EVALUATION OF *PURPUREOCILLIUM LILACINUM* AND ARBUSCULAR MYCORRHIZAL FUNGUS (AMF) ON PLANT GROWTH AND CONTROL OF EGGPLANT WILT IN ARSENIC CONTAMINATED SOIL

BY

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DHAKA-1207

DECEMBER, 2015

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BY

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Registration No. 10-03862

A Thesis
Submitted to the Faculty of Agriculture,
Sher-e-Bangla Agricultural University, Dhaka,
In partial fulfillment of the requirements
For the degree of

MASTER OF SCIENCE
IN
PLANT PATHOLOGY
SEMESTER: JULY-DECEMBER, 2015

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CERTIFICATE

This is to certify that the thesis entitled, **"EVALUATION OF *PURPUREOCILLIUM LILACINUM* AND ARBUSCULAR MYCORRHIZAL FUNGUS (AMF) ON PLANT GROWTH AND CONTROL OF EGGPLANT WILT IN ARSENIC CONTAMINATED SOIL"** submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in the partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (M. S.) IN PLANT PATHOLOGY,**embodies the result of a piece of bona fide research work carried out by "**MD. GOLAM RASUL"** bearing **Registration No. 10-03862** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: 01.12.2016
Place: Dhaka, Bangladesh

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ACKNOWLEDGEMENT

First of all I would like to thank to Almighty Allah, the most merciful and compassionate. The most gracious and beneficent to whom every praise is due and his Prophet Muhammad (SM) who is forever a Torch of knowledge and guidance for humanity as a whole with whose delighting the present endeavor has been beautiful.

Now I would like to express my earnest respect, sincere appreciation and enormous indebtedness to my reverend supervisor, Dr. F. M. Aminuzzaman, Professor, Department of Plant Pathology, Faculty of Agriculture, Sher-e- Bangla Agricultural University, Dhaka. I am grateful to his ever inspiring guidance, keen interest, helpful commentary, scholastic and constructive suggestions and polite behavior throughout the course of my study.

I wish to express my gratitude and best regards to my respected Co-Supervisor, Dr. Md. Rafiqul Islam, Professor, Department of Plant Pathology, Faculty of Agriculture, Sher-e- Bangla Agricultural University, Dhaka, for his continuous direction, constructive criticism, encouragement and valuable suggestions in carrying out the research work and preparation of this thesis.

I am also highly thankful to Dr. Md. Belal Hossain, AssociateProfessor and Chairman, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for his necessary help and co-operation during the study period.

I am highly grateful to my honorable teachers Prof. Dr. M. Salahuddin M. Chowdhury, Prof. Mrs. Nasim Akhtar, Prof. Nazneen Sultana, Associate Prof. Khadija Akhter, Associate Prof. Abu Noman Faruq Ahmmed,Associate Prof. Dr. Nazmoon Naher Tonu, Assistant Prof. Shukti Rani Chowdhury, Assistant Prof Md. Ziaur Rahman Bhuiya, Sayed Mohammad Mohsin and Hosna Ara Chowdhury Nisha, Lecturer, Department of Plant Pathology, and Professor Dr. Md. Razzab Ali, Department of Entomology, Faculty of Agriculture, Sher-e –Bangla Agricultural University, for their valuable teaching, direct and indirect advice, and encouragement and co-operation during the whole study period.

I am pleased to thank all staffs and workers of Plant Pathology Department of Sher-e-Bangla Agricultural University, Dhaka, for their valuable and sincere help in carrying out the research work.

I also thanks to my friends Sanjida Rahman, Nishat Tasnim, Kallol Das, Marzana Afrose, Nitu Ahmed, Raihana Nusrat, Shahrin Mahbuba Tanni and also thanks to my well-wishers Khalid Hasan and Amit Kumar for their help, support, inspiration and patience during my work.

I found no words to thanks my parents, for their unquantifiable love and continuous support, their never ending affection, immense strength and untiring efforts for bringing my dream to proper shape. They were constant source of inspiration, zeal and enthusiasm in the critical moment of my studies.

The Author

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ABSTRACT

The effect of eight different treatments viz. *Purpureocillium lilacinum* (T_1), Arbuscular Mycorrhizal Fungi (AMF) (T_2), *P. lilacinum* + AMF (T_3), *P.lilacinum* + arsenic (T_4), AMF + Arsenic (T_5), *P.lilacinum* + AMF + Arsenic (T_6), Arsenic (T_7) and Chemical Fungicide (T_8) were evaluated against wilt of eggplant caused by *Fusarium oxysporum*. The efficacy of the treatments varied significantly in terms of wilt incidence and plant growth. Application of treatment T_3 (*P. lilacinum* + AMF)and T_8 (Chemical Fungicide) showed the best result at 28 days of transplanting with lowest 20% of wilt incidence. Among ten growth parameters, six parameters such as root length, root fresh weight, root dry weight, shoot length, shoot fresh weight and shoot dry weight showed the best result with 16.80 cm, 5.36 g, 3.01 g, 15.70 cm, 11.60 g, 1.5 g respectively in the treatment T_3(*P. lilacinum* + AMF) which is significantly different from other treatments. In case of number of leaf/plant, treatment T_2 (AMF) showed best result with 5.00 which is significantly different from other treatments. Chlorophyll content was highest in the treatment T_8 (Chemical Fungicide) with 42.65 µg cm^{-2} which is significantly different from other treatments. Treatment T_5 (AMF+Arsenic) showed the best result in comparison to other treatments where AMF reduced 50% arsenic uptake by eggplant shoot in compare to the treatment where only arsenic was applied.

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LIST OF ABBREVIATED TERMS

ABBEVIATION	FULL WORD
AMF	Arbuscular Mycorrhizal Fungi
As	Arsenic
et al.	And others
BARI	Bangladesh Agricultural Research Institute
Cm^3	Centimeter cube
Cm^2	Centimeter square
Cm	Centimeter
μgcm^{-2}	Microgram/ Centimeter square
CV.	Cultivar
°C	Degree centigrade
Etc.	Etcetera
Ed.	Edited
Eds.	Edition
G	Gram
J.	Journal
No.	Number
PDA	Potato Dextrose Agar
LSD	Least Significant Difference
DMRT	Duncan's Multiple Range Test
%	Percent
RCBD	Randomized Completely Block Design
Res.	Research
SAU	Sher-e-Agricultural University
Viz.	Namely
Var.	Variety

CHAPTER 1

INTRODUCTION

The Eggplant, Aubergine or Brinjal (*Solanum melongena* L.), of the family Solanaceae, is grown in the subtropical and tropical regions of the world. It is one of the most common, highly productive and popular vegetable crops. It is quite popular as the poor man's crop (Gargi Chakravarty and Kalita, 2012). Eggplant is a widely grown vegetable crop in fields as well as under tunnels in Asia, Africa and America (Kalloo and Berg 1993; Sihachakr et al.1994). The eggplant is native to the Indian Subcontinent and now it is present all over the world (Yiu, 2006 and Doijode, 2001). It is grown extensively in Bangladesh, China, India, Pakistan and Philippines. It is also popular in other countries like Balkan area, France, Indonesia, Italy, Japan, Mediterranean, Turkey and United states (Bose and Som, 1986).

Worldwide production area of eggplant is approximately 1.6 million ha and production is nearly 4.2 million tons (F.A.O., 2012). China is the leading with 58 percent of the world production. In the Pakistan total area of production under this vegetable crop is about 9 thousands hectares with the 89 thousand tons of production and Pakistan ranking stands at 18th position in the world (F.A.O., 2007).

Eggplant is nutritious vegetable and have got multifarious use as a dish item (Bose and Som, 1986 and Rashid, 1993). It is largely cultivated in almost all districts of Bangladesh. It can be grown at homestead area and kitchen garden because of its popularity especially for urban people. About 8 million farm families are involved in eggplant cultivation (Islam, 2005). Eggplant is the second most

important vegetable crop next to potato in Bangladesh in respect of acreage and production (BBS, 2005). It is grown round the year both as winter (Rabi) and summer (Kharip) crops (Rashid, 1993). The eggplant is also reported to possess medicinal properties. Various plant parts are used for curing ailments such as diabetes, cholera, bronchitis, dysuria, dysentery, otitis, toothache, skin infections, asthenia and hemorrhoids. It is also ascribed narcotic, anti-asthmatic and anti-rheumatic properties (Daunay *et al*., 2003).

Eggplant is susceptible to several diseases particularly verticillium wilt (*Verticillium dahliae*), fusarium wilt (*Fusarium oxysporum* f.sp. *melongenae*) and bacterial wilt (Ralstonia solanacearum) (Kalloo and Berg 1993; Sihachakr *et al.*1994). The recommended management methods against the wilts are the rotation of crops, use of resistant varieties, solarization, soil sterility and use of fungicides (Yucel et al.2007). This crop suffers from the various diseases; about 13 different diseases so far recorded in Bangladesh (Das *et al.* 2000; Khan *et al.* 2002 and Rashid, 2000). Among those diseases wilt of eggplant has been treated as one of the major constrains in eggplant cultivation in the country (Ali, 1993).In some cases 100% of the plants are found to die in Kitchen gardens of Bangladesh due to wilt problem (Ali *et al.*,1994).

Fusarium wilt is soil borne fungal pathogen which can sustain many years in the soil without a host (Ignjatov *et al.,* 2012). *F. oxysporum*has a worldwide distribution and causes severe root rot or vascular wilt in ample range of plant families (Enya *et al.,* 2008; Lievens *et al.,* 2008; Michielse and Rep, 2009). This fungal pathogen infects the seed and early stages of seedling growth, causing seed decay and damping-off (Punja *et al.,* 2004). The soil-borne fungus invades the vascular bundles, causes severe wilting and death of the above ground parts of

plants by blocking the xylem transport system (Altınok, 2005). In order to prevent the plant diseases and to protect the crop plants against pathogens chemical control methods were in practice. In view of the high cost of chemical pesticides and their hazardous consequence use of biodegradable different alternate material like fresh plant extracts from parts gained importance during last three decades from plant disease control (Fowcett and Spenser, 1970; Mitra *et al*., 1984; Grainge and Ahamed, 1988; Jespers and Ward, 1993). Although the use of Fusarium-resistant eggplant cultivars can provide some degree of control of this disease, the occurrence and development of new pathogenic race is a continuing problem and currently there are no commercially acceptable cultivars with adequate resistance to *F. oxysporum* f. sp. *melongenae*. The recommended management methods against the wilts are the rotation of crops, use of resistant varieties, solarization, soil sterility and use of fungicides (Yucel *et al.*, 2007) and application of sawdust, *Trichoderma harzianum* T22 and grafting of eggplant with wild Solanum (*Solanum sisymbriifolium*) could be used as eco-friendly approach for management of wilt diseases and profitable production of eggplant (Faruq *et.al*. 2014) Chemical control of wilt disease is very difficult and not environmentally sound. Therefore, alternative control measures are necessary and need to be made available as soon as possible.

The application of micro-organisms as bio-control agent is important, since they may increase beneficial microbial activity which extends for a long period of time. *P. lilacinum* and AMF are considered as potential bio-control and plant growth promoting agents for many crop plants. The competition with pathogens, parasitism and the production of anti-fungal compounds are the most important mechanism in bio-control activity (Verma *et al*., 2007; Savazzini *et al*., 2009).

These antifungal micro-organisms can be relatively easily established in different types of soil and continue to persist at detectable levels for months.

On the other hand, arsenic contamination is of one of the most serious problem in Bangladesh. At present prevalence of arsenic in drinking water has been identified in 61 out of 64 districts of the country. About 20 million people in Bangladesh are using tube-wells contaminated water with arsenic over the permissible level (>50 ppb). (UNICEF, 2008).

A number of studies have also reported a correlation between arsenic in soil and reduction in crop yield. Naturally, occurring arsenic, as a water quality issue in South Asia, began to attract international attention in the early decade of the nineties. Arsenic (As) is a nonessential element in biological processes and is toxic to plants, humans and animals. Arsenic contamination of water, air and soil from both geological and anthropogenic sources is a significant environmental health concern arsenic toxicity in humans is a widespread and increasingly recognized problem, mainly resulting from accumulation of As in rice, vegetables and other grains from contaminated irrigation water (Christophersen *et al.,* 2009).

Natural, arsenic contamination of drinking water has been reported from over 70 countries worldwide, affecting an estimated 150 million people. About 50 million of these people live in Bangladesh, 30 million in India and 33 millions in other six countries of south and south-east Asia. (Heikens, 2006)

Several methods used for the control of fungal pathogens are: physical, chemical, cultural and biological methods. These methods have either one or other limitations but biological methods in the recent studies found impetus due to low effect on the plants as well as on the environment. Therefore, present study was

undertaken to evaluate the role of biological control agents such as *P. lilacinum*and AMF against *Fusarium oxysporum* of Brinjalin arsenic contaminated soil with the following objectives:

OBJECTIVES

1. To evaluate the efficacy of *Purpureocillium lilacinum* either alone or in combination with arbuscular mycorrhizal fungus (AMF) in controlling wilt of eggplant in arsenic contaminated soil.

2. To evaluate the efficacy of *Purpureocillium lilacinum* either alone or in combination with arbuscular mycorrhizal fungus on growth parameters and reducing arsenic toxicity of eggplant grown in arsenic contaminated soil.

CHAPTER 2

Review of Literature

Eggplant (*Solanum melongena* L.) is a popular solanaceous vegetable crop. Eggplant suffers from many diseases caused by fungi, bacteria, virus, nematode and mycoplasma. Of them, root disease like Fusarium wilt and Nemic wilt caused by *Fusarium oxysporum* and *Meloidogyne incognita,* respectively are responsible for devastating damage of eggplant (Ahmed and Hossain, 1985; Mian, 1986; Ali 1993).

Fusarium are generally classified as soil borne fungi that cause various vascular wilts and root and stem rots of cultivated plants (Armstrong and Armstrong, 1975; Burgess, 1981).

Walker (1969) described the symptoms of wilt caused by *Fusarium* sp. as yellowing of the lower leaves, usually affecting the leaflets unilaterally. The affected leaves die and the symptoms continue to appear on successively younger leaves. The plant as a whole is stunted and eventually goes into a permanent wilt.

Rangswami (1988) observed the symptoms of fusarium wilt that the younger leaves may die in succession and the entire plant may wilt and die in the course of a few days.

Hartman and Datnoff (1997) stated that, plants infected with the fungus *Fusarium sp.* that caused wilt and root rot have yellow leaf margins on the oldest leaves (Sherf and MacNab, 1985). Lower leaves become necrotic and drop off from the plant. Plants defoliate from lower to upper leaves as they becomes more necrotic.

Plants may wilt and die quickly. Roots become dry and the cortex and xylem turn brown.

Ramesh and Manjunath (2002) used *Trichoderma* spp., 0.2% Carbendazim and 0.3% Copper Oxychloride for management of wilt disease (caused by *Ralstonia solanacearum, Fusarium oxysporum* and *Verticillium dahliae*) on eggplant. Maximum infection was observed in plots treated with Carbendazim (17.88%) and control plot (10.65%), whereas the lowest infection was recorded in plots treated with *Trichoderma spp.* (2.78%) and Copper oxychloride (2.88%). However the highest yield was obtained with Carbendazim (11.88 t/ha)

Gonzalez-Chavez (2002)reported that, Arbuscular mycorrhizal fungi confer enhanced arsenate on *Holcus lanatus.* The role of arbuscular mycorrhizal fungi (AMF) in arsenate resistance in arbuscular mycorrhizal associations is investigated here for two *Glomus* spp. isolated from the arsenate-resistant grass *Holcus lanatus*. *Glomus mosseae* and *Glomus caledonium* were isolated from *H. lanatus* growing on an arsenic-contaminated mine-spoil soil. AMF isolates were arsenate resistant compared with nonmine isolates. That AMF have evolved arsenate resistance, and conferred enhanced resistance on *H. lanatus.*

Abdul (2005) experimented the biological control of wilt of brinjal caused by *Fusarium oxysporum* with some fungal antagonists. The experiment showed that, plants with different doses of VAM fungi, Glomus mosseae and G. fasciculatum there was observed a reduction in the pathogenic effect by *Fusarium oxysporum* and improvement in plant growth and chlorophyll content of brinjal plants. The plant length, plant weight and chlorophyll content improved greatly with the

increase in the dose of VAM fungi as compared to untreated control. The highest increase in the plant length and plant weight was observed in plants grown in pots treated with 20g of soil root inoculum of Glomus mosseae and G. fasciculatum.It was followed by plants treated with 15g, 10g and 5 g of root inoculum of VAM fungi as compared to untreated control which showed lowest plant growth.

Haseeb and Kumar (2007) reported the efficacy of bio-agents and organic amendment materials against *Fusarium oxysporum* causing brinjal wilt.This studies clearly indicate that all the bio-agents were found highly effective and neem seed powder, neem cake and mint manure were moderately effective against *F. oxysporum* and can be exploited under field conditions for further experimentation to manage this disease.

Chakraborty *et al.* (2009) conducted an experiment on integrated management of Fusarial wilt of eggplant with soil solarization. Several fungal antagonists and botanicals were tested for antimicrobial activity against the pathogen under *in vitro* and *in vivo* conditions. *Trichoderma harzianum* and *T. viride* specifically were found to have reduced the incidence of wilt disease effectively up to 86% and 83%, respectively. Soil solarization integrated with applications of *T. harzianum*, bavistin and neem was the most effective treatment.

Elahi *et al*. (2010) experimented on influence of AMF inoculation on growth, nutrient uptake, Arsenic toxicity and Chlorophyll content of eggplant grown in arsenic amended soil. The experiment was carried out to determine the influence of AMF inoculation on growth, nutrient uptake, arsenic toxicity and chlorophyll content of eggplant grown in arsenic amended pot soil. Three levels of arsenic concentrations (10ppm, 100ppm and 500ppm) were used in pot soil and eggplant

was grown in arsenic amended soils with or without mycorrhizal inoculation. Root length, shoot height, root fresh weight, shoot fresh weight, root dry weight and shoot dry weight were higher in AMF inoculated plants in comparison to their respective treatments and decreased significantly with the increase of rate of arsenic concentrations. Less arsenic content and higher chlorophyll and nutrient uptake were recorded in mycorrhiza inoculated plants in compare to non-inoculated plants. The findings of the study indicated that AMF inoculation not only reduces arsenic toxicity but also can increase growth and nutrient uptake of eggplant shoot.

Bozena and Maciej (2011) conducted an experiment on the effects of biological control on fungal communities colonizing eggplant (*Solanum melongena L*.) organs and the substrate used for eggplant cultivation. The applied biological and chemical control agents effectively reduced the populations of pathogenic fungi colonizing eggplant stems, compared with the control treatment. The lowest number of potential pathogenic species were isolated from the stems of eggplants treated with the bio-stimulator Asahi SL and the fungicide Bravo 500 SC, and from the roots of fungicide-treated plants.

Faruq *et al.* (2014) experimented on efficacy of soil application with *Trichoderma harzianum* T22 and some selected soil amendments on Fusarium wilt of eggplant (*Solanum melongena* L.). From the findings of the investigation it was concluded that *Trichoderma harzianum*, poultry waste and vermi-compost showed promising performance against wilt disease among the treatments tested in this investigation. These bio-fungicide and soil amendments could be used as an eco-friendly approach and may be advised to the farmer for profitable organic farming.

Montaser *et al.* (2014) experimented on the enhancement of growth parameters and yield components in eggplant using antagonism of *Trichoderma* spp. against *Fusarium* wilt disease.In this study, the antagonistic activity of five *Trichoderma* species (*Trichoderma spirale, T. hamatum, T. polysoprium, T. harzianum* and *T. viride*) against *F. oxysporium* f. sp. *melongenae* was evaluated using dual culture technique. *T. viride* (isolate TVM-5) and *T. hamatum* (isolate THM-2) showed the highest antagonistic activity, while *T. spirale* (TSM-1) was the lowest one. In pot experiment, the obtained data showed that all *Trichoderma* species reduced significantly area under wilt progress curve caused by *F. oxysporum* f. sp. *melongenae*. *Trichoderma viride* and *T. hamatum* recorded the highest reduction of area under wilt progress curve (AUWPC) (244.0 and 325.33 AUWPC as compared to 1125.33 in control treatment, respectively). Under field conditions results showed that, these treatments significantly reduced AUWPC and increased all tested plant growth parameters (Plant height, No. of branches plant-1) and fruit yield components (number of fruits plant-1, fruits yield plant-1, fruit weight, No of fruit Kg-1, fruit length, fruit diameters and fruits yield fed.-1) compared with control during growing seasons (2011-2012 and 2012-2013). *Trichoderma viride* and *T. hamatum* were the best biocontrol agents as manifested by the significant reduction in both disease severity and increase plant growth parameters and fruit yield components.

Bashar and Chakma (2014) determined the in vitro control of *Fusarium solani* and *Fusarium oxysporum.*Seven soil fungi *viz. Aspergillus flavus, A. fumigatus, A. niger, A. terreus, Penicillium* sp., *Trichoderma harzianum* and *T. viride* associated with the rhizosphere, non-rhizosphere and rhizoplane of brinjal plant*s* were selected to observe their antagonistic potential against the test fungi *Fusarium oxysporum* and *F. solani* . Out of seven soil fungi *T. harzianum* was found most effective to control the growth of both the test fungi.

Altinok and Erdogan (2015) determined the in vitro effect of *Trichoderma harzianum* on phytopathogenic strains of *Fusarium oxysporum.* The cultures of two strains of *Trichoderma harzianum* (T16 and T23) were examined in laboratory conditions and with pot experiments for the control of pathogenic strains of Fusarium oxysporum f. sp. melongenae (Fomg), Fusarium oxysporum f. sp. lycopersici (Fol), *Fusarium oxysporum* f. sp. *niveum* (Fon) and *F. oxysporum* f. sp. *melonis* (Fom). The T16 and T23 strains showed significant inhibition of mycelial growth in the pathogenic strains of *F. oxysporum* and the maximum inhibition were recorded when the T. harzianum strain T16 was used (72.69%). Both *T. harzianum* strains produced volatile and non-volatile metabolites that inhibited growth of *F. oxysporum* strains on PDA medium. In vitro colonization study demonstrated the root-colonizing ability of these antagonists. The interaction between *T. harzianum* isolates (T16 and T23) and pathogenic F. oxysporum hyphae showed no overgrowth, hyphal coiling, cell wall degradation or any hyphal penetration around any of the tested *F. oxysporum* hyphae. Pre-treatment of soil with T16 significantly reduced the severity of Fusarium wilt disease.

Rajinikanth *et al*. (2016) conducted an in vitro experiment on evaluation of potential bio-control agents on root knot nematode and wilt causing fungus. The experiment showed that, the effect of culture filtrates of three bio-agents, namely, *T. viride, P. fluorescens* and *P. lilacinum* showed on root-knot nematode M. incognita in in vitro. The experiments also proved the antagonism of these bio-control agents on suppression of *F. oxysporum* f.sp. *conglutinans*. For the resultant data, the effect on M. incognita and on *F. oxysporum* was an apparent indication to control the nematode induced disease complex in in vivo in cauliflower (Rajinikanth et al., 2013) by application of eco-friendly bio-control agents of bio-nematicide (*P. lilacinum*), bio-fungicide (*T. viride*) and bio-bactericide (*P. fluorescens*).

CHAPTER 3

MATERIALS AND METHODS

Pot experiments were done to study the effect of *P. lilacinum* and AMF against *Fusarium oxysporum*, the causal agent of wilt disease of Brinjal in arsenic amended soil. The materials used and the methods followed in this experiment are presented in this chapter.

3.1.Experimental site and experimental period

The present investigation was carried out during the period from September 2015 to May 2016 in the Laboratory and in the net house of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar,Dhaka-1207.

3.2.Environment of experiments

All the experimental plants were kept in the net house where the temperature was 28 ± 2º C during the "day" and 23 ± 2º C during "night" with an average temperature of 26± 2º C.

3.3.Pot Experiment

3.3.1.Crop variety used

In this experiment Brinjal Variety Shingnath was used as selected crop.

3.3.2.Collection of seeds

Brinjal variety Shingnath seed was collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur.

3.3.3. Soil collection and sterilization

Required soils were collected from agricultural farm of Sher-e-Bangla Agricultural University. Sand was also collected with soil. Then soil and sand mixed properly in a ratio of 7:3. For raising seedlings in plastic trays and for final experiment set up the mixture was autoclaved at 121ºC, 15 psi for 15 minutes on two successive days. The sterilized soil was allowed to cool to room temperature and was later used to fill the plastic trays for raising seedlings and pot for seedling transplanting.

3.3.4.Raising of seedling

Several plastic trays were filled with sterilized and fertile soil. Seeds of Shingnath was soaked in water for one night and treated with NaOCl for one minute and washed with distilled water for three times. After that the seeds were sown in plastic trays and covered with a thin layer of soil and watered. Then the trays were covered with polythene sheet and kept in sunlight for raising seedlings. Seedlings were observed regularly and watering was done as per necessity up to hardening the seedling in plastic pot.

Plate 1. Raising of eggplant seedling

3.3.5.Preparation of pots

Plastic pots of 1000 cm3 were cleaned, washed and dried up. Sterilized and fertile soil was filled in required amount into each pot. Each pot contained 800 g soil. Then the pots were arranged according to selected experimental design.

A.

B.

Plate 2. Preparing pots A. samples of sand and soil B. mixing of sand and soil

3.3.6.Treatments of the experiment

There were eight (8) treatments in the study with 10 replications. They were as follows:

T_1: *Purpureocillium lilacinum*(PL)

T_2: Arbuscular mycorrhizal fungi (AMF)

T_3: *P. lilacinum* + AMF

T_4: *P. lilacinum* + arsenic

T_5: AMF + arsenic

T_6: *P. lilacinum* + AMF + arsenic

T_7: Arsenic

T_8: Chemical Fungicide (Bavistin 50 WP)

3.3.7.Design and layout of the experiment

The experiment was laid out in Randomized Complete Block Design (RCBD) with ten replications per treatment.

3.3.8.Culturing of AMF

Spore of AMF was collected from the laboratory of Department of Plant Pathology, Sher-e-Bangla Agricultural University. Then for the multiplication of AMF spore they were cultured on maize seedlings so that they can have enough root and soil colonization. Maize seeds were surface sterilized by Murcuric Chloride and washed with sterile distilled water for three times and sown in sterilized pot soil. Pot soil was previously inoculated with AMF spore. Multiple number of pots were used for the AMF culture with maize seedlings and they were placed in the net house with proper facilities. After 8 weeks, AMF colonized root fragments of seedlings and rhizosphere soil containing spore were used for AMF inoculation.

Plate 3. Mass culture of *Glomus*sp. with trap plant maize

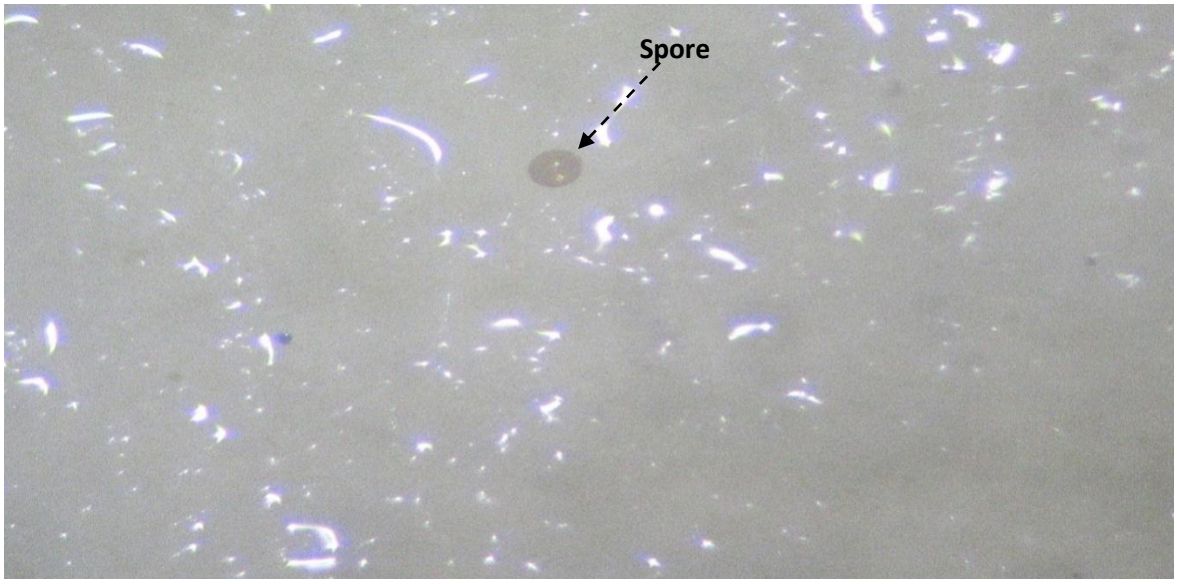


Plate 4. Spore of *Glomus* sp (40x)

3.3.9.Collection of fungal isolates

The isolates of *Purpureocillium lilacinum* and *Fusarium oxysporum* were collected from the stock culture of the Department of Plant Pathology, SAU, Dhaka.

3.3.10.Culture, mass production and harvesting of *Purpureocillium lilacinum*

Purpureocillium lilacinum was grown on Potato Dextrose Agar (PDA) medium for 8-10 days (Aminuzzaman and Liu, 2011).Within 8-10 days the fungus was transferred on mungbean husk for mass production. For mass production one hundred grams of mungbean husk free of any pesticide treatment was placed in 250 ml conical flasks and soaked in lukewarm water for 3-4 hours. Then the water was drained off, and each flask was closed with a cotton plug and covered with brown paper in two layer paper. Then flasks were placed in an autoclave for 15 minutes at 15 psi. After the flasks and contents cooled, *P. lilacinum* as a mycelial mat growing on PDA was added aseptically to one flask and shaken for better distribute of the fungus; the other flask served as an un-inoculated control. The flasks were incubated at 25-30° C for 20 days. After incubation the sterile water was added into the conical flask and the spore masses scraped away with sterile brush within laminar air flow chamber. The harvested spores were filtered through sterilized cheesecloth. The spore was harvested from each conical flask and spore was counted with a haemacytometer.

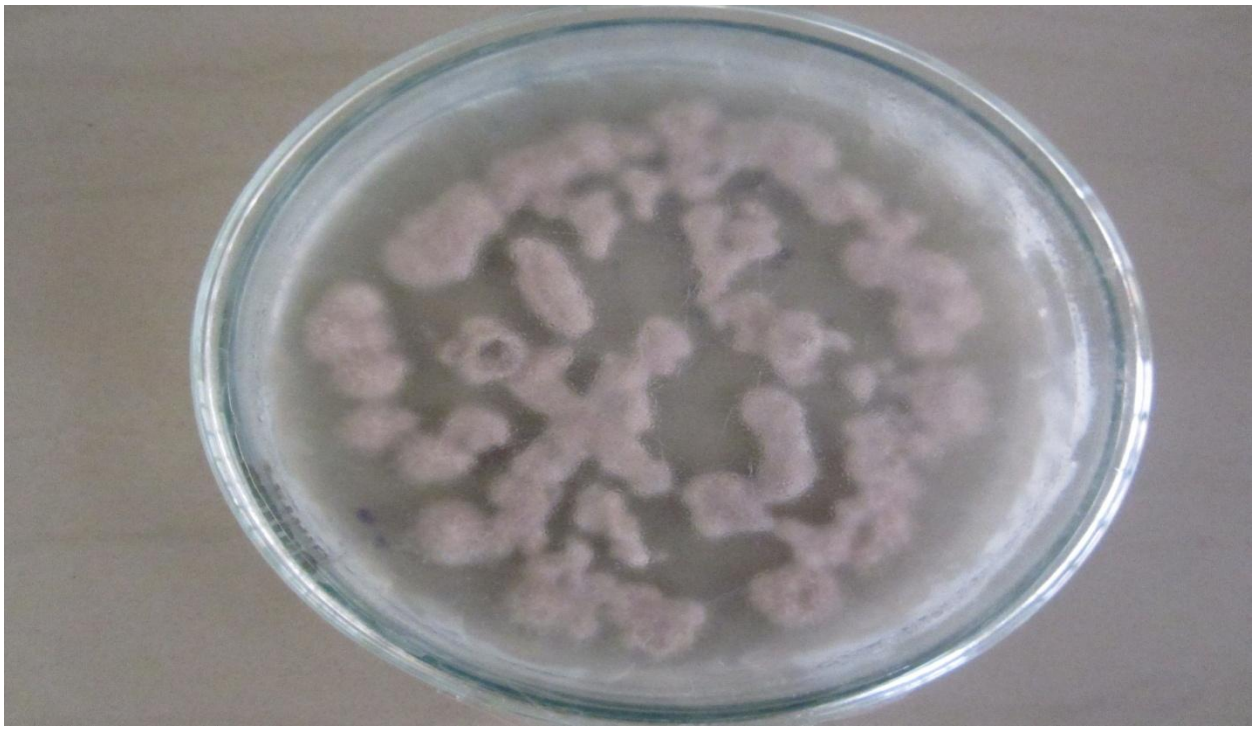
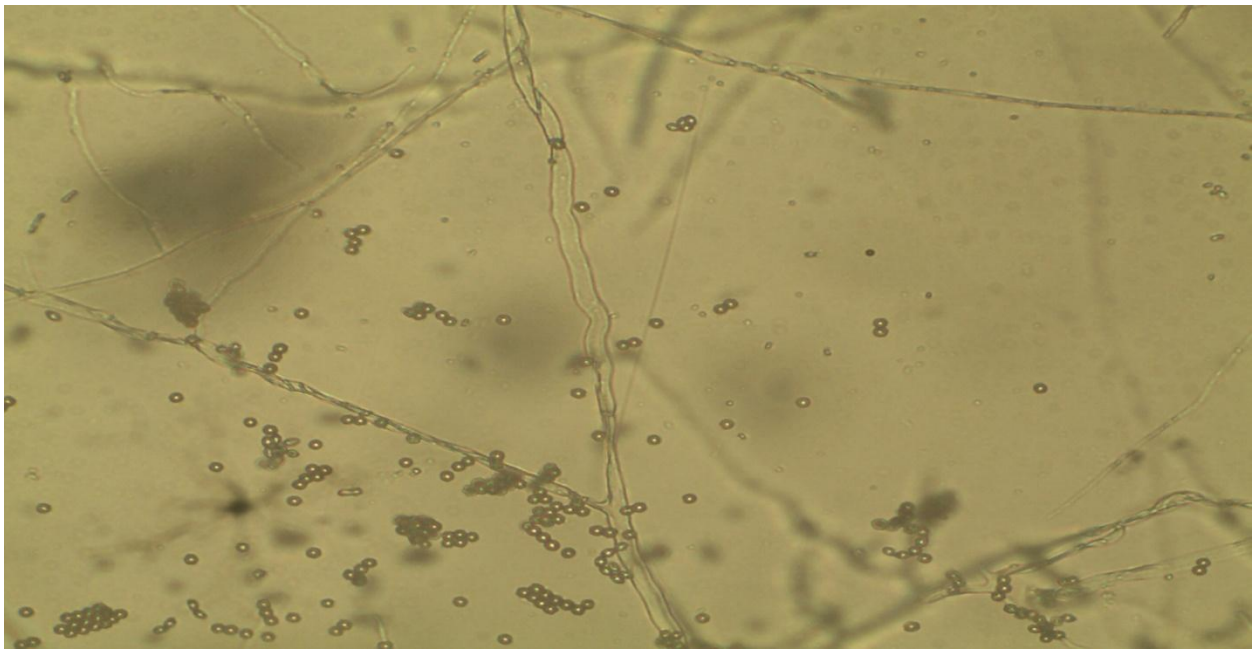
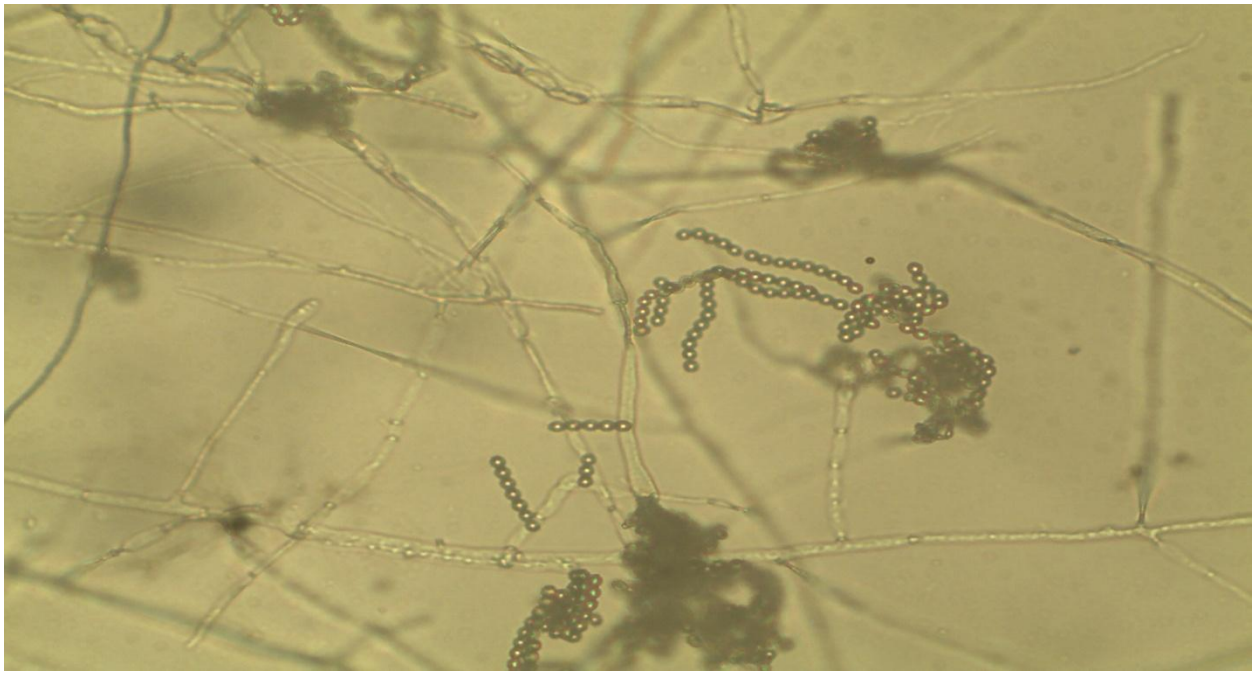


Plate 5. Pure culture of *P.lilacinum* on PDA media

Plate 6. Mass culture of *P.lilacinum* on mungbean husk



A.



B.

Plate 7. Conidiophore and conidial chain of *P. lilacinum* on (40x)

A. Scattered B. Clustered

3.3.11.Culture, mass production and harvesting of *Fusarium oxysporum*

F. oxysporum was isolated from wilted plants of brinjal collected from farmer's field. Pure culture was maintained on PDA slants at 4°C. For In vitro studies, cultures were inoculated on PDA petri plates and incubated at 27±2°C for 7 days and for pot experiment, *F. oxysporum* was multiplied on autoclaved Mungbean husk in 250 ml Erlenmeyer Flasks at 27±2°C For 12 days (Haseeb and Kumar, 2007). After incubation the sterile water was added into the conical flask and the spore masses scraped away with sterile brush within laminar air flow chamber. The harvested spores were filtered through sterilized cheesecloth. The spore was harvested from each conical flask and spore was counted with a haemacytometer.

Plate 8. Pure Culture of *F. oxysporum* on PDA

Plate 9. Mass culture of *F.oxysporum* on mungbean husk

3.3.12.Transplanting of seedlings and inoculation of *P. lilacinum, F. oxysporum* and AMF

After preparation of pot, spore of *P. lilacinum* mixed carefully into soil @ 2×10^6 CFU of *P. lilacinum*/g of soil in defined pot. Then *F. oxysporum* @ 4.5×10^5 CFU/g of soil was also applied to the defined pot soil. A separate experiment was carried out where fungal inocula were added in 100 g soil in different rate and mixed thoroughly with three replicates. 30 days old seedlings were uprooted carefully from the plastic trays and transplanted in the fungal treated pot. Only one plant was transplanted to each pot. Each of those pots were also inoculated with AMF with 25g/plant with 70% infection rate and the number of spore was 65/10g of soil. Then pots were transferred to net house. Sufficient watering was given just after transplantation with tap water daily.

3.3.13.Application of arsenic solution

50 ppm arsenic solution was prepared and applied to those plants where arsenic treatment was assigned to be applied.

3.3.14.Application of Chemical Fungicide

For the chemical control of wilt disease of eggplant we use the chemical fungicide named Bavistin 50 WP. Solution of Bavistin was prepared with 1mg/ml and applied twice @0.1% with 7 days of interval.

3.3.15.Intercultural operations

After transplantation of seedling and final experiment set up weeding and irrigation were regularly done as per necessity. General sanitation was maintained throughout the growing period.

3.3.16.Harvesting and data recording

After two months of transplanting, plants were harvested and data was recorded. The following parameters were considered for data recording:

Root length (cm)

Root fresh weight (gm)

Root dry weight (gm)

Shoot length (cm)

Shoot fresh weight (gm)

Shoot dry weight (gm)

Shoot diameter (cm)

No. of leaves/ Plant

Leaf area (cm^2)

Chlorophyll content (µg cm^{-2})

3.3.17.Plant data recorded

Shoot and root length were measured after harvest. The shoot height (cm) was measured from the base of the plant to the growing point of the youngest leaf with a measuring scale. Then the roots are harvested by cutting with an anti-cutter. Roots are carefully separated from soil, cleaned gently with water and collected in different polybag that were leveled according to different treatments. Finally the root length (cm) was taken. The length of root was measured from the growing point of root to the longest available lateral root apex. For fresh weight(g) of root and shoot was blotted dry and the weight was recorded. For dry weight (g), the shoot and root were sun dried for three days and then kept in drier machine for 4-6 hours at 40ºC temperatures. And after complete drying the weight was recorded. Shoot diameter was measured as well as number of leafs/ plant. Leaf area was measured with leaf area meter and chlorophyll content of brinjal leaves were measured by chlorophyll meter. Wilting of eggplant was also measured in

aninterval of 7 days by observing the plants

Plate 10. Measurement of Chlorophyll content of Eggplant leaf by SPAD 502 Plus Chlorophyll Meter

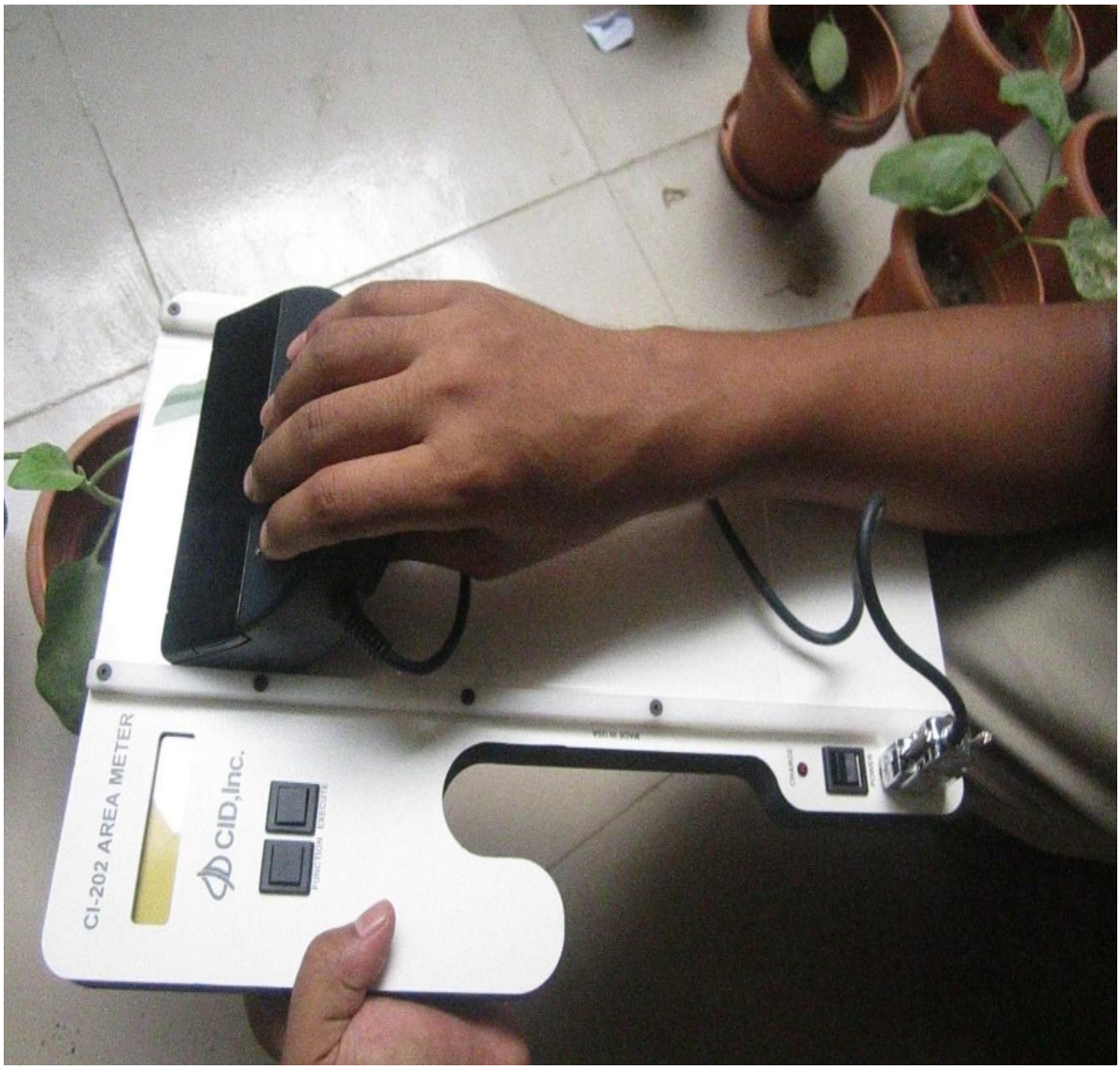


Plate 11. Leaf area measurement by CI-202 Portable Laser Leaf Area Meter

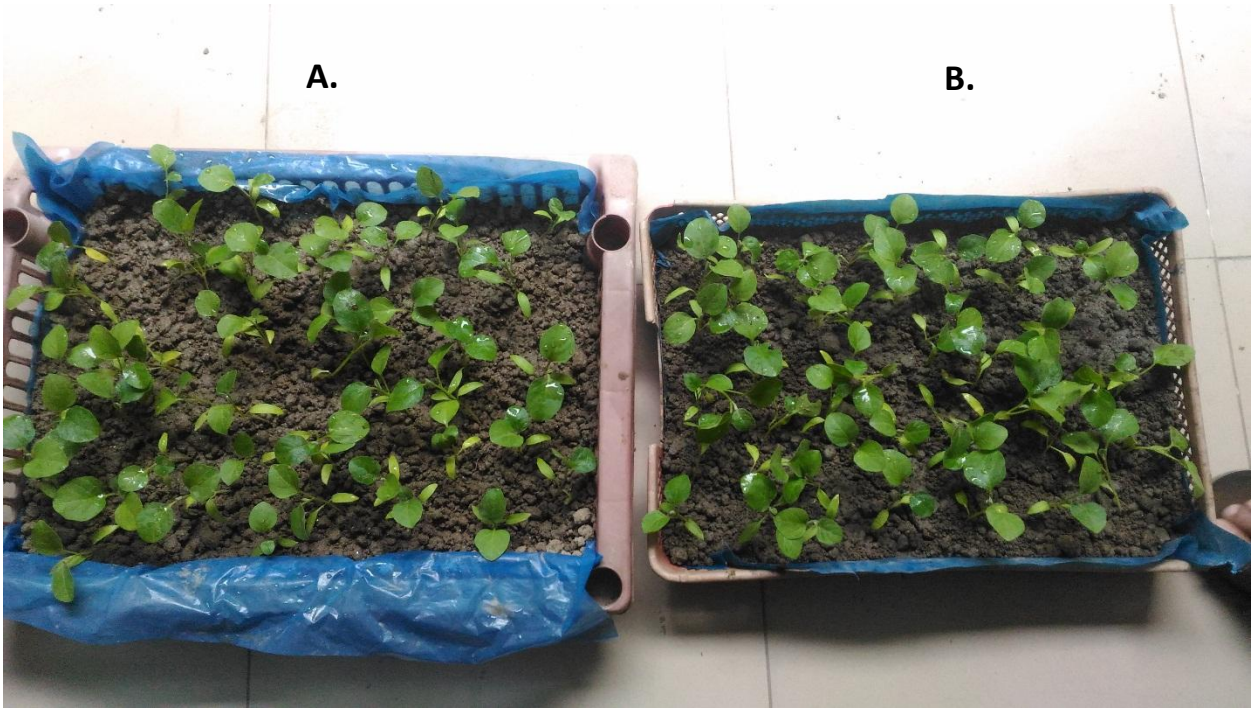


Plate 12. Observation of wilting in eggplant after 7 days. A. Tray with *Fusarium* B. Tray with bioagents &*Fusarium*

Plate 13. Observation of wilting in eggplant after 14 days. A. Tray with bioagents &*Fusarium* B. Tray with*Fusarium*

B. **A.**

Plate 14. Observation of wilting in eggplant after 21 days. A. Tray with *Fusarium* B. Tray with bioagents &*Fusarium*

A. **B.**

Plate 15. Observation of wilting in eggplant after 28 days. A. Tray with *Fusarium* B. Tray with bioagents &*Fusarium*

3.3.18. Soil colonization by *Purpureocillium lilacinum* and *F. oxysporum* (CFUg-1soil)

Samples of 1g soil from each treatment where *P. lilacinum* and *F. oxysporum* were applied was collected after harvest of the crop around the root zone. The number of colony forming unit (CFUg-1soil) per gram soil was determined using the soil dilution plate method.

Plate 16. Determination of $CFUg^{-1}$ soil of *P. lilacinum* using the soil dilution plate method

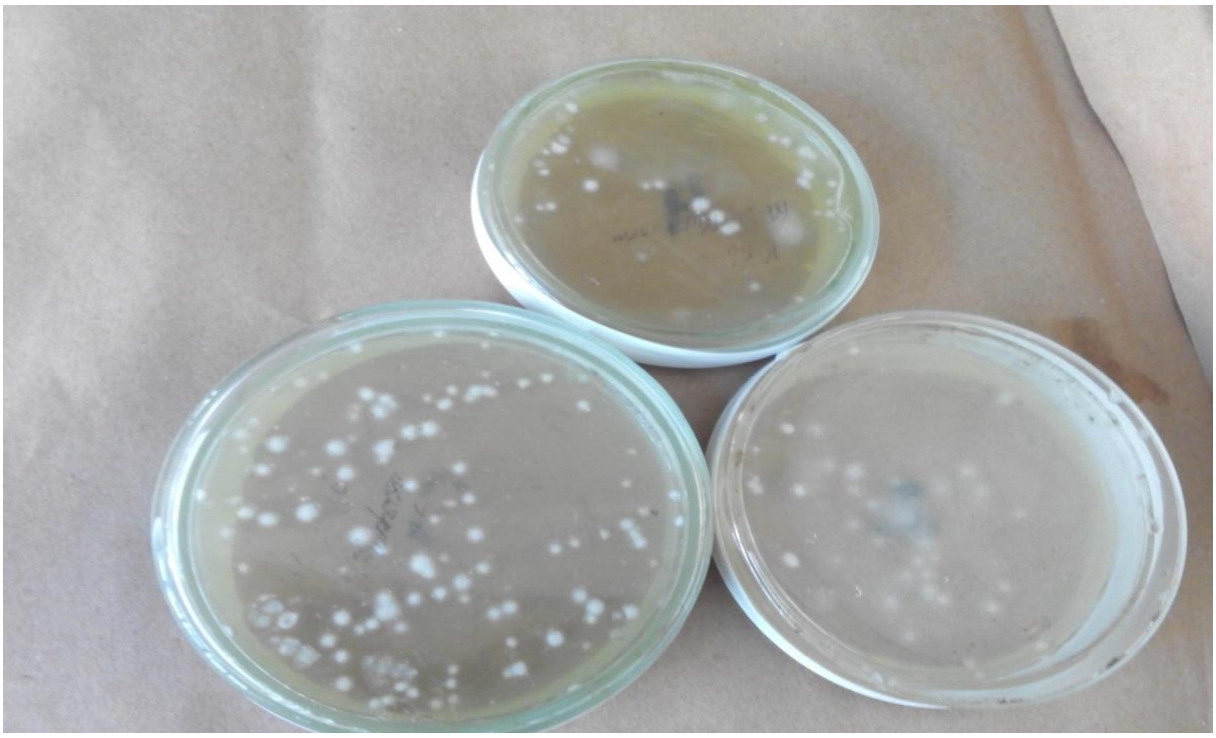


Plate 17. Determination of $CFUg^{-1}$ soil of *F. oxysporum* using the soil dilution plate method

3.3.19.Observation of roots

For the detection of the activities of AMF, root segments were prepared and placed under microscope. The presence or absence of infection of AMF in the root segments was recorded and the percent infection was calculated using the following formula:

$$\% \text{ of root infection} = \frac{\text{Number of AMF positive segments}}{\text{Total Number of segments recorded}} \times 100$$

When the root segments showed mycelium, vesicle and arbuscules or any other combination of AM fungi then that root segment was considered as infected root segment.

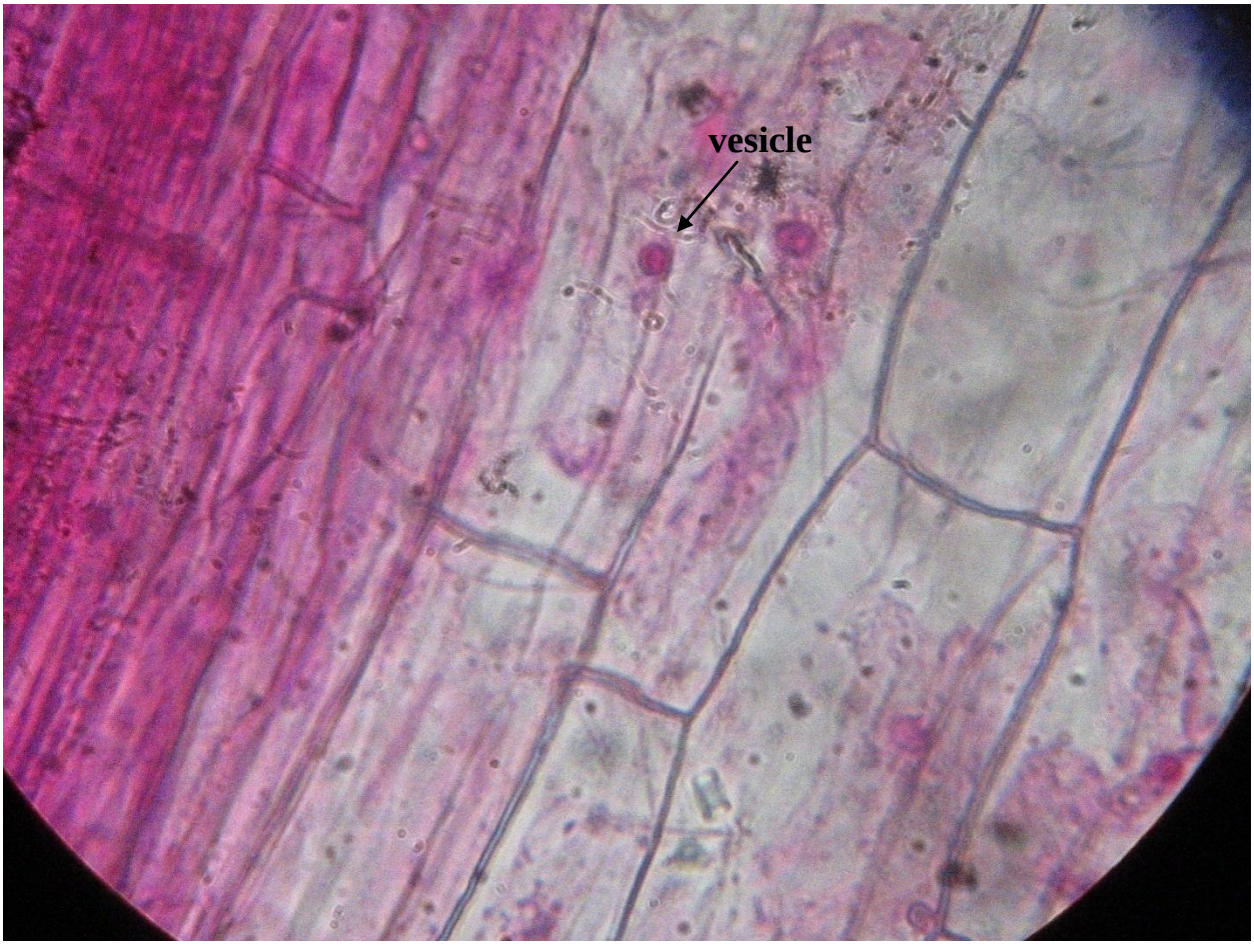


Plate 18. Observation of eggplant roots for mycorrhizal infection(40x)

3.3.20.Determination of AMF spore population in soil

We identify the spore population in soils were isolated and inoculated after confirming mycorrhizal association in the root system. The whole procedure was done in the central laboratory of department of Plant Pathology, SAU, Dhaka.

3.3.21.Analysis of data

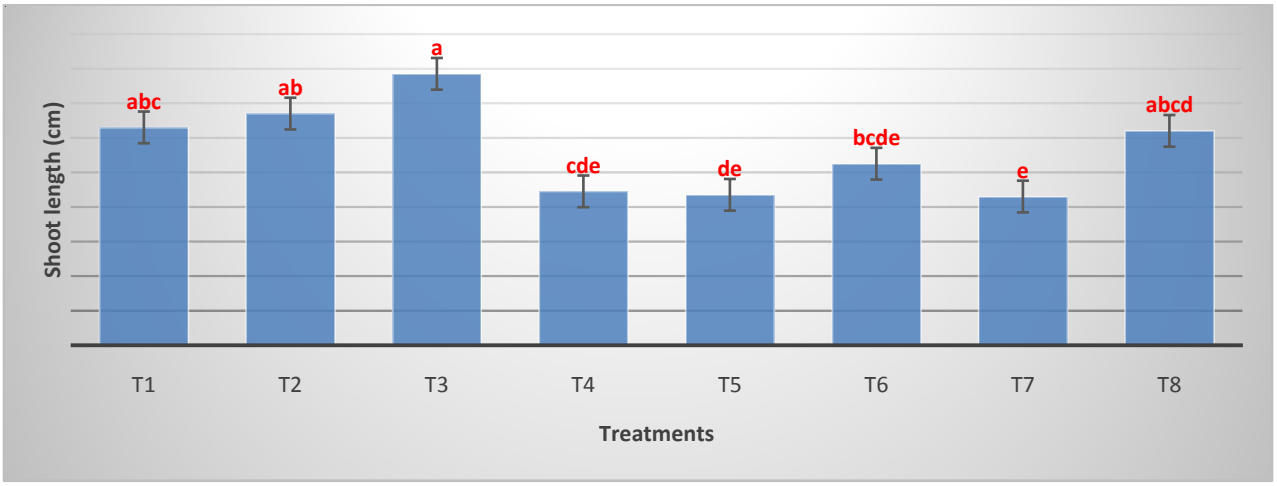
The data were statistically analyzed using analysis of variance to find out the variation of results from experimental treatments. Treatment means were compared by Duncan's New Multiple Range Test (DMRT) according to Gomez and Gomes, (1984). Data were analyzed by MSTAT-C statistical package programmed.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Shoot Length

Shoot length of eggplant influenced by the eight treatments in combination of *P.lilacinum* in arsenic amended soil challenged by *Fusarium oxysporum* is shown Figure 1. Highest shoot length was recorded from the treatment T_3 (*P. lilacinum* + AMF) followed by T_2 (AMF) and T_1 (*P. lilacinum*) which were statistically similar. The highest shoot length for treatment T_3 was 15.70 cm and for treatment T_2 and T_1 it was 13.40 cm cm and 12.60 cm cm respectively. Lowest shoot length 8.60 cm was found from the treatment T_7 (Arsenic) that was statistically similar to the treatment T_5 (AMF + Arsenic)and T_4 (*P. lilacinum* + Arsenic)where the shoot lengths were 8.70 cm and 8.90 cm respectively.

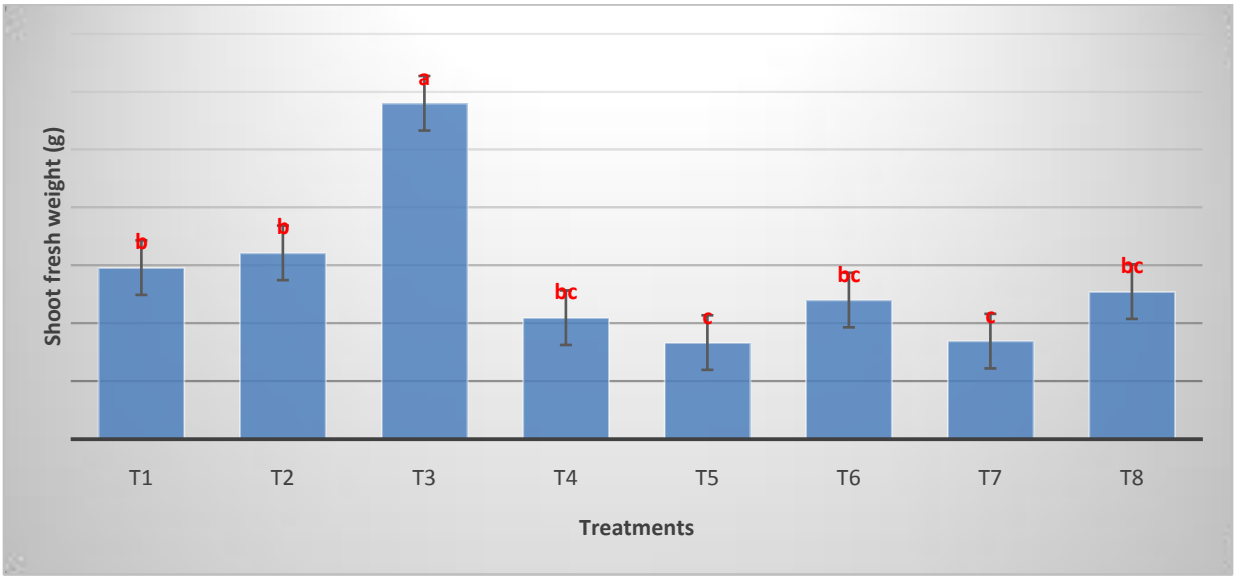


T_1=*Purpureocilliumlilacinum*, T_2=Arbuscular mycorrhizal fungi (AMF), T_3=*P. lilacinum* + AMF, T_4=*P. lilacinum* + arsenic, T_5=AMF + arsenic, T_6=*P. lilacinum* + AMF + arsenic, T_7=Arsenic, T_8=Chemical Fungicide (Bavistin 50 WP)

Figure 1. Shoot length of eggplant influenced by the eight treatments in combination of *P. lilacinum* with *Glomus* sp. in arsenic amended soil challenged by *Fusarium oxysporum*

4.2. Shoot fresh weight

Figure 2 shows the shoot fresh weight of eggplant influenced by the eight treatments in combination of *P. lilacinum* in arsenic amended soil challenged by *Fusarium oxysporum.* The highest shoot fresh weight was recorded from the treatment T_3 (*P. lilacinum* + AMF) which was 11.60 g and statistically different from the other treatments. The lowest shoot fresh weight was found at treatment T_5 (AMF + Arsenic) which was statistically similar to the treatment T_7 (Arsenic) and their value is 3.33 g and 3.38 g respectively.

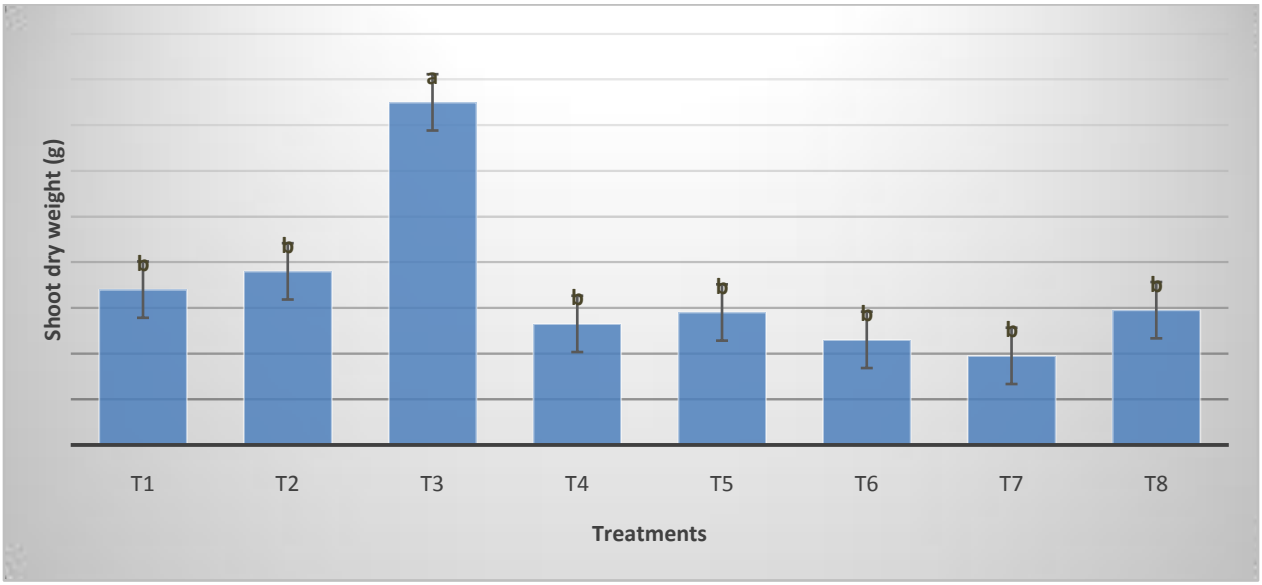


T_1=*Purpureocilliumlilacinum*, T_2=Arbuscular mycorrhizal fungi (AMF), T_3=*P. lilacinum* + AMF, T_4=*P. lilacinum* + arsenic, T_5=AMF + arsenic, T_6=*P. lilacinum* + AMF + arsenic, T_7=Arsenic, T_8=Chemical Fungicide (Bavistin 50 WP)

Figure 2. Shoot fresh weight of eggplant influenced by the eight treatments in combination of *P. lilacinum* with *Glomus* sp. in arsenic amended soil challenged by *Fusarium oxysporum*

4.3. Shoot dry weight

Shoot dry weight of eggplant influenced by the eight treatments in combination of *P.lilacinum* in arsenic amended soil challenged by *Fusarium oxysporum* is shown Figure 3. The highest shoot dry weight was recorded from the treatment T_3 (*P. lilacinum* + AMF) which was 1.5 g and not statistically similar to any of other treatments. The lowest shoot dry weight was found in treatment T_7 (Arsenic) followed by the treatment T_6 (*P. lilacinum* + AMF+ Arsenic) and their value was 0.39 g and 0.46 g respectively.

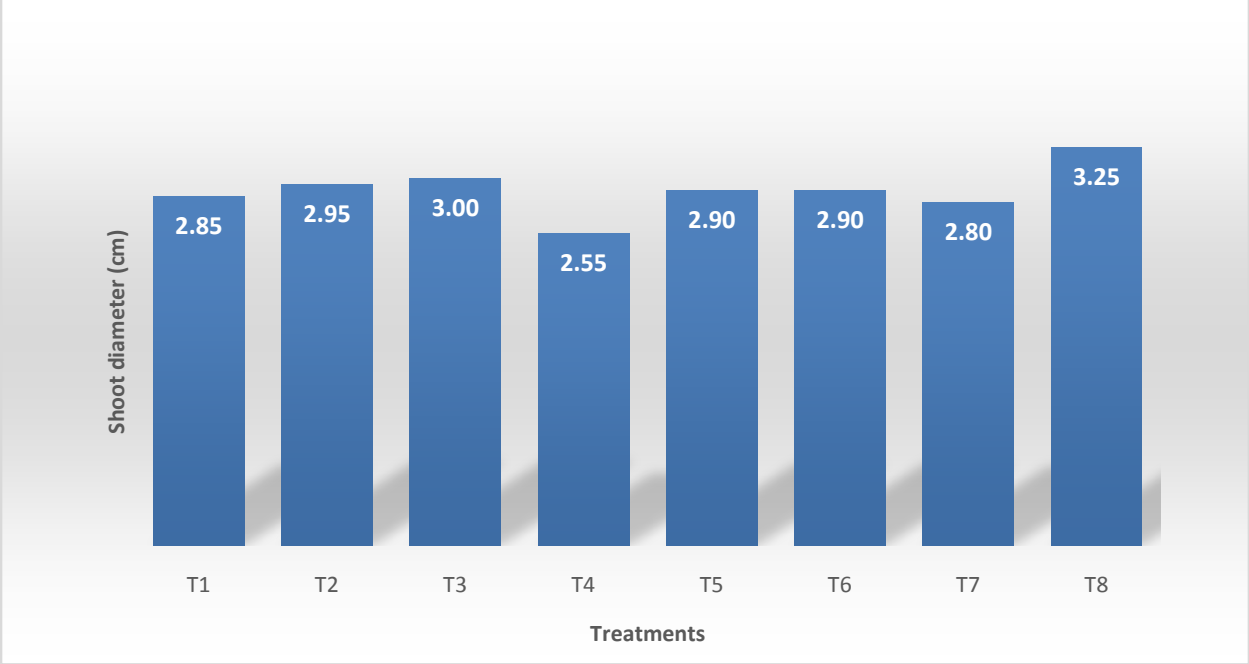


T_1=*Purpureocilliumlilacinum*, T_2=Arbuscular mycorrhizal fungi (AMF), T_3=*P. lilacinum* + AMF, T_4=*P. lilacinum* + arsenic, T_5=AMF + arsenic, T_6=*P. lilacinum* + AMF + arsenic, T_7=Arsenic, T_8=Chemical Fungicide (Bavistin 50 WP)

Figure 3. Shoot Dry Weight of eggplant influenced by the eight treatments in combination of *P. lilacinum* with *Glomus* sp. in arsenic amended soil challenged by *Fusarium oxysporum*

4.4. Shoot diameter

Figure 4 shows the shoot diameter of eggplant influenced by the eight treatments in combination of *P.lilacinum* in arsenic amended soil challenged by *Fusarium oxysporum.* Among the eight treatments the highest result was showed by the treatment T_8 (Chemical control) with a result of 3.25 cm followed by treatment T_3 (*P. lilacinum* + AMF) with 3.00 cm of diameter. Lowest result was found in treatment T_4 (*P. lilacinum* + Arsenic) with a result of 2.55 cm of diameter. All the data of shoot diameter in different treatments are statistically non-significant.

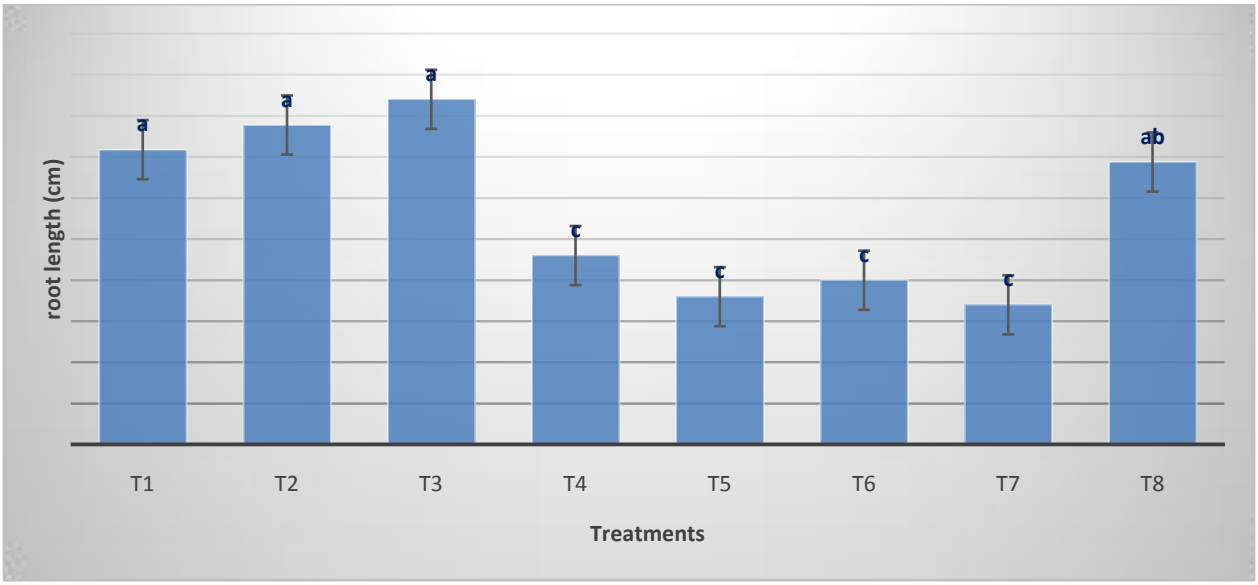


T_1=*Purpureocilliumlilacinum*, T_2=Arbuscular mycorrhizal fungi (AMF), T_3=*P. lilacinum* + AMF, T_4=*P. lilacinum* + arsenic, T_5=AMF + arsenic, T_6=*P. lilacinum* + AMF + arsenic, T_7=Arsenic, T_8=Chemical Fungicide (Bavistin 50 WP)

Figure 4. Shoot Diameter of eggplant influenced by the eight treatments in combination of *P. lilacinum* with *Glomus* sp.in arsenic amended soil challenged by *Fusarium oxysporum*

4.5. Root length

Root length of eggplant influenced by the eight treatments in combination of *P.lilacinum* in arsenic amended soil challenged by *Fusarium oxysporum* is shown Figure 5.The highest root length was recorded from the treatment T_3 (*P. lilacinum* + AMF) followed by T_2 (AMF) and T_1 (*P. lilacinum*) whichwere statistically similar.The lowest root length 6.80 cm was founded from the treatment T_7 (Arsenic) that was statistically similar to the treatment T_5 (AMF + Arsenic)and T_6 (*P. lilacinum* + AMF + Arsenic)where the root lengths were 7.20 cm and 8.00 cm respectively.

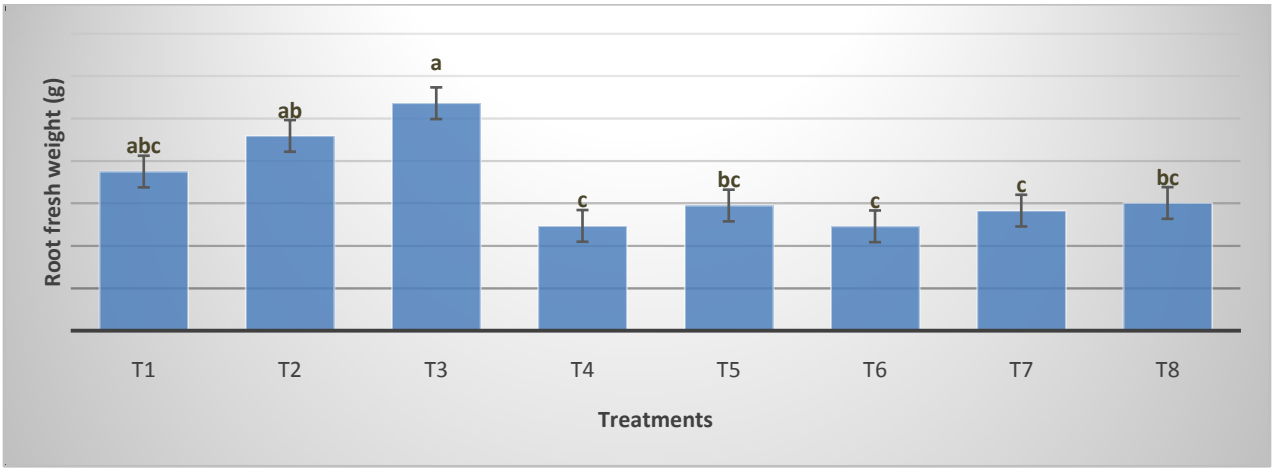


T_1=*Purpureocilliumlilacinum*, T_2=Arbuscular mycorrhizal fungi (AMF), T_3=*P. lilacinum* + AMF, T_4=*P. lilacinum* + arsenic, T_5=AMF + arsenic, T_6=*P. lilacinum* + AMF + arsenic, T_7=Arsenic, T_8=Chemical Fungicide (Bavistin 50 WP)

Figure 5. Root length of eggplant influenced by the eight treatments in combination of *P. lilacinum* with *Glomus* sp. in arsenic amended soil challenged by *Fusarium oxysporum*

4.6. Root fresh weight

Figure 6 shows the root fresh weight of eggplant influenced by the eight treatments in combination of *P.lilacinum* in arsenic amended soil challenged by *Fusarium oxysporum.* The highest root fresh weight was recorded from the treatment T_3 (*P. lilacinum* + AMF) followed by T_2 (AMF) and T_1 (*P. lilacinum*) which were statistically similar. The highest root fresh weight for treatment T_3 was 5.36 g and for treatment T_2 and T_1 it was 4.59 g and 3.75 g respectively. The lowest root fresh weight 2.46 g was founded from the treatment T_6 (*P. lilacinum* + AMF + Arsenic) that was statistically similar to the treatment T_4 (*P. lilacinum* + Arsenic)and T_7 (Arsenic) where the root fresh weight were 2.47 g and 2.83 g respectively.

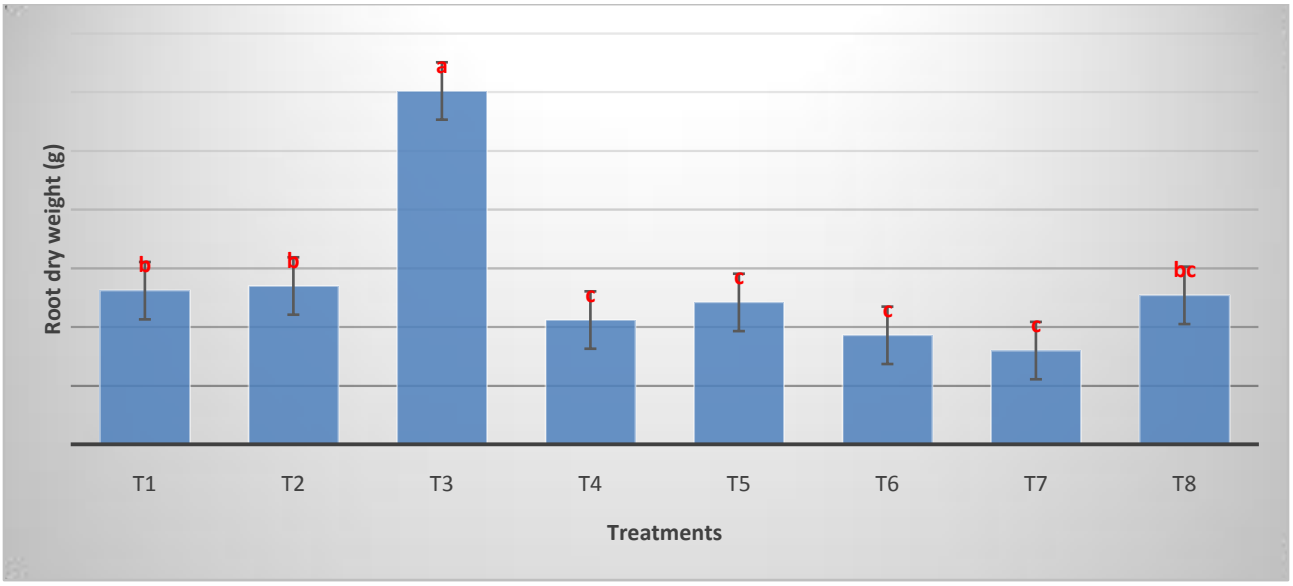


T_1=*Purpureocilliumlilacinum*, T_2=Arbuscular mycorrhizal fungi (AMF), T_3=*P. lilacinum* + AMF, T_4=*P. lilacinum* + arsenic, T_5=AMF + arsenic, T_6=*P. lilacinum* + AMF + arsenic, T_7=Arsenic, T_8=Chemical Fungicide (Bavistin 50 WP)

Figure 6. Root fresh weight of eggplant influenced by the eight treatments in combination of *P. lilacinum* with *Glomus* sp. in arsenic amended soil challenged by *Fusarium oxysporum*

4.7. Root dry weight

Root dry weight of eggplant influenced by the eight treatments in combination of *P.lilacinum* in arsenic amended soil challenged by *Fusarium oxysporum* is shown Figure 7. The highest root dry weight was recorded from the treatment T_3 (*P. lilacinum* + AMF) which was 3.01 g and not statistically similar toany of other treatments. The lowest root dry weight was found at treatment T_7 (Arsenic) which was 0.8 g and statistically different to other treatments.

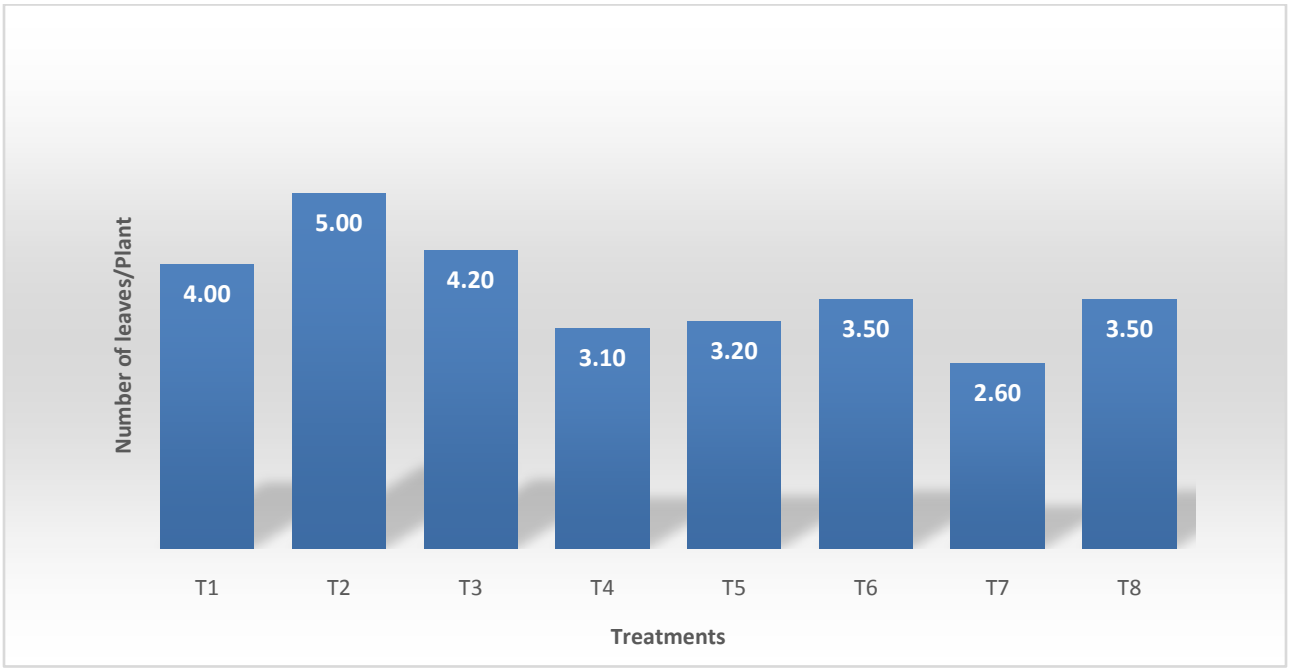


T_1=*Purpureocilliumlilacinum*, T_2=Arbuscular mycorrhizal fungi (AMF), T_3=*P. lilacinum* + AMF, T_4=*P. lilacinum* + arsenic, T_5=AMF + arsenic, T_6=*P. lilacinum* + AMF + arsenic, T_7=Arsenic, T_8=Chemical Fungicide (Bavistin 50 WP)

Figure 7. Root dry weight of eggplant influenced by the eight treatments in combination of *P. lilacinum* with *Glomus* sp. in arsenic amended soil challenged by *Fusarium oxysporum*

4.8. Number of leaves/Plant

Figure 8 shows the number of leaves/eggplant influenced by the eight treatments in combination of *P.lilacinum* in arsenic amended soil challenged by *Fusarium oxysporum.* The highest value was found in treatment T_2 (AMF) followed by the treatment T_3 (*P. lilacinum* + AMF) and treatment T_1 (*P. lilacinum*) with a result of 5.00, 4.20 and 4.00 respectively. The lowest value was showed by the treatment T_7 (Arsenic) followed by the treatment T_4 (*P. lilacinum* + Arsenic) and T_5 (AMF + Arsenic) with a result of 2.60, 3.10 and 3.20 respectively.

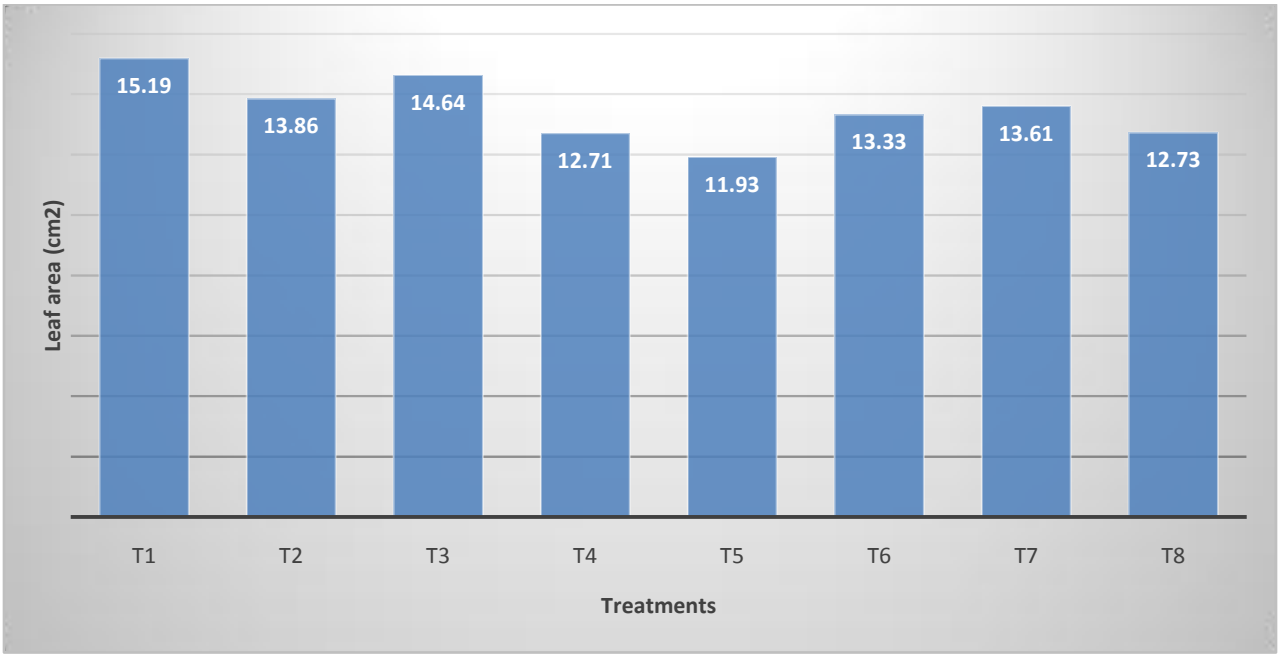


T_1=*Purpureocilliumlilacinum*, T_2=Arbuscular mycorrhizal fungi (AMF), T_3=*P. lilacinum* + AMF, T_4=*P. lilacinum* + arsenic, T_5=AMF + arsenic, T_6=*P. lilacinum* + AMF + arsenic, T_7=Arsenic, T_8=Chemical Fungicide (Bavistin 50 WP)

Figure 8. No of leaves/eggplant influenced by the eight treatments in combination of *P. lilacinum* with *Glomus* sp. in arsenic amended soil challenged by *Fusarium oxysporum*

4.9. Leaf Area

Leaf area of eggplant influenced by the eight treatments in combination of *P.lilacinum* in arsenic amended soil challenged by *Fusarium oxysporum* is shown Figure 9. Among the eight treatments highest leaf area was found in treatment T_1 (*P. lilacinum*) followed by T_3 (*P. lilacinum* + AMF)and T_2 (AMF) with a result of 15.18 cm^2, 14.64 cm^2 and 13.86 cm^2 respectively. Lowest leaf area was found in the treatment T_5 (AMF + Arsenic) with 11.93 cm^2. All the data of leaf area in all the treatments were statistically non-significant.

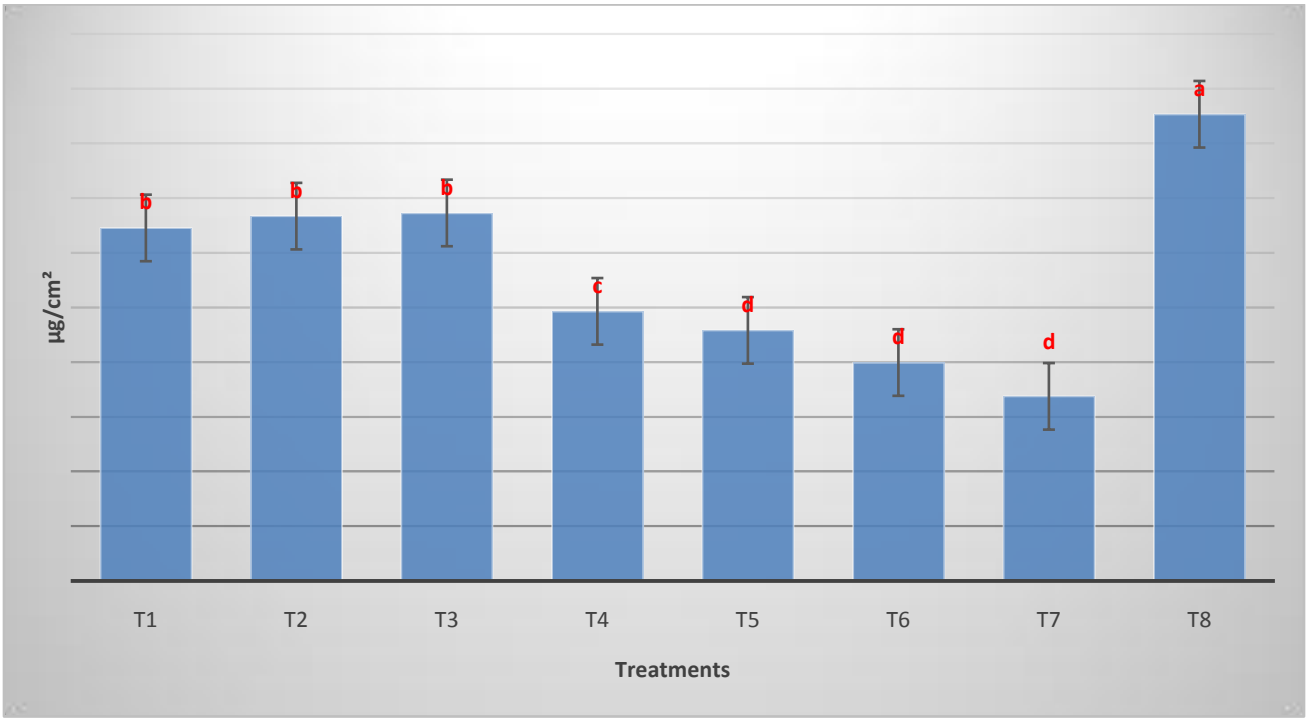


T_1=*Purpureocilliumlilacinum*, T_2=Arbuscular mycorrhizal fungi (AMF), T_3=*P. lilacinum* + AMF, T_4=*P. lilacinum* + arsenic, T_5=AMF + arsenic, T_6=*P. lilacinum* + AMF + arsenic, T_7=Arsenic, T_8=Chemical Fungicide (Bavistin 50 WP)

Figure 9. Leaf area of eggplant influenced by the eight treatments in combination of *P. lilacinum* with *Glomus* sp. in arsenic amended soil challenged by *Fusarium oxysporum*

4.10. Chlorophyll Content

Figure 10 shows the number of leaves/eggplant influenced by the eight treatments in combination of *P.lilacinum* in arsenic amended soil challenged by *Fusarium oxysporum.* The highest value was found in treatment T_8 (Chemical Control) which was statistically different from all other treatments with a result of 42.65 µg cm^{-2}.Lowest value of Chlorophyll content was found in the treatment T_7 (Arsenic) followed by the treatment T_6 (*P. lilacinum* + AMF + Arsenic) with a result of 16.87 µg cm^{-2} and 19.96 µg cm^{-2} respectively which was statistically different.

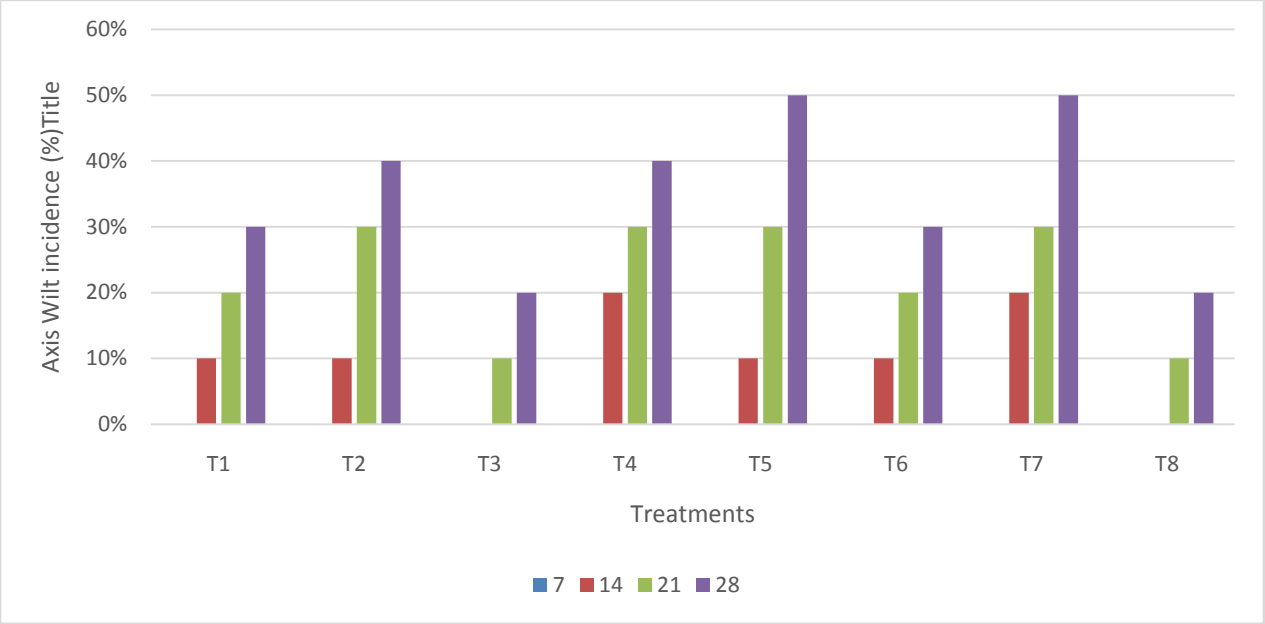


T_1=*Purpureocilliumlilacinum*, T_2=Arbuscular mycorrhizal fungi (AMF), T_3=*P. lilacinum* + AMF, T_4=*P. lilacinum* + arsenic, T_5=AMF + arsenic, T_6=*P. lilacinum* + AMF + arsenic, T_7=Arsenic, T_8=Chemical Fungicide (Bavistin 50 WP)

Figure 10. Chlorophyll content of eggplant influenced by the eight treatments in combination of *P. lilacinum* with *Glomus* sp. in arsenic amended soil challenged by *Fusarium oxysporum*

4.11. Wilt incidence

Wilt of eggplant influenced by the eight treatments in combination of *P. lilacinum* in arsenic amended soil challenged by *Fusarium oxysporum* is shown Figure 11. After 7 days it was showed that no wilting occur in any of this eight treatments. After 14 days treatment T_3 and T_8 showed zero wilting while treatment T_4 and T_7 showed highest 20% of wilting. After 21 days treatment T_3 and T_8 showed lowest wilting with 10% while treatment $T_{2,}$ $T_{4,}$ T_5 and T_7 showed the highest wilting with 30% of wilting. After 28 days treatment T_3 and T_8 showed lowest wilting with 20% while T_5 and T_7 showed the highest wilting with 50% of wilting.



T_1=*Purpureocilliumlilacinum*, T_2=Arbuscular mycorrhizal fungi (AMF), T_3=*P. lilacinum* + AMF, T_4=*P. lilacinum* + arsenic, T_5=AMF + arsenic, T_6=*P. lilacinum* + AMF + arsenic, T_7=Arsenic, T_8=Chemical Fungicide (Bavistin 50 WP)

Figure 11. Wilting of eggplant influenced by the eight treatments in combination of *P. lilacinum* with *Glomus* sp. in arsenic amended soil challenged by *Fusarium oxysporum*

4.12. CFU/g of soil of *Fusarium oxysporum* after harvest of the crop

In Figure 12, it shows CFU/g of soil for *Fusarium oxysporum* in eight different treatments. Initially 4.5×10^5 CFU/g soil of *Fusarium oxysporum* was applied to each of the treatments. After harvesting the amount of *Fusarium oxysporum*was found as:

T_1: 2.67×10^4 CFU/g soil

T_2: 2.67×10^4 CFU/g soil

T_3: 1.67×10^4 CFU/g soil

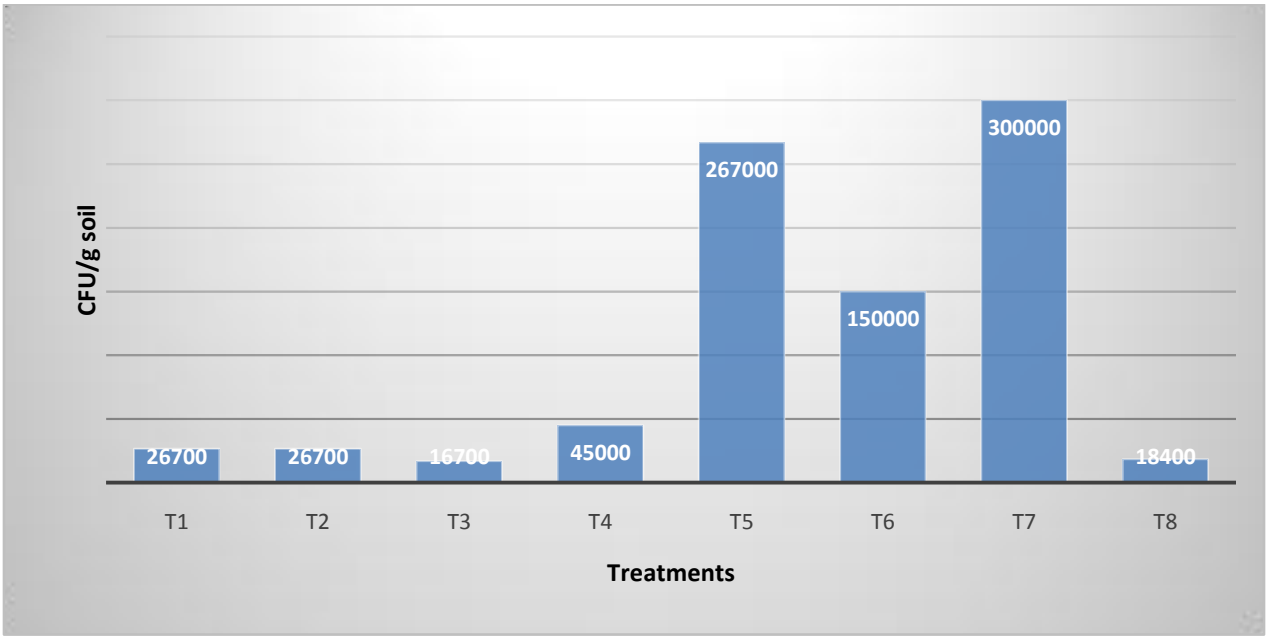
T_4: 4.5×10^4 CFU/g soil

T_5: 2.67×10^5 CFU/g soil

T_6: 1.5×10^5 CFU/g soil

T_7: 3×10^5 CFU/g soil

T_8: 1.84×10^4 CFU/g soil



T_1=*Purpureocilliumlilacinum*, T_2=Arbuscular mycorrhizal fungi (AMF), T_3=*P. lilacinum* + AMF, T_4=*P. lilacinum* + arsenic, T_5=AMF + arsenic, T_6=*P. lilacinum* + AMF + arsenic, T_7=Arsenic, T_8=Chemical Fungicide (Bavistin 50 WP)

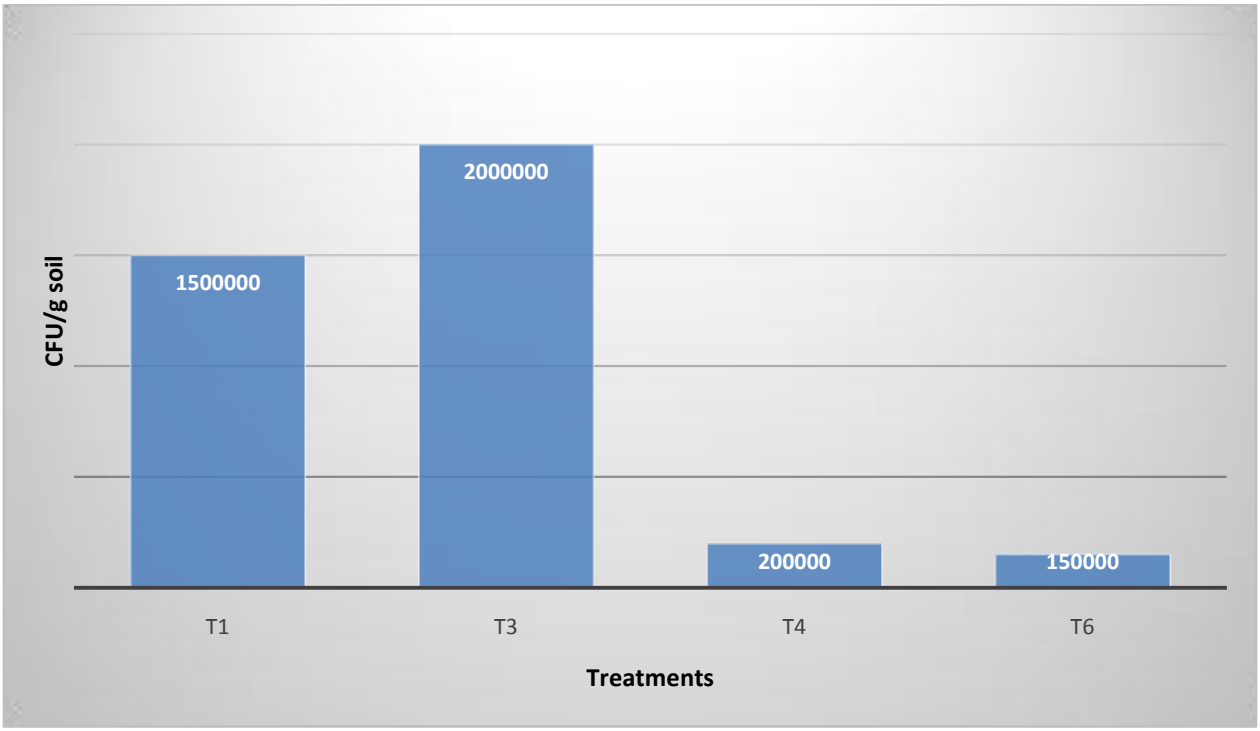
Figure 12. Amount of *Fusarium oxysporum* after harvesting in eight different treatments influenced by *P. lilacinum* with *Glomus* sp. in arsenic amended soil

4.13. CFU/g of soil of *Purpureocillium lilacinum* after harvest of the crop

In Figure 13, it shows CFU/g of soil for *Purpureocillium lilacinum* in four different treatments. Initially 2×10^6 CFU/g soil of *Purpureocillium lilacinum*was applied to each of the treatments. After harvesting the amount of *Purpureocillium lilacinum* was found like below:

T_1: 1.5×10^6 CFU/g soil T_3: 2×10^6 CFU/g soil

T_4: 2×10^5 CFU/g soil T_6: 1.5×10^5 CFU/g soil



T_1=*Purpureocillium lilacinum*, T_3=*P. lilacinum* + AMF, T_4=*P. lilacinum* + arsenic, T_6=*P. lilacinum* + AMF + arsenic

Figure 13. Amount of *Purpureocillium lilacinum* after harvesting in four different treatments combined with *Glomus* sp. challenged by *Fusarium oxysporum* in arsenic amended soil

4.14. Root and soil colonization of Arbuscular Mycorrhizal Fungi (AMF) at harvest of the crop

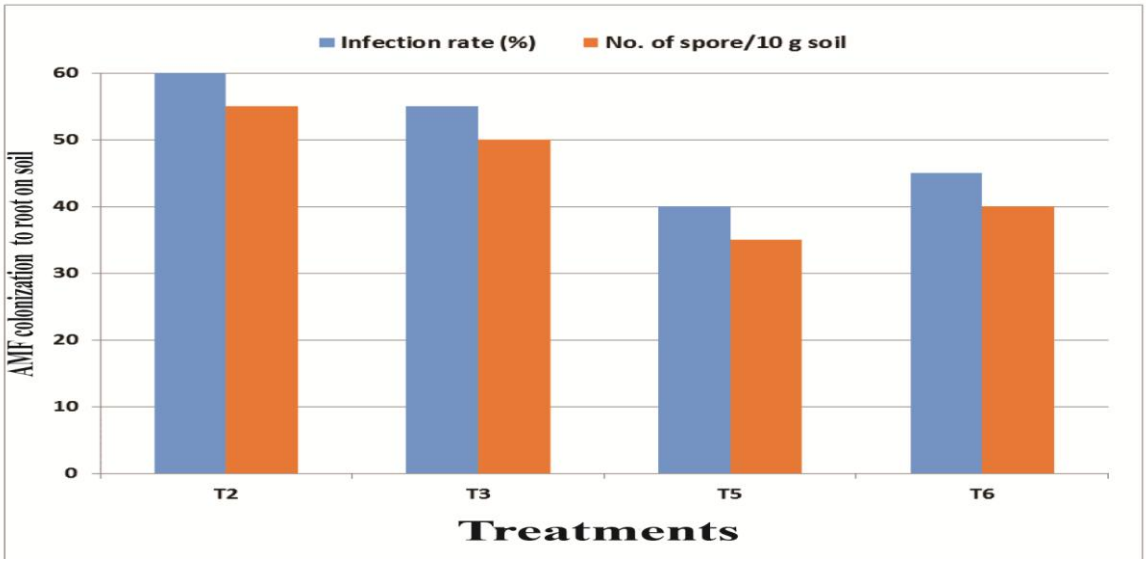
In Figure 14, it shows the infection rate and number of spore of AMF after harvesting where AMF was applied. Initial application of AMF was 25g/plant with 70% infection rate and 65 spore/10 g of soil. After harvesting it was found like below:

T_2: Infection rate- 55%, number of spore- 55/10 g soil

T_3: Infection rate- 50%, number of spore- 45/10 g soil

T_5: Infection rate- 40%, number of spore- 35/10 g soil

T_6: Infection rate- 45%, number of spore- 35/10 g soil



T_2=Arbuscular mycorrhizal fungi (AMF), T_3=*P. lilacinum* + AMF, T_5=AMF + arsenic, T_6=*P. lilacinum* + AMF + arsenic, T_7=ArsenicFigure

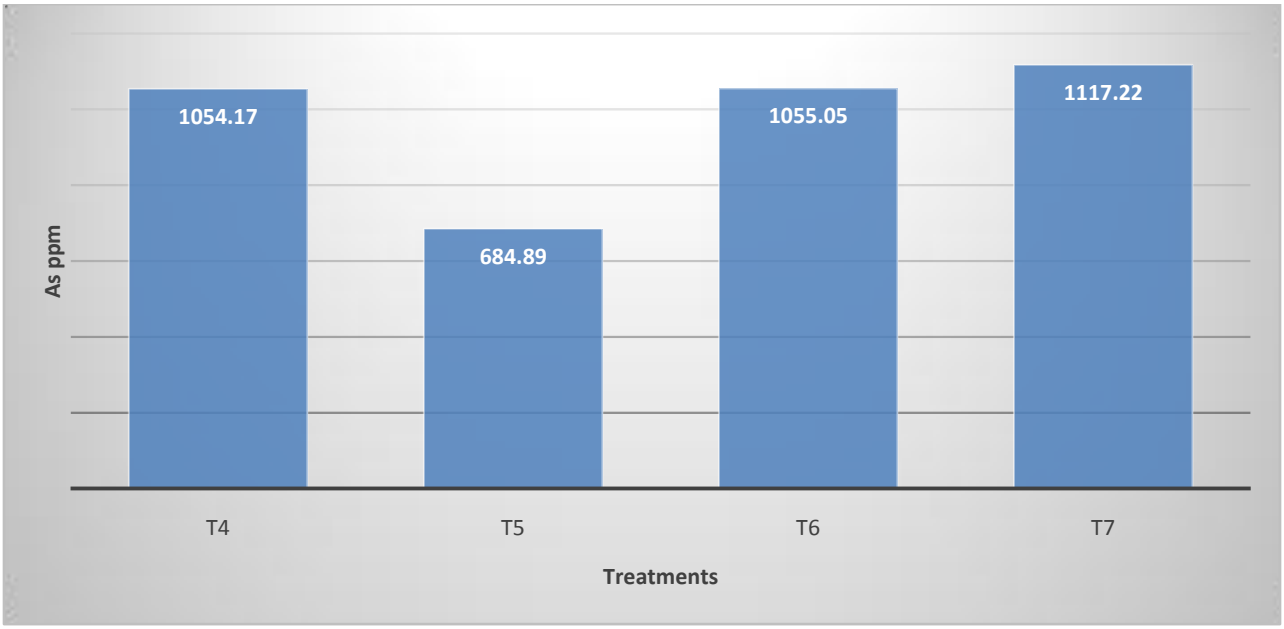
14. Infection rate and number of spore of AMF in eggplant influenced by the four treatments in combination of *P. lilacinum* in arsenic amended soil challenged by *Fusarium oxysporum*

4.15. Arsenic uptake by shoot of eggplant

In figure 15, it shows the amount of arsenic taken by dry shoot of eggplant after harvesting. 50 ppm arsenic was applied at the defined treatments. After harvesting it was found as:

T_4: 1054.17 ppm T_5: 684.89 ppm

T_6:1055.05 ppm T_7: 1117.22 ppm



T_4=*P. lilacinum* + arsenic, T_5=AMF + arsenic, T_6=*P. lilacinum* + AMF + arsenic, T_7=Arsenic

Figure 15: Effect of AMF and *P. lilacinum* on arsenic uptake by eggplant shoot.

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In this experiment eight treatments were applied where *P. lilacinum* and AMF in combined condition showed better result than the other treatments. AMF has a remarkable influence on plant growth, nutrient uptake and reducing arsenic toxicity. In arsenic amended soil AMF reduce the plant arsenic uptake in contrast to uninoculated plant. It triggers several channel to bypass the arsenic during nutrient uptake as well as produce compartment inside the cell to accumulate to reduce arsenic toxicity. AMF when artificially is inoculated in arsenic contaminated soil, it strengthens plant to face the adversity as a result it can tolerate the adverse situation and can grow as a healthy plant. Due to this mechanism plant growth and nutrient uptake is increased. It is well known that *P.lilacinum* is a nematophagus fungi but it has also a mechanism to control other fungus like *Fusarium.*

Among ten growth parameters like root length, root fresh weight, root dry weight, shoot length, shoot fresh weight, shoot dry weight, shoot diameter, leaf area, number of leaves/plant and chlorophyll content in leaves, six of the parameters showed the best result when they are treated with both *P. lilacinum* and AMF. Abdul (2005) reported the same result that plants with different doses of VAM fungi, *Glomus mosseae* and *G. fasciculatum* reduce pathogenic effect of *Fusarium oxysporum* and improve plant growth and chlorophyll content of brinjal plants. Rao *et al.* (2001) also found the same result that plant height, shoot weight, root weight and root length were significantly greater in the case of eggplants treated with both *G.mosseae and P. lilacinus*.

The growth parameter, number of leaves showed better result with single application with *P. lilacinum*. In some cases individual effect of these bio-agent on growth parameter was more or less similar like the combined effect. Haseeb and

Kumar (2007) examined the efficacy of bio-agents and it showed clearly indicate that all the bio-agents were found highly effective.

Another growth parameter like Chlorophyll content was found higher in chemical fungicide Bavistin 50WP treated plant. Ramesh and Manjunath (2002) used *Trichoderma* spp., 0.2% Carbendazim and 0.3% Copper Oxychloride for management of wilt disease (caused by *Ralstonia solanacearum, Fusarium oxysporum* and *Verticillium dahliae*) on eggplant. However the highest yield was obtained with Carbendazim (11.88 t/ha). Chlorophyll content contains the micronutrient Mn which is an important element. When chemical fungicide applied to eggplant to control pests the micronutrient Mn works as a catalyst. As a result it increases the amount of chlorophyll content in plant leaves.

In this experiment two growth parameters showed non-significant result which were leaf area and shoot diameter.

In case of wilt incidence, in this experiment the wilting percentage was observed after a regular intervals with 7 days. After 28 days of transplanting the plants which were treated with combination of both *P. lilacinum* and AMF and only chemical fungicide, showed the lowest wilting percentage while the treatment included arsenic showed the highest disease incidence. Wei Tang *et al.* (2004) observed the effect of seven fungicides against wilt pathogen *Fusarium oxysporum*. They used Prochloraz, Carbendazim, Thiram, Toclofos-Methyl, Hymexazol, Azoxystrobin and Carboxin. Where Prochloraz and Carbendazim were the most effective fungicides in inhibiting mycelial growth. The preventive effect was 87.0% after 5 mg/ml Carbendazin was added to the liquidmedia for 2 weeks with a curative effective of 34.4%.

In arsenic amended soil when treatment combination was with *Glomus* then, the arsenic uptake by eggplant is reduced upto 50%. This result was due to the

potentiality of mycorrhiza to reduce the arsenic toxicity of the plant that was revealed by Kelker *et al.*(2013) who carried out an experiment where soils with different concentrations of arsenic with and without mycorrhizal inoculums were tested in *Trigonella foenumgraceum*. The response of mycorrhiza in *T. foenumgraceum*wasdetermined in terms of percentage germination of seeds, sustainability of seedlings, fresh weight and dry weight of plants etc. It was observed that in the pot with soil contaminated with arsenic and no mycorrhizal inoculum, performance was very bad in terms of all aspects of growth, whereas in the pot where mycorrhizal inoculum was added along with contaminated soil, the performance of the plant was better. Present findings are in similar with Elahi *et al.* (2010). They carried out an experiment to determine the influence of AMF inoculation on growth, nutrient uptake, arsenic toxicity of eggplant grown in arsenic amended soil. The findings of the study indicated that AMF inoculation not only reduces arsenic toxicity but also can increase growth and nutrient uptake of eggplant shoot.

Plant uptake arsenic in two ionic forms, such as: As^{5+} and $As^{3+.}$ Eggplant when uptake arsenic in As^{5+} form it increases the toxicity of arsenic in eggplant. AMF when inoculated in eggplant root it converts arsenic As^{5+} to As^{3+} and for this conversion AMF helps eggplant to uptake less arsenic in arsenic contaminated soil.

It was seen that treatment T_5 (AMF+ Arsenic) showed the best result with almost 50% less arsenic uptake than the other three treatments where arsenic was applied as well.

CHAPTER 5

SUMMARY AND CONCLUSION

The experiment was conducted to control the *Fusarium* wilt effectively in arsenic amended soil with the influence of *P. lilacinum* and AMF. Chemical fungicide (Bavistin 50 WP) was also applied in one treatment among eight treatments. The efficacy of the controlling agents was measured on the basis of growth parameter and wilting percentage of eggplant. Among the various growth parameters the result was various and wilt incidence was also varies among treatments.

In case of root length, the treatment which is in combination with both *P. lilacinum* and AMF (T_3) showed the best result with 16.80 cm of length while the treatment which carry only arsenic (T_7) showed lowest root length with 6.80 cm of root length.

In case of root fresh weight, the treatment which is in combination with both *P. lilacinum* and AMF (T_3) showed the best result with 5.36 g of weight while the treatment which carry arsenic in combination with both *P.lilacinum* and AMF (T_6) showed lowest root fresh weight with 2.46 g of root fresh weight.

In case of root dry weight, the treatment which is in combination with both *P. lilacinum* and AMF (T_3) showed the best result with 3.01 g of weight while the treatment which carry only arsenic (T_7) showed lowest root dry weight with 0.8 g of root dry weight.

The treatment which is in combination with both *P. lilacinum* and AMF (T_3) showed the best result with 15.70 cm of length while the treatment which carry only arsenic (T_7) showed lowest shoot length with 8.60 cm of shoot length.

In case of shoot diameter, the treatment which carry chemical fungicide (T_8) showed the highest result with 3.25 cm while the treatment which is combined with *P. lilacinum* and arsenic (T_4) showed the lowest shoot diameter with 2.55 cm.

Among eight treatments, treatment T_3 (*P. lilacinum* + AMF) showed best result for shoot fresh weight with 11.60 g of weight while treatment T_5 (AMF + arsenic) showed the lowest result with 3.33 g of weight.

Among eight treatments, treatment T_3 (*P. lilacinum* + AMF) showed best result for shoot dry weight with 1.50 g of weight while treatment T_7 (Arsenic) showed the lowest result with 0.39 g of weight.

In case of no. of leaves per plant treatment T_2 (AMF) showed best result with 5.00 while treatment T_7 (Arsenic) showed the lowest result with 2.60.

Among eight treatments, treatment T_1 (*P. lilacinum*) showed best result for leaf area with 15.19 cm^2 while treatment T_5 (AMF + arsenic) showed the lowest result with 11.19 cm^2.

Chlorophyll content showed the highest result in the treatment chemical fungicide (T_8) highest result with 42.65 µg cm^{-2} while the treatment which carry only arsenic (T_7) showed the lowest result with 16.87 µg cm^{-2} of Chlorophyll.

In case of wilt percentage, treatment T_3 (*P. lilacinum* + AMF) and T_8 showed the lowest wilt percentage (20%) while treatment T_5 (AMF + Arsenic) and treatment T_7 (Arsenic) showed the highest wilting percentage (50%).

Considering the overall performance of the treatments applied in the experiment in controlling Fusarium wilt of eggplant, application of *Purpureocillium lilacinum*in combination with AMF could be used as eco-friendly approach and may be advised to the farmers for profitable production. The chemical Bavistin 50 WP

could be used for controlling the disease as the last option. However, further study need to be carried out for consecutive years for including more options as management practices in different Agro Ecological Zones (AEZs) of the country.

CHAPTER 6

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APPENDIX-1

Table 1: Influence of *Purpureocillium lilacinum* in combination with *Glomus* sp. on root length, root fresh weight, root dry weight, shoot length, shoot diameter, shoot fresh weight, shoot dry weight of eggplant in arsenic amended soil challenged by *Fusarium oxysporum*

Treatments	Root Length (cm)	Root Fresh Weight (gm)	Root Dry Weight (gm)	Shoot Length (cm)	Shoot Diameter (cm)	Shoot Fresh Weight (gm)	Shoot Dry Weight (gm)
T_1	14.35a	3.75abc	1.31b	12.60abc	2.85	5.92b	0.68b
T_2	15.55a	4.59ab	1.35b	13.40ab	2.95	6.43b	0.76b
T_3	16.80a	5.36a	3.01a	15.70a	3.00	11.60a	1.5a
T_4	9.20bc	2.47c	1.06bc	8.90cde	2.55	4.19bc	0.53b
T_5	7.20c	2.95bc	1.21bc	8.70de	2.90	3.33c	0.58b
T_6	8.00c	2.46c	0.93bc	10.50bcde	2.90	4.80bc	0.46b
T_7	6.80c	2.83c	0.8c	8.60e	2.80	3.38c	0.39b
T_8	13.75ab	3.01bc	1.27bc	12.40abcd	3.25	5.09bc	0.59b
$LSD_{(0.05)}$	4.94	1.64	0.50	3.75	NS	2.47	0.49

APPENDIX-2

Table 2: Influence of *Purpureocillium lilacinum* in combination with *Glomus* sp on leaf number, leaf area and Chlorophyll content of eggplant in arsenic amended soil challenged by *Fusarium oxysporum*

Treatments	**No. of Leaves Per Plant**	**Leaf Area (cm^2)**	**Chlorophyll Content** (μgcm^{-2})
T_1	4.00abc	15.19	32.26b
T_2	5.00a	13.86	33.34b
T_3	4.20ab	14.64	33.63b
T_4	3.10bc	12.71	24.64c
T_5	3.20bc	11.93	22.90cd
T_6	3.50abc	13.33	19.96cd
T_7	2.60c	13.61	16.87d
T_8	3.50abc	12.73	42.65a
$LSD_{(0.05)}$	1.51	NS	6.87