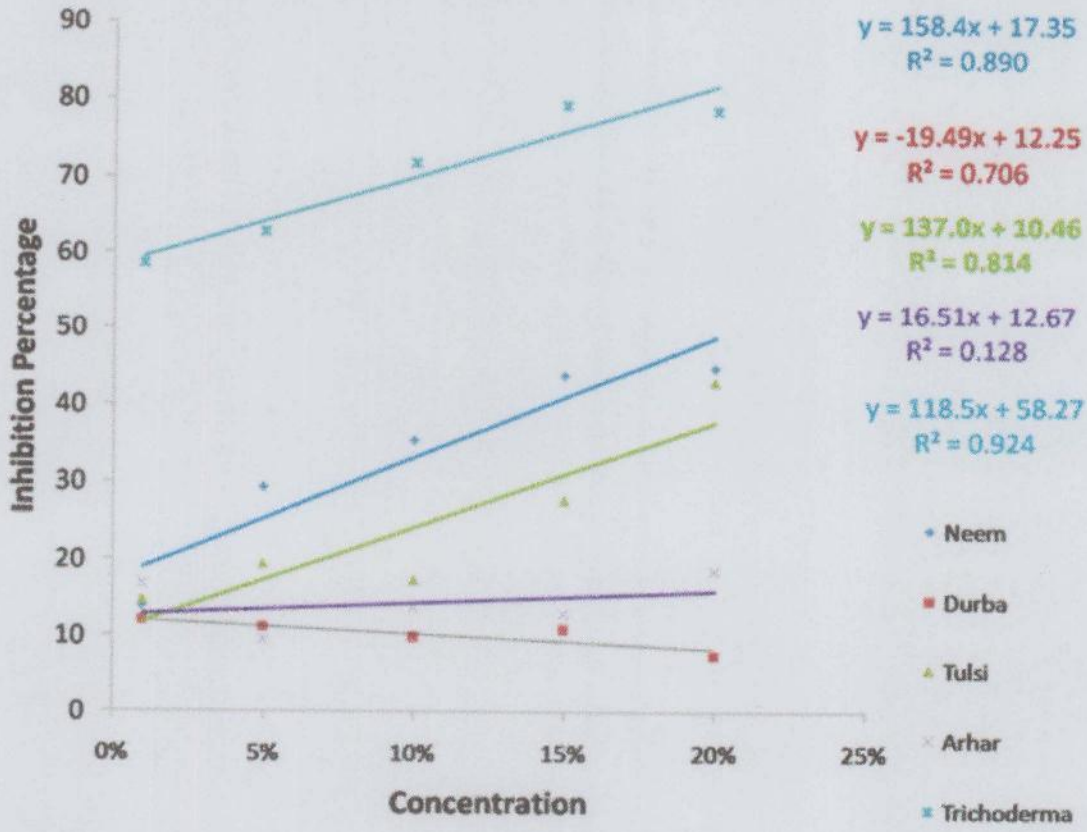

Figure 8. Functional Relationship between concentration and mycelial growth inhibition percentage *Fusarium oxysporum f.sp. lentis*

4.4 Effect of plant extracts and *Trichoderma* suspension at different concentration on Colony characteristics of *Fusarium oxysporum f.sp. lentis*

Colony characteristics at Neem leaf extract

Mycelial characteristics and colony characteristics of *Fusarium oxysporum f.sp. lentis* varied from control plate but there was no variation among different concentrations of neem leaf extracts. Cottony-fluffy mycelial growth, circular-regular margin, compact texture, whitish upper color and pinkish lower color were observed at different concentrations (Plate 5).

Colony characteristics at Durba leaf extract

There was no variation in mycelial characteristics and colony characteristics *of Fusarium oxysporum f.sp. lentis* at different concentrations except texture. Here, mycelial gowth was cottony, margin was circular and regular, texture was merged at 1%, 5%, 10% and 15% concentration but it was loose at 20% concentration while, whitish upper color and pinkish lower color was observed at different concentrations (Plate 6).

Colony characteristics at Tulsi leaf extract

Mycelial characteristics of *Fusarium oxysporum f.sp. lentis* differed at different concentrations but there was no variation among colony characteristics. In this case, mycelial growth was cottony at 1%, 5% and 10% concentration but cottony-fluffy at 15% and 20% concentration. Moveover, circular-regular margin, loose texture, whitish upper color and blakish lower color were observed at different concentration of tulsi leaf extract ((Plate 7)

Colony characteristics at Arhar leaf extract

There was no variation in mycelial characteristics and colony characteristics of *Fusarium oxysporum f.sp. lentis* at different concentrations. Cottony mycelial characteristics, circular-regular margin, loose texture, whitish upper color and pinkish lower color were reported at different concentrations of arhar leaf extract containing media (Plate 8).

Colony characteristics at *Trichoderma* suspension

There was no variation in colony characteristics of *Fusarium oxysporum f.sp. lentis* at different concentrations except mycelial characteristics and texture. Mycelial growth was cottony at 1% and 5% concentration but cottony-fluffy at 10%, 15% and 20% concentration. In addition, compact texture was reported at 1%, 5%, 10% and 20% concentration but merged texture at 15% concentration. Furthermore, circular-regular margin, whitish upper color and blakish lower color were observed at different concentration (Plate 9).

Table 6. Effect of different treatments on colony characteristics of *Fusarium oxysporum f.sp. lentis* at different concentrations

Treatment	Concentration %	Colony characteristics				
		Mycelial characteristics	Margin	Texture	Upper color	Lower color
Neem	Control	Suppressed	Circular, regular	Loose	Pinkish	Pinkish
	1%	Cottony, Fluffy	Circular, regular	Compact	Whitish	Pinkish
	5%	Cottony, Fluffy	Circular, regular	Compact	Whitish	Pinkish
	10%	Cottony, Fluffy	Circular, regular	Compact	Whitish	Pinkish
	15%	Cottony, Fluffy	Circular, regular	Compact	Whitish	Pinkish
	20%	Cottony, Fluffy	Circular, regular	Compact	Whitish	Pinkish
Durba	1%	Cottony	Circular, Regular	Merged	Whitish	Pinkish
	5%	Cottony	Circular, Regular	Merged	Whitish	Pinkish
	10%	Cottony	Circular, Regular	Merged	Whitish	Pinkish
	15%	Cottony	Circular, Regular	Merged	Whitish	Blackish
	20%	Cottony	Circular, Regular	Loose	Whitish	Pinkish
Tulsi	1%	Cottony	Circular, Regular	Loose	Whitish	Blackish
	5%	Cottony	Circular, Regular	Loose	Whitish	Blackish
	10%	Cottony	Circular, Regular	Loose	Whitish	Blackish
	15%	Cottony, Fluffy	Circular, Regular	Loose	Whitish	Blackish
	20%	Cottony, Fluffy	Circular, Regular	Loose	Whitish	Blackish
Arhar	1%	Cottony	Circular, Regular	Loose	Whitish	Pinkish
	5%	Cottony	Circular, Regular	Loose	Whitish	Pinkish
	10%	Cottony	Circular, Regular	Loose	Whitish	Pinkish
	15%	Cottony	Circular, Regular	Loose	Whitish	Pinkish
	20%	Cottony	Circular, Regular	Loose	Whitish	Pinkish
Trichoderma suspension	1%	Cottony	Circular, Regular	Compact	Whitish	Blackish
	5%	Cottony	Circular, Regular	Compact	Whitish	Blackish
	10%	Cottony, Fluffy	Circular, Regular	Compact	Whitish	Blackish
	15%	Cottony, Fluffy	Circular, Regular	Merged	Whitish	Blackish
	20%	Cottony, Fluffy	Circular, Regular	Compact	Whitish	Blackish

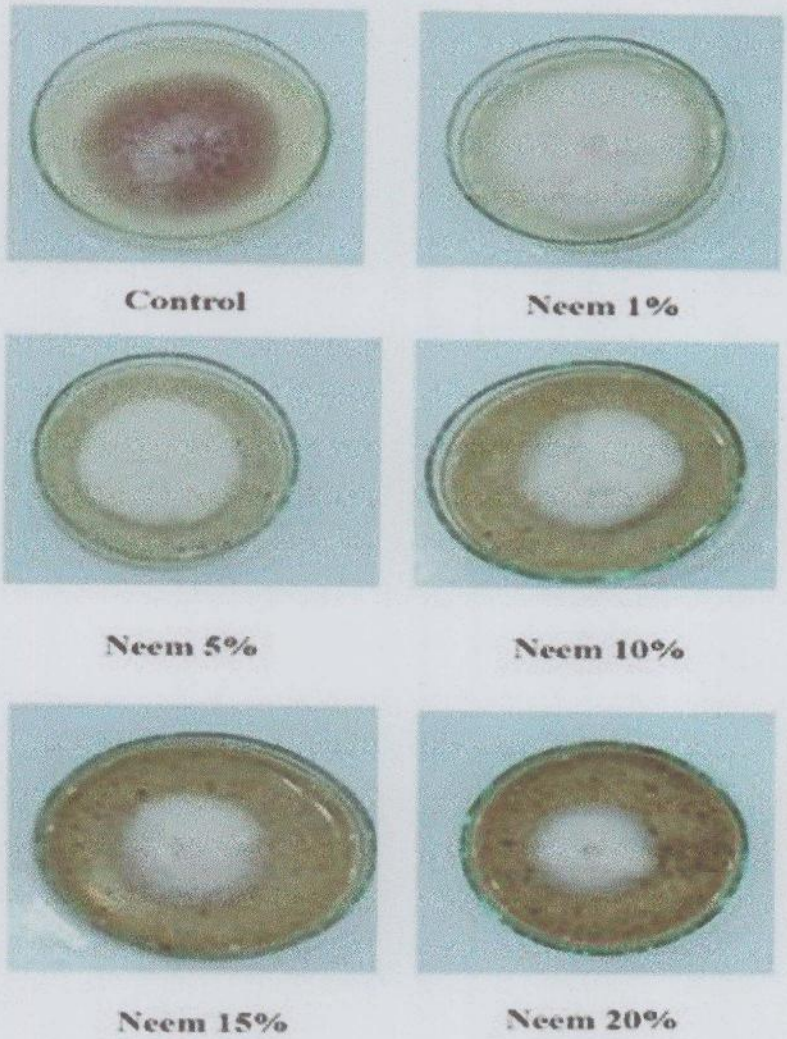

Plate 5. Mycelial growth of *Fusarium oxysporum f. sp lentis* at different concentration of neem leaf extracts

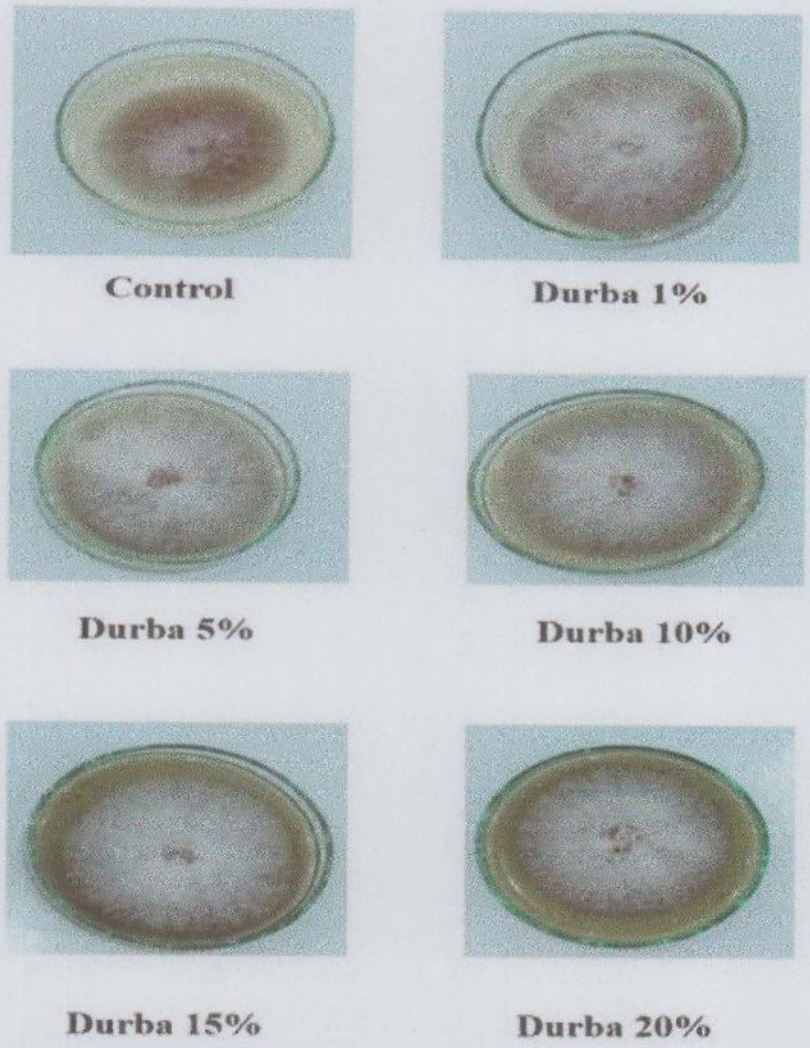

Plate 6. Mycelial growth of *Fusarium oxysporum f. sp lentis* at different concentration of durba leaf extracts

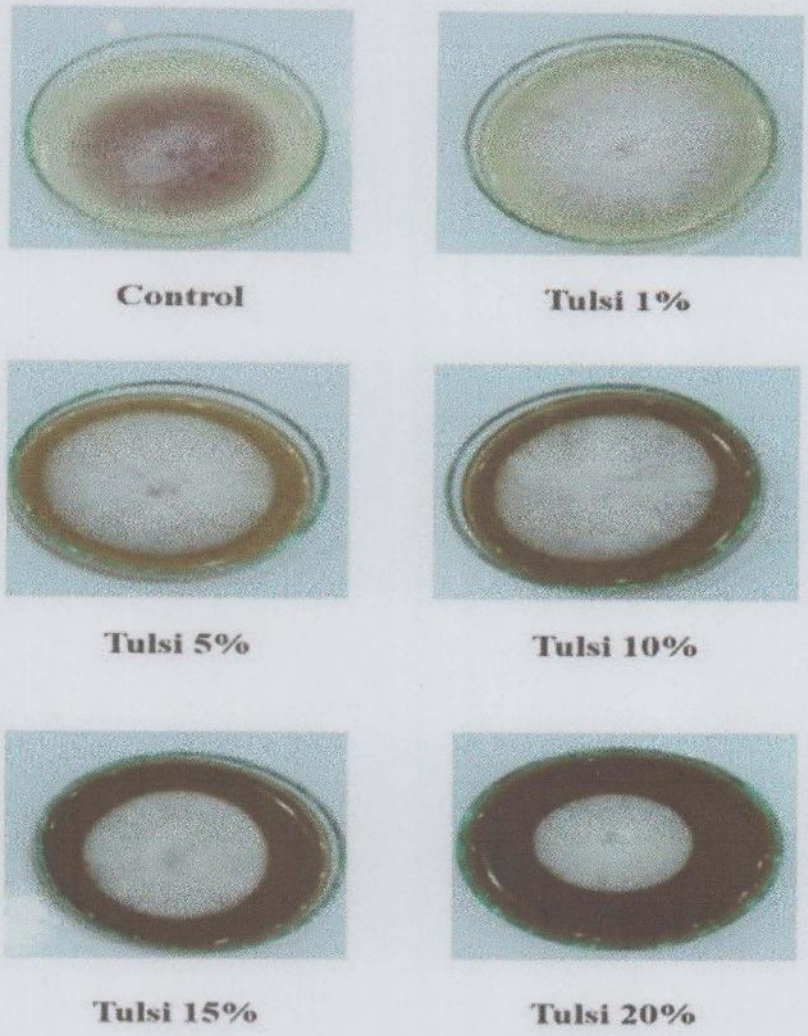

Plate 7. Mycelial growth of *Fusarium oxysporum f. sp lentis* at different concentration of tulsi leaf extracts

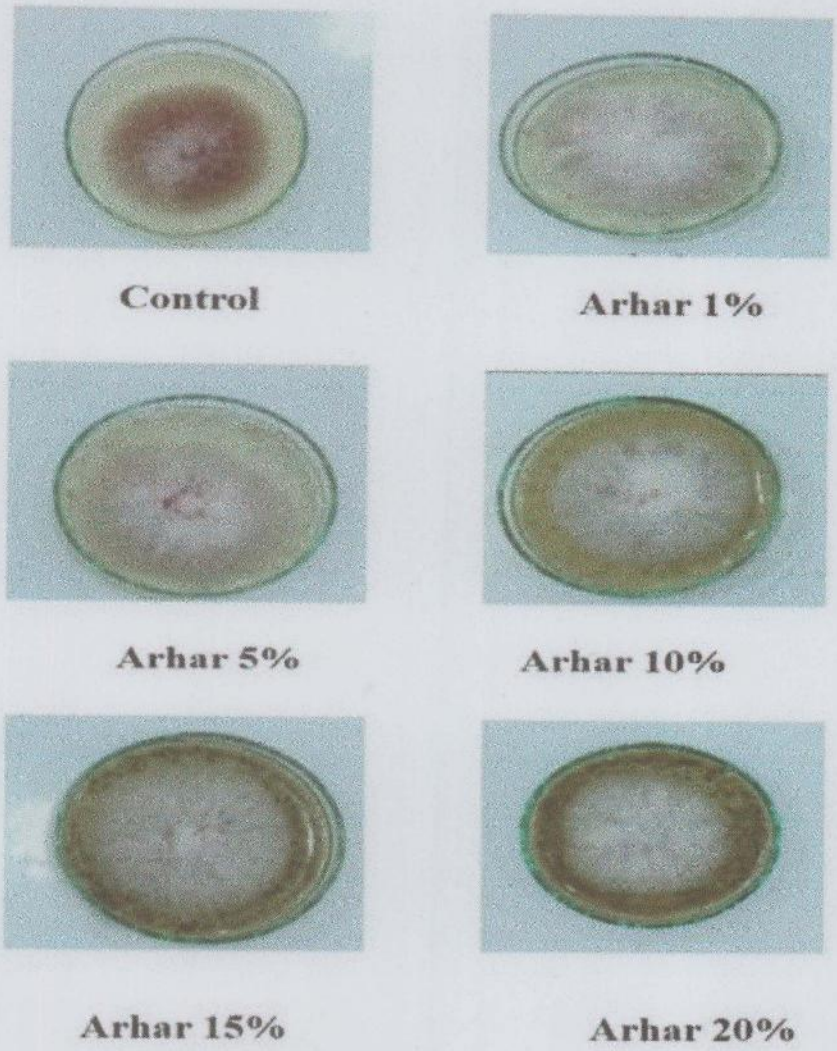

Plate 8. Mycelial growth of *Fusarium oxysporum f. sp lentis* at different concentration of arhar leaf extracts

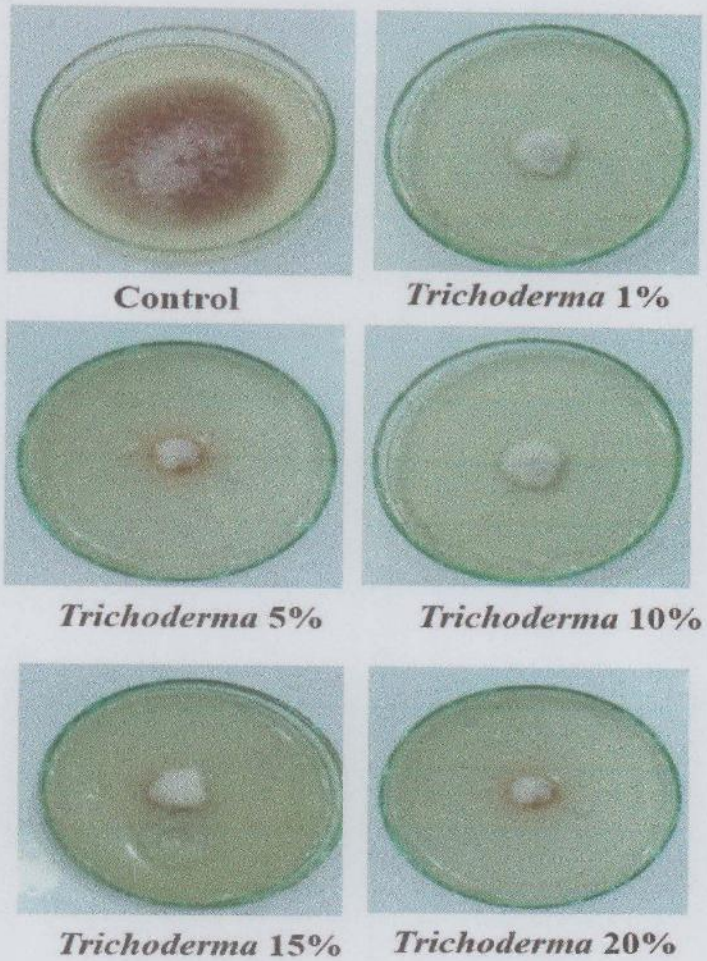

Plate 9. Mycelial growth of *Fusarium oxysporum f. sp lentis* at different concentration of *Trichoderma* suspension

4.5 Discussion

In the present study, it was evident from the first experiment that neem and tulsi leaf extracts at higher concentration (20%) showed incompatibility with *Trichoderma harzianum*. It may be due to the antimicrobial properties of neem and tulsi.

Neem leaves possessed good antibacterial activity, confirming the great potential of bio active compounds (Saradha and Subbarao, 2011). It shows antimicrobial role through inhibitory effect on microbial growth or potentiality of cell wall breakdown. The antibacterial activity of neem might be due to presence of tri-terpenoids, phenolic compounds, carotenoids, steroids, ketones and tetra-triterpenoids (Sairam et al., 2000).

Upasana et al. (2002) found that neem leaf extract was effective against *Aspergillus niger*, *Fusarium oxysporum* and *Trichoderma resii* and both dried and fresh organic extracts from leaves were effective only against *Trichoderma resii.* Another finding showed the antimicrobial role of aqueous extracts of neem leaf extracts in the inhibition of spore germination against several sporulating fungi (Kumari et al., 2013). The complete inhibition (100%) in the growth of *A. niger* obtained in assay with 20% concentration of aqueous leaf extract of neem (Bohra and Purohit, 2002).

Tulsi has long been recognized as possessing antimicrobial properties and potentially contains bioactive components. The main bioactive components are: alpha pinene, borneol, isoborenol, camphene, beta pinene, octen 3 ol, eugenol, estragol, myrcene etc. Mono terpenes, especially eugenol (23.7%) and estragole (9.6%) are present in the highest amounts in the leaf extracts (Yamani et al., 2016).

Vishwabhan et al. (2011) proposed the antimicrobial activity of tulsi by virtue of its essential oil content. Tulsi leaves extract at a concentration of 0.5%, 1%, and 2% showed a minimal zone of inhibition. However, tulsi displayed wide inhibition zones at 5% and 10% (Mallikarjun et al., 2016). The boiled extract was found significantly superior over other forms for inhibiting the growth of *R. solani, Rhizoctonia bataticola, Sclerotium rolfsii, Alternaria solani* and *A. alternata.* The growth of the species of *Fusarium oxysporum f.sp. ciceri,* and *Fusarium oxysporium f.sp. pallidoroseum* was more effectively inhibited under its crude form. (Sharma et al., 2019). For this reason neem and tulsi significantly inhibited the mycelial growth of *Trichoderma harzianum* at highert concentrations. As they have the antifungal properties to control any kind of living fungus which makes them superior than any other leaf extracts used in in vitro condition. As *Trichoderma harzianum* is an effective bio-control agent and control soil borne pathogenic fungi successfully residing in the soil. So, simultaneous application of this fungus with neem and tulsi leaf extract at higher concentration may interact with each others' activity.

On the contrary, durba and arhar leaf extract showed compatibility with *Trichoderma harzianum* and stimulated the growth of this fungus. The stimulatory effect of the autoclaved extracts indicated the availability of nutrients without active antifungal substances (Yasmin et al., 2008). The stimulatory effects of plant extracts on various pathogens are also reported (Agarwal 1978, Saha 1997 and Hossain 1993).

In the second study, we found that *Trichoderma* suspension was effective in comparison to other medicinal plant extracts against *Fusarium oxysporum f sp. lentis. Trichoderma* species are known to be effective antagonists of several plant pathogens. *Trichoderma* species have been increasingly used as biological control agents and a few of the isolates are commercially

available (Henis et al., 1984). Isolates of *Trichoderma* spp. can produce lytic enzymes (Haran et al., 1996). *Trichoderma* species are known to produce a number of antibiotics, such as trichodermin, trichodermol, harzianum A and harzianolide (Simon and Sivasithamparam, 1988). *T. harzianum* also produce antifungal compounds viz, azaphilone and butenolide that show marked in vitro inhibition of *R. solani* (Vinale et al., 2006). Grondona et al (1997) reported on the antifungal activity of *T. harzianum* against soil borne pathogens with 50% inhibition on all pathogens.

From compatibility test *Trichoderma* revealed compatibility with arhar and durba leaf extracts and these leaf extracts at higher concentration also inhibited *Fusarium oxysporum f sp. lentis.* So these botanicals can be applied simultaneously.

Colony characteristics

Fusarium oxysporum f. sp. lentis exhibited variations in colony characteristics such as mycelial growth, color, margin and texture. Mycelial growth was cottony and fluffy. Colony colors varied from pinkish, whitish and blackish. Colony margins were circular and regular. Colony textures were loose and compact. Similarly, Saxena and Singh (1987) recorded various types of pigmentations (yellow, brown, crimson) in culture of the pathogen. Chauhan (1962) found variation with respect to their mycelium type, colony colour, toxin production and pathogenicity of *Fusarium oxysporum. Fusarium* isolates were highly variable in their colony growth pattern, size of colony and pigmentations. The current findings were well supported by Dubey et al. (2010).

CHAPTER V
SUMMARY AND CONCLUSIONS

Two experiments were conducted in the Plant Protection Laboratory of Agrotechnology Discipline, Khulna University, Khulna. Firstly the compatibility of *Trichoderma harzianum* with four different botanicals (neem, tulsi, arhar, durba leaf extracts) at five concentrations (1%, 5%, 10%, 15% and 20%) was evaluated. Then the effectiveness of *Trichoderma harzianum* suspension along with these four botanicals was evaluated against a pathogenic fungi *Fusarium oxysporum f. sp. lentis* the causal agent of *Fusarium* wilt of lentil. The experiment was conducted following Completely Randomized Design (CRD) with five replications. Radial mycelial growth and colony characteristics were recorded and mycelial growth inhibition percentage was calculated on the basis of measured mycelial growth.

Trichoderma harzianum revealed compatibility with neem and tulsi leaf extracts at lower concentration (1% to 10%) and arhar and durba leaf extracts at all the concentrations. The minimum mycelial growth (45.5 mm) and the highest inhibition percentage (43.12) was reported from tulsi leaf extract at 20% concentration.

In case of *Fusarium oxysporum f. sp. lentis*, all the treatments at different concentration significantly inhibited the mycelial growth of the pathogen ($p<0.01$). Where neem leaf extracts and tulsi leaf extracts revealed the highest percentage inhibition (45.04% and 43.16%, respectively) at 20% concentration and the lowest (13.84% and 14.66%, respectively) at 1% concentration. Arhar leaf extracts also provided the highest percentage inhibition (18.62%) at 20% concentration but the lowest percentage inhibition (9.56%) at 5% concentration.

Furthermore, *Trichoderma* suspension revealed the highest inhibition percentage (79.44%) at 15% concentration which was statistically similar to 20% concentration (78.92%) and the lowest percentage inhibition (58.66%) at 1% concentration. On the contrary, durba leaf extracts represented the highest percentage inhibition (11.96%) at 1% concentration and the lowest (7.42%) at 20% concentration.

Among all the treatments, *Trichoderma* suspension inhibited the radial mycelial growth of *Fusarium oxysporum f.sp lentis* significantly at 15% and 20% concentration in comparison to other bio-agents. Furthermore, the media containing 15% and 20% concentration of *Trichoderma* suspension provided the best performance with 79.44% radial mycelial growth inhibition of the target fungus. Whereas, the control plate represented no mycelial growth inhibition.

Regression equation between different concentrations of all the treatments and mycelial growth inhibition percentage of both the fungi revealed positive functional relationship except in case of durba leaf extract.

From the above result of this experiment it can be concluded that:

- *Trichoderma harzianum* revealed compatibility with arhar and durba leaf extracts at all concentration
- On the contrary, *Trichoderma harzianum* exhibited incompatibility with neem and tulsi leaf extract at 20% concentration
- Among all the botanicals *Trichoderma* suspension was very much effective against *Fusarium oxysporum f. sp. lentis* at 15% and 20%
- Neem and tulsi extracts inhibited the fungal growth at higher concentration
- Positive significant functional relationship was observed between inhibition (%) and concentration in all cases except durba.

CHAPTER VI

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