

1-13/14

ROLE OF MYCORRHIZA ON GROWTH AND NUTRIENT UPTAKE BY DIFFERENT AGRICULTURAL CROPS IN ARSENIC AMENDED SOIL

BY
FERDOUS-E-ELAHI

শেরেবাংলা কৃষি বিশ্ববিদ্যালয়
সংযোজন নং 58
তারিখ 25/01/10

Sher-e-Bangla Agricultural University
Library

Accession No. 37382

Sign: *[Signature]* Date: 12-12-13



571.92
E112
2008

**DEPARTMENT OF PLANT PATHOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA-1207**

x, 124 p.

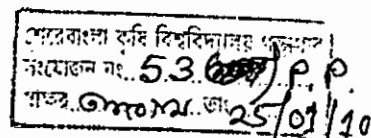
DECEMBER, 2008



37382

**ROLE OF MYCORRHIZA ON GROWTH AND
NUTRIENT UPTAKE BY DIFFERENT AGRICULTURAL
CROPS IN ARSENIC AMENDED SOIL**

BY



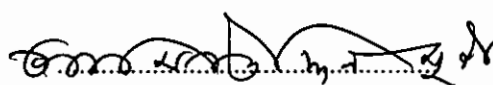
REGISTRATION NO. 03-01148


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' 2008**



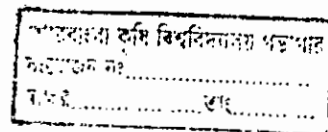
Approved by:


.....
(Prof. Dr. Md. Amin Uddin Mridha)
Vice Chancellor
Pabna University of
Science and Technology
Supervisor


.....
(Dr. F. M. Aminuzzaman)
Assistant Professor
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Co-Supervisor


.....
(Dr. M. Salahuddin M. Chowdhury)
Chairman
Examination Committee
Department of Plant Pathology

Prof. Dr. Md. Amin Uddin Mridha
Vice Chancellor
Pabna University of
Science and Technology
Pabna, Bangladesh
Phone- 0088028931063 (Off.), Mobile: 01731896661
E-mail: mridha52@gmail.com



Ref: _____

Date: _____

CERTIFICATE

This is to certify that thesis entitled, "ROLE OF MYCORRHIZA ON GROWTH AND NUTRIENT UPTAKE BY DIFFERENT AGRICULTURAL CROPS IN ARSENIC AMENDED SOIL" 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, embodies the results of a piece of bona fide research work carried out by FERDOUSE ELAHI, Registration No. 03-01148 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma in anywhere.

I further certify that any help or sources of information, as have been availed of during the course of this investigation, have duly been acknowledged.

Dated: December, 2008
Place: Dhaka, Bangladesh


(Prof. Dr. Md. Amin Uddin Mridha)
Supervisor

DEDICATED

TO



MY BELOVED

PARENTS

SOME COMMONLY USED ABBREVIATIONS AND SYMBOLS

Abbreviations	=	Full word
%	=	Percentage
°C	=	Degree Celsius
AMF	=	Arbuscular Mycorrhizal Fungi
As	=	Arsenic
BADC	=	Bangladesh Agricultural Development Corporation
BBS	=	Bangladesh Bureau of Statistics
BRRRI	=	Bangladesh Rice Research Institute
cm	=	Centi-meter
%CV	=	Percentage of Coefficient of Variation
DAS	=	Days After Sowing
DMRT	=	Duncan's Multiple Range Test
<i>et al.</i>	=	And others
g	=	Gram (s)
hr.	=	Hour (s)
J.	=	Journal
K	=	Potassium
kg	=	kilogram (s)
L	=	Liter
N	=	Nitrogen
N	=	Normal
NS	=	Non Significant
P	=	Phosphorus
ppm	=	Parts Per Million
PSI	=	Pressure Per Square Inch
S	=	Sulphur
VAM.	=	Vesicular Arbuscular Mycorrhiza
µg	=	Microgram

ACKNOWLEDGEMENT

All the praises and gratitude are due to the omniscient, omnipresent and omnipotent Almighty Allah, who has kindly enabled the author to complete this research work and complete this thesis successfully for increasing knowledge and wisdom.

The author sincerely desires to express her deepest sense of gratitude, respect, profound appreciation and indebtedness to her research Supervisor, Professor Dr. Md. Amin Uddin Mridha, Vice Chancellor, Pabna University of Science and Technology, Pabna, Bangladesh for his kind and scholastic guidance, untiring effort, valuable suggestions, inspiration, co-operation and constructive criticisms throughout the entire period of the research work and the preparation of the manuscript of this thesis.

The author expresses heartfelt gratitude and indebtedness to her Co-supervisor, Dr. F. M. Aminuzzaman, Assistant Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for his co-operation, criticisms on the manuscript and helpful suggestions for the successful completion of the research work.

The author expresses her deepest respect and boundless gratitude to Mrs. Nasim Akhtar, Professor and Chairman, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for her sympathetic co-operation and inspiration throughout the research work.

The author is thankful to Professor Mohammad Hossain Bhuiyan, Co-ordinator (CASR) for providing necessary facilities and conducive atmosphere to accomplish his research work.

Special thanks and indebtedness to all the respective teachers of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for their valuable teaching, sympathetic co-operation and inspiration throughout the period of the study.

The author thankfully remembers the students of the Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for their cooperation in the entire period of study. The author also extends her thanks to all the staff of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for their help and co-operation during the research work.

The author also likes to give thanks to all of her friends for their support and inspiration throughout her study period in Sher-e-Bangla Agricultural University, Dhaka.

The author also express thanks to A. K. M. Yousuf Hanun, Nasir, Shahidul, Gazi, Hasnan, Nibir da, Mintu vai, Ibrahim vai, Momtaz Vai, Srabony, Shukti, Bony apu, Romana Papri apu, Esna, Atifa and Bonita apu for their cordial support, co-operation and inspiration in preparing this thesis.

Finally, the author found no words to thank her parents and her elder brothers and sisters for their unquantifiable love and continuous support, their sacrifice, never ending affection, immense strength and untiring efforts for bringing her dream to proper shape. They were constant source of inspiration, zeal and enthusiasm in the critical moment of her studies.

Dated: December, 2008.

The author



Place: Sher-e-Bangla Agricultural University, Dhaka

CONTENTS

CHAPTER	TITLE	PAGE NO.
	ABBREVIATIONS	i
	ACKNOWLEDGEMENT	ii
	LIST OF CONTENTS	iv
	LIST OF TABLES	v
	LIST OF FIGURES	ix
	ABSTRACT	x
I	INTRODUCTION	1
II	REVIEW OF LITERATURE	6
	2.1 Role of Mycorrhiza in different agricultural crops	6
	2.2 Arsenic and Mycorrhiza interaction	12
	2.3 Arsenic and chlorophyll interaction	16
III	MATERIALS AND METHODS	19
	3.1. Study of root colonization	19
	a. Selection of location	19
	b. Period of collection	19
	c. collection of root samples	19
	d. Cleaning and preservation of roots Field experiment	20
	e. Staining of roots	20
	f. Observation of roots	20
	g. Estimation of intensity of infection of AMF	21
	3.2. Study of spore population in soil	21
	3.3 Plant growth in polybag	21
	a. Collection of soil	22
	b. Preparation of soil	22
	c. Mycorrhizal assessment	22
	d. Staining of roots	22
	e. Preparation of inoculums	22
	f. Preparation of arsenic solution	24
	g. Selection of crops	24
	h. Collection of crops	24
	3.4 Role of arsenic on seed germination	25
	a. Treatments	25

	b. Preparation of seedling bags	25
	c. Sowing of seeds	26
	d. Intercultural operation	26
	e. Harvesting	26
	f. Data recording	27
	g. Assessment of root colonization	27
	h. Chemical analysis of plant sample	28
	i. Nutrient analysis	28
	ii. Arsenic analysis	29
	iii. Chlorophyll analysis	32
	i. Statistical analysis	32
IV	RESULTS	33
	4.1. Study of root clonization	33
	a. Saiyad pur Village	33
	b. Rup pur village	34
	c. Masum dia Village	36
	d. Vati koa Village	38
	4.2 Role of arsenic on seed germination	39
	A. Brinjal	41
	B Tomato	54
	C. Chili	67
	D. Danta	80
	E. Okra	94
V	DISCUSSION	107
VI	SUMMARY AND CONCLUSION	113
VII	REFERENCES	116



LIST OF TABLES

Table No.	Title of the Tables	Page No.
1	Percent intensity of infection of AMF on root of different agricultural crops collected from Saiyad pur village of Pabna district	34
2	Percent intensity of infection of AMF on root of different agricultural crops collected from Rup pur village of Pabna district	36
3	Percent intensity of infection of AMF on root of different agricultural crops collected from Masum dia village of Pabna district	38
4	Percent intensity of infection of AMF on root of different agricultural crops collected from Vati koa village of Pabna district	39
5	Influence of AMF inoculation on germination percentage, number of leaves, shoot height and root length of brinjal at different growth periods in soil amended with different concentrations of arsenic solution	43
6	Influence of AMF inoculation on fresh and dry weight of shoot and root of brinjal at different growth periods in soil amended with different concentrations of arsenic solution	46
7	Influence of AMF inoculation on percent vigourity of brinjal at different growth periods in soil amended with different concentrations of arsenic solution	48
8	Influence of AMF inoculation on nutrient uptake and arsenic uptake by shoot and chlorophyll content by leaves of brinjal at 60 DAS in soil amended with different concentrations of arsenic solution	51

9	Root colonization (%) in brinjal by the influence of AMF inoculation at different growth periods in soil amended with different concentrations of arsenic solution	52
10	Influence of AMF inoculation on germination percentage, number of leaves, shoot height and root length of tomato at different growth periods in soil amended with different concentrations of arsenic solution	56
11	Influence of AMF inoculation on fresh and dry weight of shoot and root of tomato at different growth periods in soil amended with different concentrations of arsenic solution	60
12	Influence of AMF inoculation on percent vigourity of Tomato at different growth periods in soil amended with different concentrations of arsenic solution	62
13	Influence of AMF inoculation on nutrient uptake and arsenic uptake by shoot and chlorophyll content by leaves of tomato at 60 DAS in soil amended with different concentrations of arsenic solution	65
14	Root colonization (%) in tomato by the influence of AMF inoculation at different growth periods in soil amended with different concentrations of arsenic solution	66
15	Influence of AMF inoculation on germination percentage, number of leaves, shoot height and root length of chili at different growth periods in soil amended with different concentrations of arsenic solution	70
16	Influence of AMF inoculation on fresh and dry weight of shoot and root of chili at different growth periods in soil amended with different concentrations of arsenic solution	73

17	Influence of AMF inoculation on percent vigourity of chili at different growth periods in soil amended with different concentrations of arsenic solution	75
18	Influence of AMF inoculation on nutrient uptake and arsenic uptake by shoot and chlorophyll content by leaves of chili at 60 DAS in soil amended with different concentrations of arsenic solution	77
19	Root colonization (%) in chili by the influence of AMF inoculation at different growth periods in soil amended with different concentrations of arsenic solution	79
20	Influence of AMF inoculation on germination percentage, number of leaves, shoot height and root length of danta at different growth periods in soil amended with different concentrations of arsenic solution	83
21	Influence of AMF inoculation on fresh and dry weight of shoot and root of danta at different growth periods in soil amended with different concentrations of arsenic solution	87
22	Influence of AMF inoculation on percent vigourity of danta at different growth periods in soil amended with different t concentrations of arsenic solution	88
23	Influence of AMF inoculation on nutrient uptake and arsenic uptake by shoot and chlorophyll content by leaves of danta at 60 DAS in soil amended with different concentrations of arsenic solution	91
24	Root colonization (%) in danta by the influence of AMF inoculation at different growth periods in soil amended with different concentrations of arsenic solution	82

25	Influence of AMF inoculation on germination percentage, number of leaves, shoot height and root length of okra at different growth periods in soil amended with different concentrations of arsenic solution	96
26	Influence of AMF inoculation on fresh and dry weight of shoot and root of okra at different growth periods in soil amended with different concentrations of arsenic solution	100
27	Influence of AMF inoculation on percent vigourity of okra at different growth periods in soil amended with different concentrations of arsenic solution	102
28	Influence of AMF inoculation on nutrient uptake and arsenic uptake by shoot and chlorophyll content by leaves of okra at 60 DAS in soil amended with different concentrations of arsenic solution	105
29	Root colonization (%) in okra by the influence of AMF inoculation at different growth periods in soil amended with different concentrations of arsenic solution	106



LIST OF FIGURES

Figure No.	Title of the figures	Page No.
1	<i>Leucas aspera</i> plant	23
2	<i>Glomus intraradices</i> found in <i>Leucas aspara</i> plant	23
3	Percent AMF infection of different Agricultural crops root collected from Saiyad pur village of Pabna district	33
4	Percent AMF infection of different Agricultural crops root collected from Rup pur pur village of Pabna district	35
5	Percent AMF infection of different Agricultural crops root collected from Masum dia village of Pabna district	37
6	Percent AMF infection of different Agricultural crops root collected from Vati koa village of Pabna district	39
7	Role of mycorrhiza on germination and seedling growth of brinjal in arsenic treated soil	44
8	Role of mycorrhiza on germination and seedling growth of tomato in arsenic treated soil	53
9	Role of mycorrhiza on germination and seedling growth of chili in arsenic treated soil	67
10	Role of mycorrhiza on germination and seedling growth of danta in arsenic treated soil	80
11	Role of mycorrhiza on germination and seedling growth of okra in arsenic treated soil	93



Role of Mycorrhiza on growth and nutrient uptake by different agricultural crops in arsenic amended soil

By

Registration No. 03 01148

ABSTRACT

The present experiment was carried out to determine the role of mycorrhizal fungi on growth and nutrient uptake by different agricultural crops in arsenic amended soil. This experiment was also performed to know the mycorrhizal status of crops collected from different arsenic affected area of Pabna district. Five crops viz. Tomato, Okra, Brinjal, Chili, and Danta were grown in arsenic amended soils with or without mycorrhizal inoculation. Three levels of arsenic concentrations (10ppm, 100ppm and 500ppm) were used. At higher concentration of arsenic, the seed germination was affected more than the other treatments. In case of all the five crops; Tomato, Okra, Brinjal, Chili, and Danta the germination percentage, plant height, shoot height, root length, number of leaves, both fresh and dry weight of shoots and roots, mycorrhizal root colonization, percent vigor and the amount of chlorophyll were higher in AMF inoculated plants in comparison to the non inoculated plants and decreased significantly with the increase rate of arsenic concentrations. Less arsenic content and higher nutrient uptake were recorded in mycorrhiza inoculated plants.

Chapter 1

Introduction

CHAPTER 1

INTRODUCTION

Arsenic is a ubiquitous metalloid that is introduced in to the environment from both anthropogenic and geochemical sources (Smith *et al.*, 1998). In Bangladesh, arsenic contamination of groundwater is believed to cause arsenic-related disorders in 80% of the population (Alam *et al.*, 2002; Das *et al.*, 2004).

During the last seven years, clinical symptoms relating to arsenic toxicity have been detected in millions of rural Bangladeshis. Arsenic can be introduced to food through plant uptake in soil contaminated by groundwater or irrigation water. It enters the living biota through biogeochemical and biochemical pathways. Livestock feeding on arsenic contaminated feeds will accumulate this element with potential of arsenic to be transferred to humans. In Bangladesh, the groundwater arsenic contamination problem is the worst in the world. High levels of As in groundwater are causing widespread poisoning in Bangladesh. The World Health Organization (WHO) recommends a safe limit for As in drinking water of $10 \mu\text{g L}^{-1}$. A recent survey looked at the As concentrations of drinking water from deep wells in 64 districts in the country and found that 59 had concentrations $>10 \mu\text{g L}^{-1}$ and 43 had concentrations $>50 \mu\text{g L}^{-1}$. Contaminated groundwater is also used for irrigation of paddy rice, and other agricultural crops.

Brinjal (*Solanum melongena*) belongs to the family Solanaceae is commonly used as a popular vegetable in Bangladesh. It grows well during winter in Bangladesh. Moreover, in Bangladesh, about 28827 hectares of land was under Brinjal cultivation and total production was about 222110 metric tons (BBS, 2007).

Tomato (*Lycopersicon esculentum*) is a vegetable fruit crops belonging to the family of Solanaceae. Nowadays, it is a popular vegetable and grown in winter throughout the country. In Bangladesh, about 19417 hectares of land was under tomato cultivation and total production was about 136935 metric tons (BBS, 2007).

Okra (*Abelmoscus esculentus*) belongs to the family of Malvaceae. It contains Ca, Protein, Beta carotene and Vit-C. In Bangladesh, about 9384 hectares of land was under Okra cultivation and total production was about 38715 metric tons (BBS, 2007).

Chili (*Capsicum frutescens*) belongs to the family of Solanaceae. It contains Vit- A and Vit-C. It is a very popular and essential spice in our country. In Bangladesh, about 294245 acres of land was under chili (winter) cultivation and 22362 hectares of land was under summer variety cultivation. Moreover, total production for winter and summer varieties were about 130715 and 23240 metric tons respectively (BBS, 2007).

Danta (*Amaranthus oleraceus*) is a leafy vegetable belonging to the family of Amaranthaceae. In Bangladesh, about 9089 hectares of land was under Danta cultivation and total production was about 56715 metric tons (BBS, 2007).

Arbuscular mycorrhiza (AM) fungi are vital components of nearly all terrestrial ecosystems, forming mutually beneficial (mutualistic) symbiosis with the roots of around 80% of vascular plants and often increasing phosphate (P) uptake and growth. Since the association is mutualistic, both organisms benefit from the association. The fungus receives carbohydrates (sugars) and growth factors from the plant, which in turn receives many benefits, including increased nutrient absorption. In this association, the fungus takes over the role of the plant's root hairs and acts as an extension of the root system.



Arbuscular mycorrhiza (AM) is known to play an important role in promoting and sustaining vegetable productivity even under adverse environmental conditions (Smith and Read, 1997). A major function of these fungi is to increase the surface area of plant root systems, greatly facilitating uptake of soil water and nutrients, especially in harsh conditions. In particular AM fungi can greatly enhance the uptake of PO_4 , as well as NH_4^+ , K^+ , and NO_3^- (Marschner and Dell, 1994; Hayman, 1983). The external fungal hyphae act as a bridge transporting slow diffusing nutrients like P more effectively than those of non-mycorrhizal ones.

The positive role of the vesicular-arbuscular mycorrhizal (VAM) fungi in P uptake and plant growth response under P-deficient conditions has been well established for many agricultural systems (Mosse, 1973). In the tropics many crops are grown in infertile acid soils, where their establishment is frequently limited by low levels of available phosphorus. In such soils, an efficient mycorrhizal association can increase phosphorus uptake and crop yield (Howeler *et al.*, 1987). Vesicular-arbuscular mycorrhizas are also important for N uptake to stimulate the growth and nutrition of plants and are of great ecological importance with regards to N-nutrition of plant, especially non-fixing species (Barea, 1991).

Mycorrhiza helps in the formation of soil aggregation and aggregate stability (Miller and Jastrow, 1994). Arbuscular mycorrhizae increase plant productivity by increasing the rate of photosynthesis (Masri, 1997; Syvertsen and Graham, 1999) and providing protection against toxic metals (Bonifacio *et al.*, 1999). As a chemical analogue of phosphate, As competes with P in the soil, and during plant uptake from the external because both elements are taken up via the phosphate transport systems (Meharg and

Macnair, 1990; Cao *et al.*, 2003). On the other hand, phosphate may also have a direct effect on As speciation in soil and may enhance As phytoavailability (Melamed *et al.*, 1995; Peryea and Kammereck, 1997).

It is well known that arbuscular mycorrhizal (AM) fungi are ubiquitous in natural and agricultural ecosystems (Harley, 1989; Smith and Read, 1997). Some studies have shown that higher plants that have adapted to As-polluted soils are generally associated with mycorrhizal fungi (Meharg and Cairney, 1999; sharples *et al.*, 2000, Gonzalez *et al.*, 2002). Recently it has been demonstrated that mycorrhizas and phosphate fertilizers can protect plants grown in As-contaminated soils. The mechanisms proposed include the tolerance of higher plants to arsenate through down regulated arsenate/ phosphate transporters in the epidermis and root hairs (Meharg and Macnair, 1992; Gonzalez-Chavez *et al.*, 2002), to reduce the uptake of As, and upregulated low affinity of phosphate transporters located in the membrane fraction of mycorrhizal roots (Harrison *et al.*, 2002), to take up more P for better growth. Certain Arbuscular mycorrhiza (AM) fungi have been shown to provide host plants with some tolerance of toxic conditions; including high metal concentrations (Sharples *et al.*, 2000; Bradley *et al.*, 1981, 1982). There is growing evidence that AM fungal infection can exert protective effects on host plants under conditions of trace element/metal/metalloid contamination. When considering the toxicity of arsenic to plants, the role of mycorrhizal associations must also be considered, as one of the principal roles of mycorrhizal fungi is phosphorus uptake (Smith and Read, 1997). This could potentially be a problem on arsenic contaminated substrates because of enhanced acquisition of arsenate. However, there is also growing evidence that

mycorrhizal fungi may alleviate metal or metalloid toxicity to the host plant by acting as a barrier to uptake (Leyval *et al.*, 1997). Sharples *et al.* (2000) showed that the ericoid mycorrhizal fungus *Hymenoscyphus ericae* acted as an As and Cu filter to maintain low As concentration in plant tissues, while improving P nutrition of the host plant in an As/Cu contaminated mine site. It has been widely reported that ectomycorrhizal and ericoid mycorrhizal fungi can increase the tolerance of their host plants to heavy metals when the metals are present at toxic levels (Bradley *et al.*, 1981, 1982; Jones and Hutchinson 1988). The underlying mechanism is thought to be the binding capacity of fungal hyphae which immobilize the metals in or near the roots and thus depresses translocation to the shoots (Bradley *et al.*, 1981; Brown and Wilkins 1985; Wasserman *et al.*, 1987).

At higher concentration of arsenic solution the seed germination is affected more than the other treatments. All the physical and chemical parameters show positive response to AMF inoculation. Higher nutrient uptake less arsenic content is recorded in mycorrhiza inoculated plants (Saha, 2008).

The present research work was designed with the following objectives:

- To assess the mycorrhizal status of some standing crops grown in different arsenic affected area of Pabna in Bangladesh.
- To assess the role of arsenic on seed germination, seedling growth and nutrient uptake of some agricultural crops.
- To assess the interaction of arsenic and mycorrhiza on different physical and chemical growth parameters of some agricultural crops.

CHAPTER 2

REVIEW OF LITERATURE

To know the role of AMF inoculation on agricultural crops growth in many areas of the world a number of studies were done. Mycorrhizal fungi are vital components of nearly all terrestrial ecosystems, forming mutually beneficial (mutualistic) symbioses with the roots of around 80% of vascular plants and often increasing phosphate (P) uptake and growth. Some of the published reports relevant to research topic from various sources of home and abroad have been reviewed in this chapter.

2.1. Role of mycorrhiza in different agricultural crops

Krishna and Bagyaraj (1982) studied the effect of VA mycorrhizal and soluble phosphorus on *Abelmoscus esculentus* (L.). They reported that root, shoot and total plant dry weight were significantly greater in mycorrhizal plants than in non-mycorrhizal controls. Mycorrhizal dependency was found to decrease with increase in added soluble P.

Sylvia and Neal (1990) reported that the flow of carbon to the soil mediated by mycorrhizae serves several important functions. It can increase plant tolerance to salinity and it can decrease plant susceptibility to diseases (Jalali and Chand, 1988).

Matsubara *et al.* (1994) reported that the growth of 17 species of vegetables was noticeably enhanced by VAMF inoculation to roots. The degree of growth enhancement varied with the host-fungus combination. VAMF inoculation caused both

leaf sheaths and leaf blades to thicken in Welsh onion and enhanced the formation of shoots and crowns in asparagus. Fresh weights of shoot and root increased when the plants were inoculated with VAMF. In most vegetables, the increase in fresh weight of roots was caused by an increase of the number of roots, They also informed that *mycorrhizal dependency* (ratio of total dry weight of 10 VAMF-inoculated plants to total dry weight of 10 non-inoculated plants) was maximum in Liliaceae (Welsh onion and asparagus) among 7 families with VAM fungus infection.

Edathii *et al.* (1994) evaluated the VAM status of tomato, brinjal (aubergenic) and chilli (*Capsicum*) during the initial establishment period in natural field conditions and in pot culture using non sterile soil. The soil had a low nutrient status and no manorial application was made during the 60-d study. VAM colonization in roots was max. at 45, 50 and 60 days after germination of brinjal, tomato and capsicum seeds, respectively under field conditions and on the 60th day in pot culture.

Trimble and Knowles (1995) divulged the growth response of greenhouse cucumber (*Cucumis sativus* L.) to infection by vesicular-arbuscular mycorrhizal (VAM) fungi. Plants were highly receptive to colonization by *Glomus mosseae*, *G. dimorphicum* and *G. intraradices*. Growth rates of primary yield components (e.g., stem and leaf dry weights, leaf area) of VAM-infected plants were greater than those of noninfected plants at all levels of P nutrition. The VAM-enhanced growth was similar to that induced by increases in P nutrition.



Nedumpara and Mercy (1996) reported on the Vesicular Arbuscular Mycorrhizal (VAM) association with many vascular plant species and the contribution of VAM fungi on uptake N, P, K by crop plants. Colonization by VAM fungi significantly enhanced P uptake and plant growth. There was no effect of VAM fungi on plant growth in high P soil. In low P soil the positive effects of VAM fungi on plant growth due to enhanced P uptake were more important than any negative.

Eltrop and Marschner (1996) studied on the growth, nitrogen uptake and mineral nutrient concentrations in the plant tissues in non-mycorrhizal and mycorrhizal seedlings grown under controlled condition. The concentrations of N, P, K, Ca and Mg tended to be higher in the smaller mycorrhizal than in the larger non-mycorrhizal plants. A significant increase in mineral nutrient concentration in mycorrhizal compared with non-mycorrhizal plants was found.

Tarafdar and Praveen (1996) studied the effect of different vesicular arbuscular mycorrhizal fungi (VAMF) on crops (*Vigna aconitifoli*) under field conditions. Plants growth and nutrient uptake of non-inoculated plants were compared with the growth and nutrient uptake of VAMF-inoculated plants. After 8 weeks of growth, percent root infection by VAMF was increased 29-fold in inoculated plants. At maturity of crop, shoot biomass, N, P, K, Zn and Cu concentration were significantly improved in all cases of inoculated plants.

Sreeramulu *et al.* (1996) noted a greater number of AM spores with root zones of inoculated *Amaranthus viridis* and *Trigonella foenuni* than that of uninoculated plants. Mridha *et al.* (1999) recorded spore density in some vegetable crops viz *Amaranthus gangeticus*, *Coriandrum sativum*, *Curcubita moschata*, *Cucumis sativus*, *Capsicum frutescens* and *Lablab purpureus*. They observed a larger number of spore populations in the rhizosphere zone of these crops.

Mridha *et al.* (1999) studied AM colonization in some crops of Bangladesh. They observed high levels of colonization in the members of Leguminosae family and no colonization in Amaranthaceae, Chenopodiaceae and Cruciferae.

Mahmud *et al.* (1999) worked with different agricultural crops of Bangladesh and the relationship with Vesicular Arbuscular Mycorrhizal (VAM) fungi. They identified *Acaulospora*, *Entrophosphora*, *Gigaspora*, *Glomus* and *Scutellospora*. *Glomus* species were the most common followed by *Gigaspora* and *Scutellospora* in vegetables and rice.

George (2000) reported that arbuscular mycorrhizal (AM) fungi can greatly affect the plant uptake of mineral nutrients. It may also protect plants from harmful elements in soil. The contribution of AM fungi to plant nutrient uptake is mainly due to the acquisition of nutrients by the extraradical mycorrhizal hyphae. Many mycorrhizal fungi can transport nitrogen, phosphorus, zinc, and copper to the host plant, but other nutrients can also be taken up and translocated by the hyphae. Among

the nutrients, phosphorus is often the key element for increased growth or fitness of mycorrhizal plants because phosphorus is transported in hyphae in large amounts compared to the plant phosphorus demand.

Gaur and Adholeya (2000) carried out an experiment on onion, potato and garlic inoculated with AM fungi. They reported that inoculation response in terms of yield increase was maximum in onion (70%) whereas garlic and potato showed 30% and 48% increases, respectively.

Mridha and Xu (2001) studied the genus diversity of AM fungi in some vegetable crops in Bangladesh. They identified *Acaulospora*, *Entrophosphora* and *Glomus* abundantly. But *Gigaspora* and *Sclerocystis* were poor in number.

Karagiannidis *et al.*, (2002) studied the effect of the arbuscular mycorrhizal fungus (AMF) *Glomus mossecte* and the soil-borne *Verticillium dahliae* and their interaction on root colonization, plant growth and nutrient uptake in eggplant and tomato seedlings grown in pots. Root colonization by the AMF as well as spore formation was higher (34.6 and 30.5%, respectively) in the eggplant than in tomato. The mycorrhiza treatments increased fresh and dry weight and mean plant height in tomato by 96, 114 and 21% compared to controls.

Giri *et al.* (2005) assessed the effect of two Arbuscular Mycorrhizal (AM) fungi, *Glomus fasciculatum* and *G. macrocarpum* on shoot and root dry weights and

nutrient content of *Cassia siamea* in a semi-arid wasteland soil. Under nursery conditions, mycorrhizal inoculation improved growth of seedlings. Root and shoot dry weights were higher in mycorrhizal than non-mycorrhizal plants. The concentration of P, K, Cu, Zn and Na was significantly higher in AM inoculated seedlings than in non-inoculated seedlings. On transplantation to the field, the survival rate of mycorrhizal seedlings (75%-90%) was higher than that of non-mycorrhizal seedlings (40%). Combination of AMF and pseudomonads proved to be better. Present findings indicated that microbial gene pool especially the key helpers for the maintenance of soil health residing in the vicinity of roots, was positively affected by using pseudomonads and AMF.

Islam (2006) carried out an experiment on the role of Arbuscular Mycorrhizal (AM) fungi on growth and nutrient uptake of some legumes. He observed growth response was positive to AMF in all the selected legumes. The seedling emergence, plant height, shoot length and root length of inoculated legumes were comparatively higher than that of uninoculated legumes.

Akond *et al.* (2008) carried out an investigation for fifteen plant species, cultivated widely as vegetable crops in mycorrhizal fungi. Fourteen out of the fifteen species were having developed VA-Mycorrhizal colonization in their root tissues with a range of 7% to 98% variations in root infections and spore densities were found statistically significant. Plant species had a significant role in root tissue colonization by Mycorrhizal fungi



Ali (2008) reported that AMF has great influence on growth of some agricultural crops like brinjal, tomato, okra, danta and chili. Mycorrhiza enhanced disease reduction in all the selected agricultural crops and also significantly influenced the nutrient uptake capacity of crops over control.

2.1. Arsenic and mycorrhiza interaction

The literatures concerning arsenic and mycorrhizal interaction of Bangladesh are very frugal. However, some of the published reports related to research topic from various sources of home and abroad have been presented in this chapter.

Gonzalez *et al.* (2002) studied the role of arbuscular mycorrhizal fungi (AMF) in arsenate resistance which was isolated from the arsenate-resistant grass *Holcus lanatus*. Resistant and nonresistant *G. mosseae* both suppressed high-affinity arsenate/phosphate transport into the roots of both resistant and nonresistant *H. lanatus*. Resistant AMF colonization of resistant *H. lanatus* growing in contaminated mine spoil reduced arsenate uptake by the host. They conclude that AMF have evolved arsenate resistance, and conferred enhanced resistance on *H. lanatus*.

Liu *et al.* (2005) conducted a glasshouse pot experiment to study the effect of arbuscular mycorrhizal (AM) colonization by *Glomus mosseae* on the yield and arsenate uptake of tomato plants in soil. Mycorrhizal colonization was little affected by As application and declined only in soil amended with 150 mg As kg/1). Shoot As concentration increased with increasing As addition up to 50 mg kg/1) but decreased with mycorrhizal colonization. Mycorrhizal plants had higher shoot and root P/As

ratios at higher As application rates than did non-mycorrhizal controls. Mycorrhizal colonization may have increased plant resistance to potential As toxicity at the highest level of As contamination.

Agely *et al.* (2005) reported that Chinese brake fern (*Pteris vittata* L.) is a hyperaccumulator and mycorrhizal symbiosis may be involved in As uptake by this fern. This is because arbuscular mycorrhizal (AM) fungi have a well-documented role in increasing plant phosphorus (P) uptake and ferns are known to be colonized by AM fungi. They found that the AM fungi not only tolerated As amendment, but their presence increased frond dry mass at the highest As application rate. These data indicate that AM fungi have an important role in arsenic accumulation by Chinese brake fern.

Ahmed *et al.* (2006) examined the effects of As and inoculation with an AM fungus, *Glomus mosseae*, on lentil. Plant height, leaf number, pod number, plant biomass and shoot and root P concentration/offtake increased significantly due to mycorrhizal infection. Plant height, leaf/ pod number, plant biomass, root length, shoot P concentration/offtake, root P offtake and mycorrhizal infection decreased significantly with increasing As concentration. However, mycorrhizal inoculation reduced As concentration in roots and shoots. This study shows that growing lentil with compatible AM inoculum can minimize As toxicity and increase growth and P uptake.

Trotta *et al.* (2006) reported that As treatment produced a dramatic increase of As concentration in pinnae and a much lower increase in roots of both mycorrhizal and control plants. Mycorrhization increased pinnae dry weight and leaf area, strongly reduced root As concentration, and increased the As translocation factor. The concentration of phosphorus in pinnae and roots was enhanced by both fungi.

Leung *et al.* (2006) conducted a greenhouse trial to investigate the role of arbuscular mycorrhiza in aiding arsenic uptake and tolerance by *Pteris vittata* and *Cynodon dactylon*. The infectious percentage of mycorrhizas and the average biomass of shoots in infected *P. vittata* increased according to the increase of As levels when compared to control. The indigenous mycorrhizas enhanced As accumulation in the As mine populations of *P. vittata* and also sustained its growth by aiding P absorption. For *C. dactylon*, As was mainly accumulated in mycorrhizal roots and translocation to shoots was inhibited.

Kim *et al.* (2006) reported that inoculation of AM fungi (*Glomus mosseae*) to plants resulted high yield and increase arsenic resistance to its toxicity and has a potential applicability to enhance the efficiency of phytostabilization in soils highly contaminated with arsenic.

Xia *et al.* (2007) examined the effects of arbuscular mycorrhizal fungus (*Glomus mosseae*) and phosphorus addition on arsenic uptake by maize plants from an As-contaminated soil. The results indicated that addition of P inhibited root

colonization, shoot and root biomass and development of extraradical mycelium. Root length, dry weight and shoot and root As concentrations both increased with mycorrhizal colonization under the zero-P treatments. AM fungal inoculation decreased shoot As concentrations when no P was added. AM colonization therefore appeared to enhance plant tolerance to As in low P soil, and have some potential for the phytostabilization of As-contaminated soil.

Dong *et al.* (2007) reported that, in a compartmented cultivation system, white clover and ryegrass were grown together in arsenic (As) contaminated soil. The influence of AM inoculation on plant growth, As uptake, phosphorus nutrition, and plant competitions were investigated. Results showed that both plant species highly depended on mycorrhiza for surviving the As contamination.

Chen *et al.* (2007) used a compartmented pot cultivation system to investigate the roles of *Glomus mosseae* in plant phosphorus and As acquisition by *Medicago sativa* and P-As interactions. The results indicate that fungal colonization increased plant dry weight and also substantially increased both plant P and As contents. The decreased shoot As concentrations were largely due to "dilution effects" that resulted from stimulated growth of AM plants and reduced As partitioning to shoots.

Ultra *et al.* (2007) set up an experiment to find out the effects of arbuscular mycorrhiza (AM) and phosphorus application on arsenic toxicity in As-contaminated soil. The treatments consisted of a combination of two levels of AM (*Glomus aggregatum*) inoculation and two levels of P application. AM inoculation as well as P

application reduced As toxicity symptoms and increased plant growth. Shoot As concentrations were reduced by AM inoculation but enhanced by P application.

Saha (2008) conducted three experiments to justify the effect of mycorrhiza on seed germination in soil amended with different concentrations of arsenic solution. He conducted the experiments in plastic tray, blotter plate and poly bags. Seedling emergence, shoot height, root length, fresh and dry weight of shoot, fresh and dry weight of root and also nutrient uptake by shoot of plants is increased in mycorrhiza inoculated plants than that of inoculated plants.

Akhtar (2008) conducted an experiment on the effect of mycorrhizal fungi on growth and nutrient uptake by few crops in arsenic amended soil. She found that 10 ppm arsenic solution + mycorrhiza treatment showed the highest performance and 500 ppm arsenic solution treatment showed the lowest performance in all the selected crops. The experiment exposed that with the increase of arsenic concentration plants show the decrease growth performance.

2.2. Arsenic and chlorophyll interaction:

Jain and Grade (1997) investigated on the effect of As on chlorophyll synthesis with a view to gain some insights into the possible mechanism. Supply of 0.01 to 1.00 mM Na arsenate to the greening maize leaf segments decreased the chlorophyll content as well as chlorophyllase activity. Supply of arsenate also reduced total RNA, protein and acid soluble thiole content of the tissue.

Mitevae (2002) set up an experiment on accumulation and effect of arsenic on tomatoes. Changes in plant growth and in pigment content were studied in tomatoes (*Lycopersicon esculentus*), cultivated on soils, polluted with arsenic (As) in sublethal doses (15, 25, 50 and 100 ppm). An elongation of root system and increase of stem height and stem weight was observed at lower arsenic concentration (15 and 25 ppm) especially at 15 ppm. The higher element concentrations (50 to 100 ppm) led to a decrease in growth of both the vegetative and root system. The significant decline of the pigments in plants treated with higher doses is an indication of poor conditions of those plants and the lack of adaptive adjustment to high As levels.

Semra (2002) studied on influence of Arbuscular Mycorrhiza on some physiological growth parameters of pepper. The effect of Arbuscular Mycorrhizal fungus *Glomus intradices* on the physiological growth parameters of pepper (*Capsicum annuum* L.CV centinel-150) plants was investigated. Phosphorus and dry matter contents, chlorophyll (chl) concentrations (chl a, chl b and chl a+b), amounts of some reducing sugars were investigated in the shoots and leaves of mycorrhizal and non mycorrhizal plants. All parameters increased in mycorrhizal pepper plants by 12%-47% compared with those of the non mycorrhizal plants. It is concluded that P concentration was positively correlated with all chlorophyll and sugar contents.

Rahman *et al.* (2007) conducted a glass house experiment to investigate the effect of soil arsenic on photosynthetic pigments, chlorophyll-a and chlorophyll-b

and their correlations with rice yield and growth. Both chl a and b contents in rice leaf decreased significantly ($P < 0.05$) with the increase of soil Arsenic concentrations. Chlorophyll content and rice growth and yield suggesting that Arsenic toxicity affects the photosynthesis which ultimately results I the reduction of rice growth and yield.

Milivojovic *et al.* (2006) studied on the effect of arsenic (32-96 μ M) on the phosphorus content and chl flurescence in soybean (*Glycine max* Merrill) grown in the nutrient solution with or without phosphorus. The increased concentration of As led to the decrease in P content in plant organs.

Chapter 3

Materials and Methods

CHAPTER 3

Materials and Methods

3.1 Study of root colonization

Root samples of some standing agricultural crops were collected from different arsenic affected locations of Pabna district for the detection of AMF infection and intensity of infection.

a. Selection of location

Five different arsenic affected locations of Pabna district were selected for the present investigation. Root samples were collected from different agricultural fields, side of the road, pond side and both high and low lands.

b. Period of collection

Root samples were collected in July, 2008 from all the selected locations to observe the variation of AMF infection and intensity of infection in different crops.

c. Collection of root samples

Root samples were collected at a depth of 10 - 15 cm. During the collection of roots, the soil around the selected plant was loosened, fine roots with some coarse roots were collected with a sharp knife, Special care was taken to separate the fine roots from the soil. The collected roots were put into tagged polyethylene bags and brought to the laboratory for study.



d. Cleaning and preservation of roots

Collected root samples were freed from adhering soil and washed carefully. Fine roots were cut into small segment of approximately 1 cm for determination of AMF colonization. From these, 100 segments were randomly selected for staining. Some segments were preserved in 50% ethyl alcohol solution in vials and kept in refrigerator for future use. The roots were stained by the following methods of Koske and Gemma (1989).

e. Staining of roots

The roots of each plant species were stained according to Koske and Gemma (1989) with some modifications (Mridha *et al.*, 1999). The root pieces were boiled in 2.5% KOH solution for 30 minutes at 90° C temperatures. Later on, the root segments were washed in water for several times and acidified with 1% HCl solution for 24 hours. Heavily pigmented roots were bleached by 10% H₂O₂ for 20 to 60 minutes. Again, these segments were boiled for 30 minutes in 0.05% aniline blue at a temperature of 90°C. Subsequently the roots were destined at room temperature in acidic glycerol.

f. Observation of roots

The stained root segments were mounted in acetic glycerol on slides and the cover slip was placed and slightly pressed. The roots were observed under the microscope. The presence or absence of infection in the root segments was recorded and the percent infection was calculated using the following formula:

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

(Read *et al.*, 1976)

At least 50 segments were examined for each sample. A root segment was considered to be infected if it showed mycelium, vesicle and arbuscules of any other combination of Arbuscular mycorrhizal fungi.

g. Estimation of intensity of infection of AMF

For the determination of intensity of infection of AMF, we used 0-3 scale. 0 indicate that there was no infection. The intensity of infection of AMF was estimated as, 1 if only mycelium were present; 2 mycelium and vesicle were present and 3 mycelium, vesicle and arbuscules were present. (Mridha and Dhar, 2007)

3.2. Study of spore population in soil

After confirming mycorrhizal association in root system, spore population was identified in soils and then isolated. However, a few number of mycorrhizal spore was isolated so that by using this data any variation was not found to make any table. The identification was done in the mycorrhiza laboratory, Department of Botany, Chittagong University.

3.3. Plant growth in poly bag

The poly bag experiment was performed in the net house of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from March 2008 to July 2008 to study the role of mycorrhiza on growth of few crops in arsenic amended soil.

a. Collection of soil

Soil was collected from the Agronomy field of Sher-e-Bangla Agricultural University campus from a depth of 5 to 10 inch.

b. Preparation of soil

After collecting the soil, clods were broken and weeds, stones, gravels, roots, and other unwanted materials were removed. Soil was prepared for the experiment containing 10% sand and 90% soil.

c. Mycorrhizal assessment

A survey programme was conducted in the Agronomy field of Sher-e-Bangla Agricultural University in the February 2008 to collect natural inoculum of Mycorrhiza. Root samples of different plants species growing under natural condition in different places of the Agronomy field were collected for the observation of occurrence of vesicular arbuscular mycorrhizal (AMF) association with the root systems.

d. Staining of roots

Same as previous staining procedure of roots of study of root colonization (3.1.e).

e. Preparation of inoculums

Leucas aspera roots were collected from Agronomy field along with rhizosphere soil (Fig.1 and Fig.2). The presence of AM fungi within the root sample was confirmed



Figure 1. Dronapushpi (*Leucas aspera*)

SHETODRONE

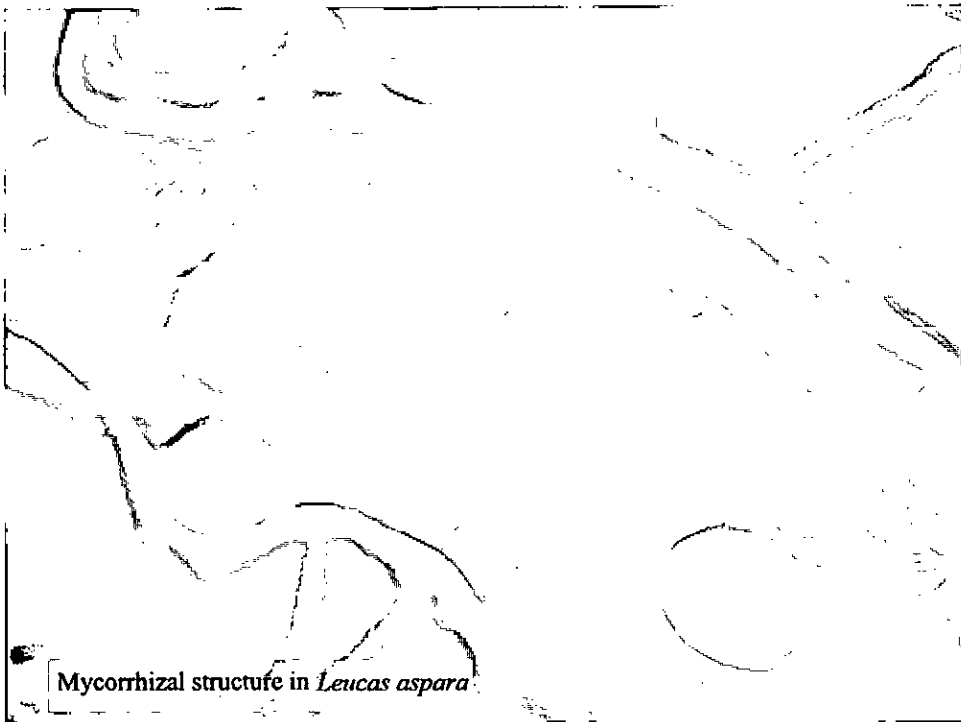


Figure 2. *Glomus intraradices* found in *Leucas aspara* plant

using the staining procedure of Koske and Gemma (1989). Collected root samples were cut into small pieces with the help of chopper. Half of rhizosphere soils and root samples were sterilized in the autoclave at 121°C at 15 PSI for 45 minutes and used it as base materials for sterilized mycorrhiza inoculated pots.

f. Preparation of arsenic solution

For preparation of 1000 ppm 1 liter arsenic solution at first 4 gm Sodium hydroxide was taken in a 100 ml measuring cylinder. Sodium hydroxide was diluted with distilled water and the volume of the cylinder rose up to the 100 ml mark. Then 1.32 gm arsenic powder was taken in another 1000 ml measuring cylinder and dilute with Sodium hydroxide. 10% HCL was taken in a beaker. Then HCL was added into the 1000 ml measuring cylinder to make it acidic. At lasts the volume of the flask rise up to 1000 ml mark with distilled water.

g. Selection of crops

Five different agricultural crops (Danta, chili, tomato, okra and brinjal) were selected for these experiments.

h. Collection of seeds:

Seeds of different agricultural crops namely Danta, Tomato, Chili, Brinjal and Okra were collected from BADC (Bangladesh Agricultural Development Corporation).

3.4. Role of arsenic on seed germination

a. Treatments:

There were seven treatments in this study which were as follows:

T₁: Control

T₂: 10 ppm arsenic solution

T₃: 10 ppm arsenic solution + mycorrhiza

T₄: 100 ppm arsenic solution

53
25/01/10
T₅: 100 ppm arsenic solution + mycorrhiza

T₆: 500 ppm arsenic solution

T₇: 500 ppm arsenic solution + mycorrhiza

b. Preparation of seedling bags

The polythene bags of 12"×10" size were bought from the market, which has the capacity to fill with 2 kg soil. The bags were perforated at the bottom portion by the perforator to remove excess water. Soil was sterilized by formaldehyde (0.05%) and used it as base soil. Soil was taken into the perforated seedling bags. At first $\frac{2}{3}$ rd portion of the seedling

37382
bags were filled with substratum. Then a layer of both inoculum i.e. root inoculum 25 g and soil inoculum 100 g, were placed in each treated bag. For each crop 5 replications i.e., 5 for inoculated and 5 for non-inoculated were prepared. Both 25 g roots and 100 g soil (rhizosphere) with inoculum were used in non-inoculated bags to maintain the same nutrient status between the inoculated and non-inoculated bags. The inoculum layer of each bag was covered with a thin soil (substratum) layer of 2 cm below the surface in which seeds were sown. 175 polythene bags (7 × 5 × 5) were prepared for five crops in the present study.

c. Sowing of seeds

For each crop 5 replications were maintained and each replication consists same number of seed of same crop plant. For different crops, different number of seeds was sown in the bags based on seed size. For okra 8 seeds/bag, tomato 20 seeds/bag, brinjal 20 seeds/bag, chilli 20 seeds/bag and danta 20 seeds/bag was sown. After 15 days, 5 seedlings in each bag were retained and other seedlings were removed.

d. Intercultural operation

The seedling bags were irrigated whenever necessary. Intercultural operations (weeding, mulching, and thinning) were done when necessary to ensure the normal growth of the crops. The bags were carefully observed regularly to record any change of plant growth.

e. Harvesting

When the seedlings were 30, 45 and 60 days old then those were harvested. In this case 3 seedlings bags from inoculated and 3 seedlings bags from non-inoculated were harvested randomly for each crop. At first polythene bags were removed very carefully with sharp knife. The roots were washed with tap water to remove the adhering soil. Shoots and roots were separated with the help of sharp scissors and were preserved after necessary processing for determining shoot mass and root mass. Shoots and roots were then dried in an oven for 72 hours at 70°C until the samples gave constant weight.



f. Data recording

Data were recorded on seedling emergence (%) (7 DAS, 10 DAS and 15 DAS), shoot fresh and dry weight (g) (30 DAS, 45 DAS, 60 DAS), root fresh and dry weight (g) (30 DAS, 45 DAS, 60 DAS), shoot and root length (cm) (30 DAS, 45 DAS, 60 DAS), per cent vigourity and root colonization. Vigor index was calculated by using the following formula:

$$\text{Vigor index} = (\text{Mean root length} + \text{Mean shoot length}) \times \text{percent germination}$$

(Abdul and Anderson 1972)

g. Assessment of root colonization

Preserved root samples were assessed. Roots were taken out of the vial and washed 2-3 times with clear water and cut into small segments of approximately 1 cm length for the determination of percent of AM colonization. The root pieces were stained according to Koske and Gamma (1989) with some modifications (Mridha and Xu, 2001). The percentage of AM colonization was estimated by root slide technique (Read *et al.*, 1976). Fifty root segments were examined. The stained root pieces were mounted in acidic glycerol on slides and the cover slip was place and slightly pressed. The roots were observed under a microscope. A root segment was considered as positively colonized when it showed mycelium, arbuscules and vesicles or any other combination of these structural characteristics of AM colonization. The presence or absence of infection in colonization was calculated as follows:

$$\% \text{ Root colonization} = \frac{\text{Number of AM positive segments}}{\text{Total number of segments}} \times 100$$

h. Chemical analysis of plant sample

i) Nutrient analysis:

➤ Preparation of plant sample

Plant (shoot) samples were dried in oven at 70°C for 72 hours and then ground the samples and sufficient amount of sample for each treatment was kept in desiccators for chemical analysis.

➤ Digestion of plant samples with nitric-perchloric acid mixture

An amount of 0.5 g of sub-sample was taken into a dry clean 100 ml Kjeldahl flask, 10 ml of di-acid mixture (HNO_3 , HClO_4 in the ratio of 2:1) was added and kept for few minutes. Then, the flask was heated at a temperature rising slowly to 200°C. Heating was instantly stopped as soon as the dense white fumes of HClO_4 occurred and after cooling, 6 ml of 6N HCl were added to it. The content of the flask was boiled until they became clear and colorless. This digest was used for determining P, K and S.

Phosphorous

Phosphorous in the digest was determined by ascorbic acid blue color method (Murphy and Riley, 1962) with the help of a Spectrophotometer (LKB Novaspec, 4049).

Potassium

Potassium content in the digested plant sample was determined by flame photometer.

Sulphur

Sulphur content in the digest was determined by turbidimetric method as described by Hunt (1980) using a Spectrophotometer (LKB Novaspec, 4049).

Nitrogen

Plant samples were digested with 30% H₂O₂, conc. H₂SO₄ and a catalyst mixture (K₂SO₄: CuSO₄.5H₂O: Selenium powder in the ratio of 100: 10: 1, respectively) for the determination of total nitrogen by Micro-kjeldahl method. Nitrogen in the digest was determined by distillation with 40% NaOH followed by titration of the distillate absorbed in H₃BO₃ with 0.01 N H₂SO₄ (Bremner and Mulvaney, 1982).

ii) Arsenic analysis

➤ Dilution of digested samples

After digestion, the samples were diluted individually with deionized water separately in 20 ml calibrated volumetric flask. After dilution, each sample was filtered individually with filter paper (Whatman 42) into correspondingly marked sterile 30ml screw capped sterile glass vials and preserved at 4⁰C in a refrigerator until tested for arsenic.

➤ Detection of arsenic in plant samples

Arsenic was detected in the laboratory of Pharmacology Department, Bangladesh Agricultural University, Mymensingh with Hydride Generation Atomic Absorption Spectrophotometer (HG-AAS; PG-990, PG Instruments Ltd. UK; Arsenic was detected by forming AsH₃ at below p^H 1.0 after the reaction of As with a solution of potassium borohydride (KBH₄=53.94, BDH Chemicals Ltd., Poole England, UK.), sodium hydroxide (NaOH, M=40,000 g/mol, Merck KGaA, Darmstadt, Germany) and 10% HCl. In this test, standard was maintained as As^V ranging from 0 to 12.5 µg/L.

➤ **Preparation of working solutions**

Trace element grade chemicals were used in the preparation of working solutions.

➤ **Preparation of carrier liquid**

Carrier liquid consisted of 10% HCl in deionized water. To make this solution, 100 ml of concentrated HCl was taken in a 1000 ml calibrated volumetric flask and then deionized water was added up to the mark of 1000 ml of the flask and thereby the required solution was prepared.

➤ **Preparation of potassium borohydride solution**

Potassium borohydride solution used in the detection of As in HG-AAS contained 1.5% potassium bromohydride and 0.3% sodium hydroxide in deionized water. This solution was freshly prepared immediately before detection by taking 7.5 g of potassium bromohydride and 1.5 g of sodium hydroxide in a 500ml calibrated volumetric flask, and then deionized water was added part by part with frequent gentle shaking the flask for dissolving the solute to the solvent. Addition of deionized water was continued up to the 500ml mark of the volumetric flask, and thereby potassium borohydride solution was made

➤ **Preparation of arsenic standard solutions**

Arsenic standard solutions were prepared with the As concentration of 0, 2.5, 5.0, 7.5, 10.0 and 12.5 $\mu\text{g/L}$ in 10% HCl in separate 25ml calibrated volumetric flasks following proper acid washing and drying from 1000 $\mu\text{g As/L}$ working solution of As_2O_5 (arsenic pentaoxide).

➤ Operation of AAS-HG and taking reading

After preparation of samples and all the necessary chemical solutions along with arsenic standard solutions, the HG-AAS was started by switching the power and argon gas was allowed to flow to the hydride generation unit of the HG-AAS with flow rate between 120 to 140 ml/minute by regulating the pressure meter of the gas cylinder. Operation of the hydride generation was started by switching the operation button. Then the HG-AAS was calibrated as per manufacturer's instruction mentioned in the HG-AAS operation manual.

Before the formal determination, for cleaning of water and air in the fluid measurement system, the sample suction tube was inserted first into the carrier liquid. Then the operation process was carried out for two times by switching the hydride generation (HG). The waste liquid in the burette used for cleaning was drained quickly through the gas-liquid separation tube. Respective sucking tubes were inserted into potassium borohydride solutions and the carrier liquid. Then the blank solution was calibrated before measuring the sample solution. The first two data was ignored.

The carrier liquid was carrying the sample solution and the potassium bromohydride began their permanent flow and the reaction was taken place after their convergence. The carrier gas (argon gas) into the gas-liquid separation tube brought along the resultant, and the mixed gas entered the electric quartz absorption tube atomizer. The As in the sample was first ionized into arsine and then atomized.

Reading was taken with the help of a computer connected to the HG-AAS by using manufacturer supplied 'AAwin software' (Atomic Absorption Spectrophotometer PC-Software). The reading of the tested sample was displayed on

the computer monitor in a pre-customized Microsoft excel sheet provided by the AAWin software as numerical number with giving a peak of As concentration on the respective part of the software displayed sheet on the computer monitor. Readings of As concentrations of the samples were taken in ppb.

After finishing the detection of one sample, the waste liquid was driven out automatically through HG outlet and taken into a waste liquid container. The procedures were the same as those of the traditional flow injection method. After the determination was finished, in order to clean every tube, all the three suction tubes were inserted into the distilled water, the operating procedures were carried out twice, taken them out, air was sucked to expel water and the operating procedures were carried out once.

iii) Chlorophyll Extraction

One gram fresh plant samples (leaves) were cut into small pieces and inserted into test tubes. Then 100 ml (80%) acetone was added in air tight condition to avoid losses. The test tubes were kept for 24-48 hrs in normal temperature. Data was recorded by Spectrophotometer.

Total chlorophyll = Absorbance (chlorophyll a + chlorophyll b) × Correction Factor

➤ Detection of chlorophyll in plant leaf samples

Chlorophyll was detected in the Plant Physiology Laboratory, Bangladesh Rice Research Institute (BRRI), Gazipur with the help of Spectrophotometer

i. Statistical analysis

All data were analyzed in the computer using MSTAT package program and mean difference was measured by DMRT.

Chapter 4

Results

CHAPTER 4

RESULTS

4.1. Study of root colonization

Different agricultural crops root were collected from different villages of Pabna area to know the intensity of infection of arbuscular mycorrhizal fungi

a. Saiyadpur village

The percentage of AM-fungal association in different agricultural crops root collected from Saiyadpur village of Pabna district is presented in Figure 3. The percent root infection was ranged within 0 -18%. Among the five plant species the highest root infection was observed in *Cassia tora* (18%). No infection was recorded in *Cucurbita moschata* and *lagenaria vulgaris*.

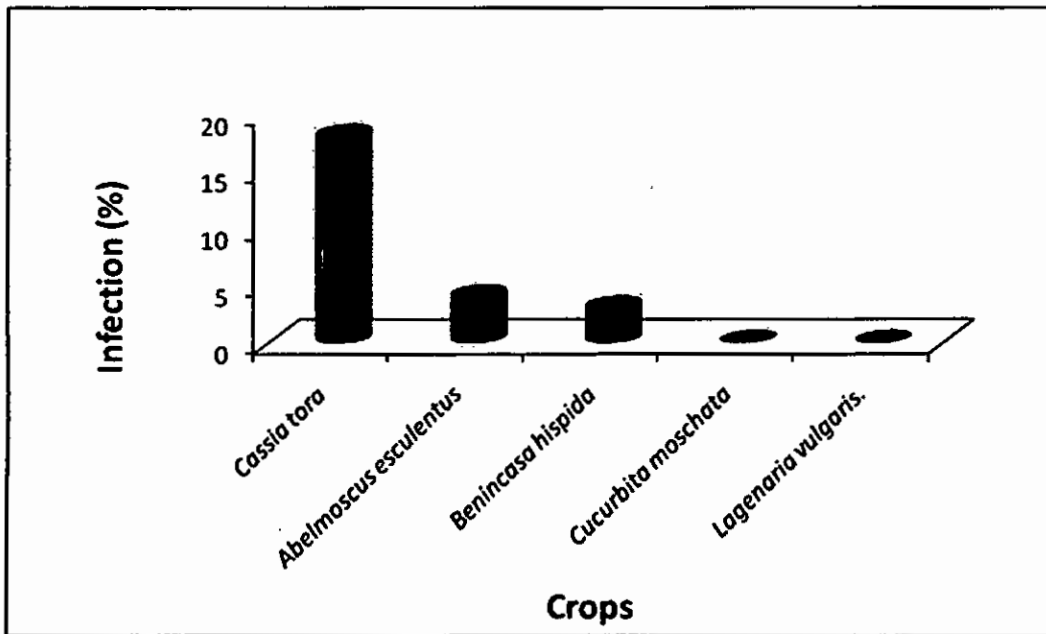


Figure3. Percent AMF infection of different agricultural crops root collected from Saiyadpur village of Pabna district.

Percentage of intensity of infection of AMF on different agricultural crops root collected from Saiyad pur village of Pabna district is presented in Table 1. Intensity of infection was also varied at different scale. *Cassia tora* root collected from the field showed 8% intensity of infection in scale 1, 7% in scale 2 and 3% in scale 3 whereas, *Abelmoscus esculentus* root sample collected from the field showed 1%

intensity of infection in scale 1 and 3% in scale 2 and no intensity of infection was found in scale-3. *Benincasa hispida* roots collected from the field showed 2% intensity of infection in scale 1 and 1% in scale 2 and no intensity of infection was found in scale-3 whereas, *Cucurbita moschata* and *Lagenaria vulgaris* roots sample collected from different fields showed no intensity of infection in scale 1, 2 and 3.

Table 1. Percent intensity of infection of AMF on root of different agricultural crops collected from Saiyad pur of Pabna district

Name of the plant species	Intensity of infection (%)			
	0	1	2	3
<i>Cassia tora</i>	82	8	7	3
<i>Abelmoscus esculentus</i>	96	1	3	0
<i>Benincasa hispida</i>	97	2	1	0
<i>Cucurbita moschata</i>	100	0	0	0
<i>Lagenaria vulgaris.</i>	100	0	0	0

0-3 scale: 0= no infection, 1= only mycelium present, 2= mycelium + vesicle present, 3= mycelium + vesicle + arbuscule present

b. Ruppur village

The percentage of AM-fungal association in different agricultural crops root collected from Ruppur village of Pabna district is presented in Figure 4. To determine the infection percentage of seven different plant species were collected from Rup pur village of pabna area. The percent root infection was ranged within 0-20%. Among the seven plant species the highest root infection was observed in *Capsicum frutescens* (20%) and the lowest root infection observed in *Sesamum indicum*. No infection was recorded in *Trichosanthes anguina* and *Luffa acutangula*.

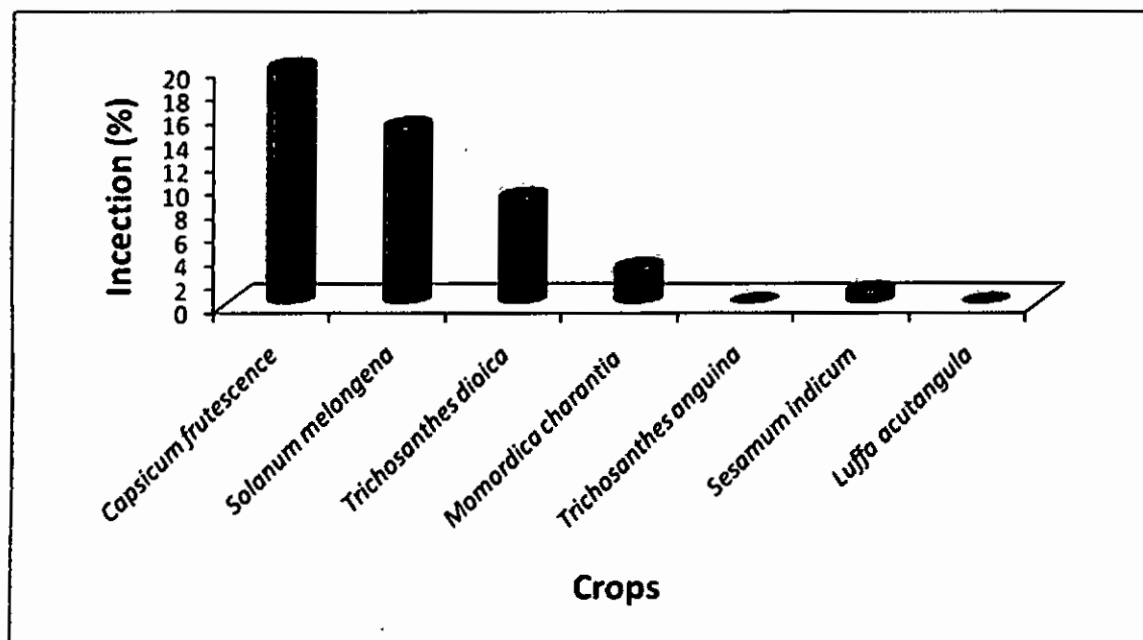


Figure 4. Percent AMF infection of different agricultural crops root collected from Rup pur pur village of Pabna district.

Percentage of intensity of infection of AMF on different agricultural crops root collection from Rup pur village of Pabna district is presented in Table 2. 0-3 scale was used to determine the intensity of infection. In scale 1 intensity of infection was recorded in 6 cases. Among them the lowest percentage was found in *Sesame* and that was 1% and the highest was found in *Solanum melongena* and that was 10%. In scale 2 Intensity of infection was also recorded in 4 cases. In scale 3 *capsicum frutescence* and *Solanum melongena* showed 7% and 2% intensity of infection respectively.

Table 2. Percent intensity of infection of AMF on root of different agricultural crops collected from Ruppur of Pabna district

Name of the plant species	Intensity of infection (%)			
	0	1	2	3
<i>Capsicum frutescence</i>	80	7	6	7
<i>Solanum melongena</i>	85	10	3	2
<i>Trichosanthes dioica</i>	91	8	1	0
<i>Momordica charantia</i>	98	2	1	0
<i>Trichosanthes anguina</i>	100	0	0	0
<i>Sesamum indicum</i>	99	1	0	0
<i>Luffa acutangula</i>	100	0	0	0

0-3 scale: 0= no infection, 1= only mycelium present, 2= mycelium + vesicle present 3= mycelium + vesicle + arbuscule present

C. Masumdia village

The percentage of AM-fungal association in different agricultural crops root collected from Masumdia village of Pabna district is presented in Figure 5. To determine the infection percentage of five different plant species were collected from Rup pur village of pabna area. The percent root infection was ranged within 0%-18 percentage. Among the five plant species the highest root infection was observed in *Amaranthus tricolor* (18%) and the lowest root infection was observed in *Leucas aspera*. No infection was recorded in *corcorus capsularis*.



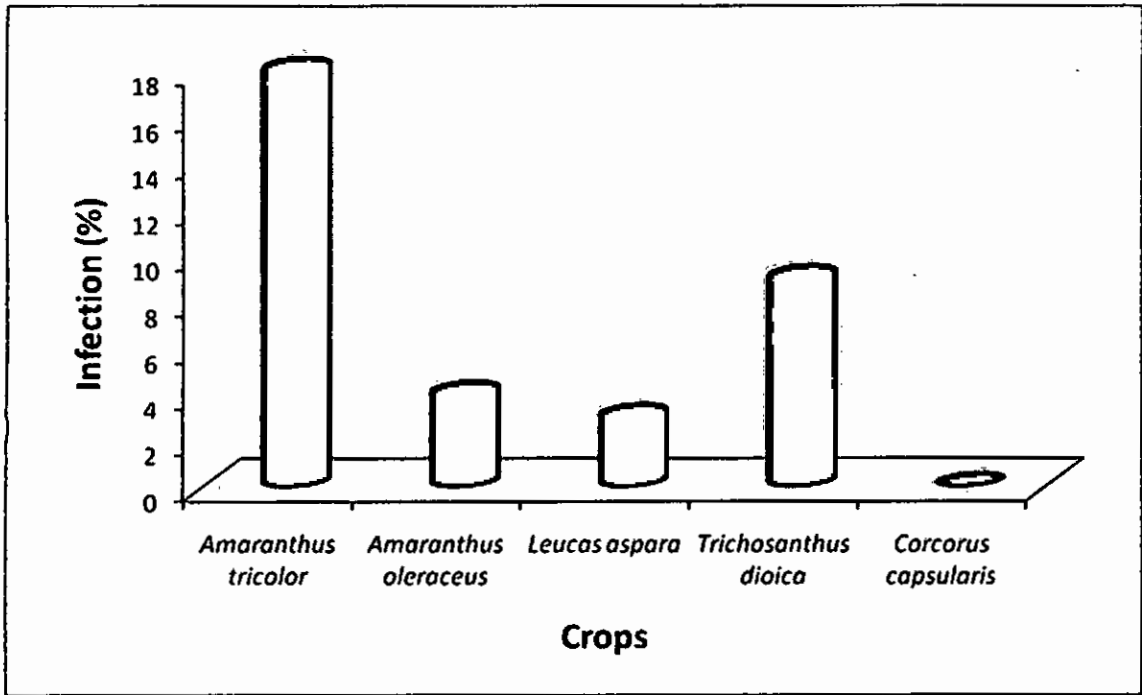


Figure 5. Percent AMF infection of different agricultural crops root collected from Masumdia village of Pabna district.

Percentage of intensity of infection of AMF on different agricultural crops root collection from Masum dia village of Pabna district is presented in Table 3. 0-3 scale was used to determine the intensity of infection. Intensity of infection was also varied at different scale. In scale 1 intensity of infection was recorded in 4 cases. Among them the lowest percentage was found in *Amaranthus tricolor* and that was 1% and the highest was found in *Trichosanthus dioica* and that was 8%. In scale 2 Intensity of infection was also recorded in 4 cases. In scale 3 only *Amaranthus tricolor* showed 9% of infection whereas other crops showed no intensity of infection in the same scale.

Table 3. Percent intensity of infection of AMF on root of different agricultural crops collected from Masumdia of Pabna district

Name of the plant species	Intensity of infection (%)			
	0	1	2	3
<i>Amaranthus tricolor</i>	82	1	8	9
<i>Amaranthus oleraceus</i>	96	2	2	0
<i>Leucas aspara</i>	98	2	1	0
<i>Trichosanthus dioica</i>	91	8	1	0
<i>Corcorus capsularis</i>	100	0	0	0

0-3 scale: 0= no infection, 1= only mycelium present, 2= mycelium + vesicle present, 3= mycelium + vesicle + arbuscule present

d. Vatikoa village

The percentage of AM-fungal association in different agricultural crops root collected from Vatikoa village of Pabna district is presented in Figure 6. To determine the infection percentage of five different plant species were collected from vatikoa village of pabna area. The percent root infection was ranged within 0%-21percentage. Among the five plant species the highest root infection was observed in *Brassica napus* (21%) and the lowest root infection was observed in *Corcorus capsularis*. No infection was recorded in *Lagenaria vulgaris* and *Abelmoscus esculentus*.

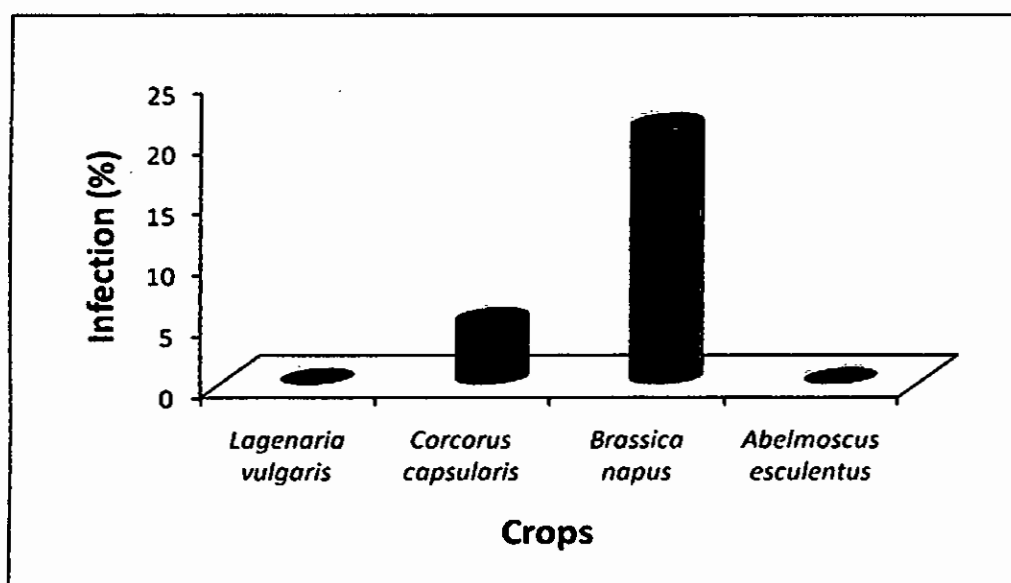


Figure 6. Percent AMF infection of different agricultural crops root collected from Vatikoa village of Pabna district.

Percentage of intensity of infection of AMF on different agricultural crops root collection from vatikoa village of Pabna district is presented in Table 4. Intensity of infection was also varied at different scale. The highest percentage of intensity was found in *Brassica napus* and that was 17 in scale 1 and 4 in scale 2. There was no intensity of infection was found in 'scale 3'.

Table 4. Percent intensity of infection of AMF on root of different agricultural crops collected from Vatikoa of Pabna district

Name of the plant species	Intensity of infection (%)			
	0	1	2	3
<i>Lagenaria vulgaris</i>	100	0	0	0
<i>Corcorus capsularis</i>	95	5	0	0
<i>Brassica napus</i>	79	17	4	0
<i>Abelmoscus esculentus</i>	100	0	0	0

0-3 scale: 0= no infection, 1= only mycelium present, 2= mycelium + vesicle present, 3= mycelium + vesicle + arbuscule present

4.2. Role of Mycorrhiza on plant growth in arsenic amended soil

Poly bag Experiment

Role of AMF on plant growth of some agricultural crops grown in soil amended with different concentrations of arsenic solution is studied in this section.

A. Brinjal

➤ Seedling emergence

The influence of AMF inoculation on seedling emergence of brinjal seeds sown in soil amended with different concentrations of arsenic is shown in table 5. The seed germination was recorded after 7, 10 and 15 days of sowing. The seedling emergence varied at different concentrations but with increase of incubation period the emergence increased. Higher seed germination was recorded in treatment T₃ in all the three recorded time (Figure 7). Among the treatment T₂, T₄ and T₆ higher germination was recorded in treatment T₂. In the same way among the treatment T₃, T₅ and T₇ higher germination was recorded in treatment T₃, where mycorrhiza was added with 10 ppm arsenic solution. In comparison to 10 ppm arsenic + mycorrhiza gave better germination than 100 ppm arsenic + mycorrhiza treatment.

➤ Number of leaves

The influence of AMF inoculation on number of leaves of brinjal at different growth periods in soil amended with different concentrations of Arsenic solution is also presented in Table 5. The number of leaves of brinjal was recorded after 10, 20 and 30 days of sowing. The highest number of leaves of brinjal was recorded in treatment T₃ and the lowest number of leaves of Brinjal was recorded in treatment T₇ at 10 DAS. Similar results were also obtained at 20 DAS and 30 DAS. It was observed that the number of leaves per plant of mycorrhizal treatment is more than the other treatment. Among the treatments it was revealed that with the increase of arsenic concentration the number of leaves of brinjal decreasing.



➤ Shoot height

The influence of AMF inoculation on shoot height of brinjal seeds sown in soil amended with different concentrations of arsenic solution are shown in Table 5. The shoot height was varied significantly at different concentrations. The shoot height was recorded after 30, 45 and 60 days after sowing. The highest shoot height was recorded in T₃ treatment after 60DAS. In all the three recorded periods it was observed that the shoot height of brinjal decreased with the increase of arsenic concentration and shoot height was higher in mycorrhiza inoculated poly bags than non-inoculated.

Root length

Root length of brinjal influenced by AMF inoculation in soil amended with different concentrations of arsenic solution is presented in Table 5. Data were recorded after 30, 45 and 60 days of sowing. Root length of brinjal ranges from 14.53cm-19.37cm, 20.27cm-22.37cm and 18.53cm-28.07cm at 30 DAS, 45DAS and 60DAS respectively. In comparison to all the treatments mycorrhizal treatment T₃ (10 ppm arsenic solution + mycorrhiza) gave the highest result and those were 22.87cm and 28.07cm at 45DAS and 60DAS respectively and the lowest root length of brinjal was recorded in case of treatment T₄ (100 ppm arsenic solution) and those were 14.53cm, 20.27cm and 21.37cm at 30 DAS, 45DAS and 60DAS respectively. It was revealed from the table the root length of brinjal was the lowest in only arsenic treated poly bags but when mycorrhiza is added with those treatments the root length of brinjal increased. Among the treatment T₂, T₄ and T₆ treatment T₆ gave the lowest result so it was clearly exposed that with the increase of arsenic concentration the root length of brinjal decreasing.

Table 3. Percent intensity of infection of AMF on root of different agricultural crops collected from Masumdia of Pabna district

Name of the plant species	Intensity of infection (%)			
	0	1	2	3
<i>Amaranthus tricolor</i>	82	1	8	9
<i>Amaranthus oleraceus</i>	96	2	2	0
<i>Leucas aspara</i>	98	2	1	0
<i>Trichosanthus dioica</i>	91	8	1	0
<i>Corchorus capsularis</i>	100	0	0	0

0-3 scale: 0= no infection, 1= only mycelium present, 2= mycelium + vesicle present, 3= mycelium + vesicle + arbuscule present

d. Vatikoa village

The percentage of AM-fungal association in different agricultural crops root collected from Vatikoa village of Pabna district is presented in Figure 6. To determine the infection percentage of five different plant species were collected from vatikoa village of pabna area. The percent root infection was ranged within 0%-21percentage. Among the five plant species the highest root infection was observed in *Brassica napus* (21%) and the lowest root infection was observed in *Corchorus capsularis*. No infection was recorded in *Lagenaria vulgaris* and *Abelmoscus esculentus*.

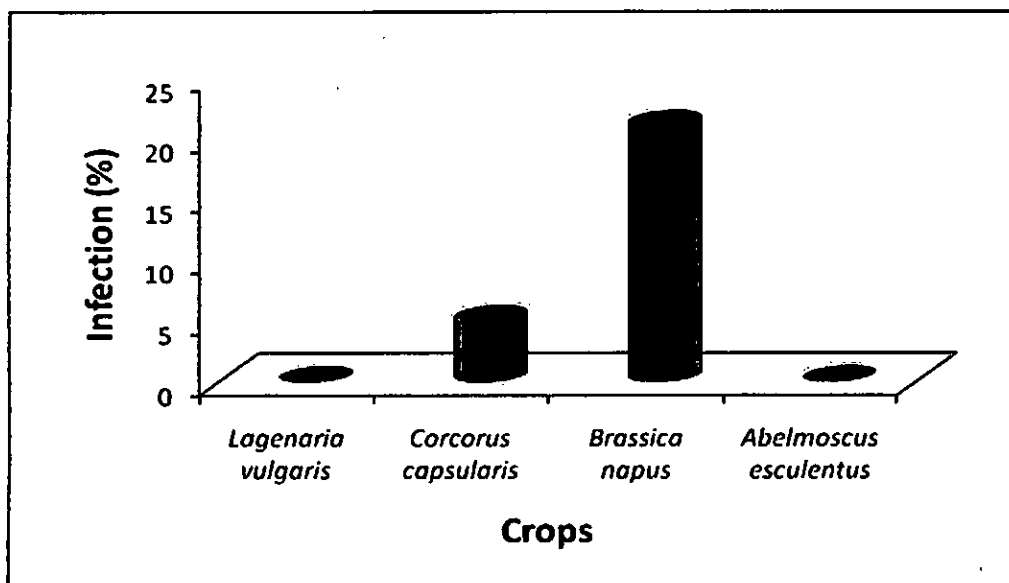


Figure 6. Percent AMF infection of different agricultural crops root collected from Vatikoa village of Pabna district.

Percentage of intensity of infection of AMF on different agricultural crops root collection from Vatikoa village of Pabna district is presented in Table 4. Intensity of infection was also varied at different scale. The highest percentage of intensity was found in *Brassica napus* and that was 17 in scale 1 and 4 in scale 2. There was no intensity of infection was found in 'scale 3'.

Table 4. Percent intensity of infection of AMF on root of different agricultural crops collected from Vatikoa of Pabna district

Name of the plant species	Intensity of infection (%)			
	0	1	2	3
<i>Lagenaria vulgaris</i>	100	0	0	0
<i>Corcorus capsularis</i>	95	5	0	0
<i>Brassica napus</i>	79	17	4	0
<i>Abelmoscus esculentus</i>	100	0	0	0

0-3 scale: 0= no infection, 1= only mycelium present, 2= mycelium + vesicle present, 3= mycelium + vesicle + arbuscule present

4.2. Role of Mycorrhiza on plant growth in arsenic amended soil

Poly bag Experiment

Role of AMF on plant growth of some agricultural crops grown in soil amended with different concentrations of arsenic solution is studied in this section.

A.Brinjal

➤ Seedling emergence

The influence of AMF inoculation on seedling emergence of brinjal seeds sown in soil amended with different concentrations of arsenic is shown in table 5. The seed germination was recorded after 7, 10 and 15 days of sowing. The seedling emergence varied at different concentrations but with increase of incubation period the emergence increased. Higher seed germination was recorded in treatment T₃ in all the three recorded time (Figure 7). Among the treatment T₂, T₄ and T₆ higher germination was recorded in treatment T₂. In the same way among the treatment T₃, T₅ and T₇ higher germination was recorded in treatment T₃, where mycorrhiza was added with 10 ppm arsenic solution. In comparison to 10 ppm arsenic + mycorrhiza gave better germination than 100 ppm arsenic + mycorrhiza treatment.

➤ Number of leaves

The influence of AMF inoculation on number of leaves of brinjal at different growth periods in soil amended with different concentrations of Arsenic solution is also presented in Table 5. The number of leaves of brinjal was recorded after 10, 20 and 30 days of sowing. The highest number of leaves of brinjal was recorded in treatment T₅ and the lowest number of leaves of Brinjal was recorded in treatment T₇ at 10 DAS. Similar results were also obtained at 20 DAS and 30 DAS. It was observed that the number of leaves per plant of mycorrhizal treatment is more than the other treatment. Among the treatments it was revealed that with the increase of arsenic concentration the number of leaves of brinjal decreasing.



➤ Shoot height

The influence of AMF inoculation on shoot height of brinjal seeds sown in soil amended with different concentrations of arsenic solution are shown in Table 5. The shoot height was varied significantly at different concentrations. The shoot height was recorded after 30, 45 and 60 days after sowing. The highest shoot height was recorded in T₃ treatment after 60DAS. In all the three recorded periods it was observed that the shoot height of brinjal decreased with the increase of arsenic concentration and shoot height was higher in mycorrhiza inoculated poly bags than non-inoculated.

Root length

Root length of brinjal influenced by AMF inoculation in soil amended with different concentrations of arsenic solution is presented in Table 5. Data were recorded after 30, 45 and 60 days of sowing. Root length of brinjal ranges from 14.53cm-19.37cm, 20.27cm-22.37cm and 18.53cm-28.07cm at 30 DAS, 45DAS and 60DAS respectively. In comparison to all the treatments mycorrhizal treatment T₃ (10 ppm arsenic solution + mycorrhiza) gave the highest result and those were 22.87cm and 28.07cm at 45DAS and 60DAS respectively and the lowest root length of brinjal was recorded in case of treatment T₄ (100 ppm arsenic solution) and those were 14.53cm, 20.27cm and 21.37cm at 30 DAS, 45DAS and 60DAS respectively. It was revealed from the table the root length of brinjal was the lowest in only arsenic treated poly bags but when mycorrhiza is added with those treatments the root length of brinjal increased. Among the treatment T₂, T₄ and T₆ treatment T₆ gave the lowest result so it was clearly exposed that with the increase of arsenic concentration the root length of brinjal decreasing.

Table 5. Influence of AMF inoculation on germination percentage, number of leaves, shoot height and root length of brinjal at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Germination percentage			Number of leaves			Shoot height (Cm)			Root length (Cm)		
	7 DAS	10 DAS	15 DAS	10 DAS	20 DAS	30 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
T ₁	12.6 0 AB	15.00 A	15.20 AB	1.00 A	3.33 AB	5.33 A	17.67 BC	18.73 C	20.73 AB	15.67 CD	20.53 A	21.63 C
T ₂	14.6 0 AB	14.60 A	15.00 AB	1.33 A	3.66 AB	5.66 A	17.97 BC	17.37 C	17.83 C	16.50 BCD	20.70 A	22.80 D
T ₃	14.8 0 A	15.20 A	18.00 A	1.33 A	3.66 AB	5.00 A	18.90 ABC	18.97 BC	22.20 A	17.40 ABC	22.87 A	28.07 A
T ₄	11.2 0 B	12.00 A	12.20 AB	1.00 A	1.66 BC	2.66 B	18.13 BC	18.03 C	18.03 C	14.53 D	20.27 A	21.37 D
T ₅	13.8 0 AB	14.40 A	14.60 AB	1.33 A	4.00 A	5.66 A	21.67 A	20.77 A	19.60 BC	19.17 A	22.37 A	18.53 D
T ₆	13.2 0 AB	14.80 A	15.40 AB	1.33 A	4.00 A	5.33 A	20.10 AB	21.43 A	19.80 AB	18.47 A	20.77 A	21.03 C
T ₇	11.8 0 AB	12.40 A	13.20 B	1.00 A	1.00 C	2.00 B	16.90 C	19.00 BC	18.57 BC	19.37 A	21.73 A	23.67 B
LSD	3.16 4	NS	2.933	NS	2.091	1.677	2.762	1.827	2.325	2.446	3.188	1.832
CV (%)	8.44	9.60	5.18	19.04	13.08	6.74	9.43	5.01	9.32	6.24	4.45	8.89

T₁ = Control

T₂ =10 ppm arsenic solution

T₃=10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ =100 ppm arsenic solution+ mycorrhiza

T₆ =500 ppm arsenic solution

T₇ =500 ppm arsenic solution+ mycorrhiza

➤ **Fresh weight of shoot**

The influence of AMF inoculation on fresh weight of shoot of brinjal at different growth periods in soil amended with different concentrations of arsenic solution is presented in Table 5. Fresh weight of shoot of brinjal ranges from 6.71-10.77, 7.030-11.24 and 8.900-19.18 at 30 DAS, 45 DAS and 60 DAS respectively. The highest fresh weight of shoot of Brinjal was recorded in treatment T₃ followed by treatment T₂, T₅ and the lowest fresh weight of shoot of brinjal was recorded in treatment T₇ at 30 DAS. Similar results were also obtained at 45 DAS and 60 DAS. In case of treatment T₂ and T₄ it was clearly showed that with the increase of arsenic concentration the fresh weight of shoot of brinjal decreased. In case of only arsenic treatment T₂ (10 ppm arsenic solution) the fresh weight of shoot of brinjal at 60DAS was 13.75gm but it increased to 19.18 gm when we inoculated mycorrhiza with that 10 ppm arsenic solution.

➤ **Fresh weight of root**

The role of AMF inoculation on fresh weight of root of brinjal, which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 6. There was a remarkable variation of fresh weight of root of brinjal among the 7 different treatments. The variation of fresh weight of root of brinjal was recorded due to the effect of mycorrhiza inoculation against arsenic solution. Result revealed that treatment T₃ (10 ppm arsenic solution + mycorrhiza) gave the highest results and those were 8.97gm, 8.99gm and 10.93gm at 30 DAS, 45 DAS and 60 DAS respectively which is significantly better in comparison to each of the other treatments.

Treatment T₁ (Control) showed the second highest fresh weight of root of brinjal. The lowest Fresh weight of root of Brinjal was recorded from the only arsenic treated treatments (T₂, T₄ and T₆) and fresh weight of root of Brinjal decreased when the rate of arsenic concentration increased. The variation of fresh weight of root of brinjal was recorded due to the effect of mycorrhiza inoculation against arsenic solution.

➤ **Dry weight of shoot**

The role of AMF inoculation on dry weight of shoot of brinjal, which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 6. There was a remarkable variation of dry weight of shoot among the 7 different treatments. The variation of dry weight of shoot of brinjal was recorded due to the effect of mycorrhiza inoculation against arsenic solution. Result revealed that treatment T₃(10 PPM arsenic solution + mycorrhiza) gave the highest 1.67gm 1.93gm 1.99gm at 30 DAS, 45 DAS and 60 DAS respectively which is significantly different and better in comparison to each of the other treatments. Treatment T₁ (Control) showed the second highest dry weight of shoot of brinjal. The dry weight of shoot of brinjal decreased when the rate of arsenic concentration increased.

➤ **Dry weight of root**

The role of AMF inoculation on dry weight of root of brinjal, as affected by with different concentrations of arsenic solution is shown in Table 6. There was a remarkable variation of dry weight of root among the 7 different treatments. The variation of dry weight of root of brinjal was recorded due to the effect of mycorrhiza inoculation against arsenic solution. Result revealed that treatment T₃ (10 ppm arsenic

solution + mycorrhiza) gave the highest dry weight of root of brinjal and those were 0.53gm 1.16gm 1.74gm at 30 DAS, 45 DAS and 60 DAS respectively which is significantly different and better in comparison to each of the other treatments. Treatment T₁ (Control) showed the second highest dry weight of root of brinjal. Dry weight of root of brinjal decreased when the rate of arsenic concentration increased.

Table 6. Influence of AMF inoculation on fresh and dry weight of shoot and root of brinjal at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Fresh weight of shoot (g)			Fresh weight of root (g)			Dry weight of shoot (g)			Dry weight of root (g)		
	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
T ₁	8.77 B	9.57 ABC	11.4 7 BC	8.79 A	8.57 A	8.73 B	1.58 A	1.42 A	1.69 A	0.50 A	0.98 A	1.61 C
T ₂	10.7 7 A	10.5 3 AB	13.7 5 B	8.46 A	8.57 AB	10.4 5 B	1.40 A	1.40 A	1.45 A	0.26 AB	0.73 A	1.40 ABC
T ₃	8.43 BC	11.2 4 A	19.1 8 A	8.97 A	8.99 AB	10.9 3 A	1.67 A	1.93 A	1.99 A	0.53 A	1.16 A	1.74 A
T ₄	6.93 D	7.58 BC	12.9 9 B	5.67 0 B	5.89 B	6.75 C	1.57 A	1.39 A	1.58 A	0.14 B	0.82 A	1.08 AB
T ₅	6.88 D	10.1 0 AB	13.4 1 B	6.21 B	7.31 AB	7.21 C	1.36 A	1.27 A	1.58 A	0.29 AB	0.85 A	1.25 BC
T ₆	7.91 C	8.63 ABC	10.5 5 B	6.75 B	7.63 AB	7.55 C	1.06 A	1.21 A	1.40 A	0.39 AB	0.63 A	0.49 D
T ₇	6.71 D	7.03 C	8.90 0 C	6.31 B	6.73 AB	6.91 C	1.40 A	1.03 A	1.54 A	0.26 AB	0.52 A	0.62 D
LSD	0.73 35	2.79 2	3.44 3	1.53 3	2.04 2	9.19 0	0.57 65	0.96 79	0.401 8	0.313 2	NS	0.38 98
CV (%)	5.07	5.68	4.36	6.08	6.87	9.98	5.50	6.90	7.77	8.81	7.89	5.78

T₁ = Control

T₂ =10 ppm arsenic solution

T₃=10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ =100 ppm arsenic solution+ mycorrhiza

T₆ =500 ppm arsenic solution

T₇=500 ppm arsenic solution+ mycorrhiza



➤ **Percent vigourity:**

The role of AMF inoculation on vigourity percentage of brinjal, as affected by different concentrations of arsenic solution is presented in Table 7. There was a remarkable variation of plant vigor of brinjal by the 7 different treatments. Result revealed that treatment T₃ (10 ppm Arsenic solution + mycorrhiza) gave the highest percent vigourity of brinjal and those were 22.41%, 28.27% and 31.36% at 30 DAS, 45 DAS and 60 DAS respectively which is significantly different and better in comparison to other treatments. Treatment T₁ (Control) showed the second highest percent vigourity. The lowest vigourity percentage of brinjal was recorded from the 500 ppm arsenic treated treatment (T₆) and percent vigourity of Brinjal decreased when the rate of arsenic concentration increased.

➤ **Percent vigourity:**

The role of AMF inoculation on vigourity percentage of brinjal, as affected by different concentrations of arsenic solution is presented in Table 7. There was a

remarkable variation of plant vigor of brinjal by the 7 different treatments. Result revealed that treatment T₃ (10 ppm Arsenic solution + mycorrhiza) gave the highest percent vigourity of brinjal and those were 22.41%, 28.27% and 31.36% at 30 DAS, 45 DAS and 60 DAS respectively which is significantly different and better in comparison to other treatments. Treatment T₁ (Control) showed the second highest percent vigourity. The lowest vigourity percentage of brinjal was recorded from the 500 ppm arsenic treated treatment (T₆) and percent vigourity of Brinjal decreased when the rate of arsenic concentration increased.

Table7. Influence of AMF inoculation on percent vigourity of brinjal at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Vigourity (%)		
	30 DAS	45 DAS	60 DAS
T ₁	20.47 B	25.99 AB	27.81 B
T ₂	19.55 B	24.73 BC	24.22 C
T ₃	22.41 A	28.27 A	31.62 A
T ₄	19.70 B	22.34 C	23.18 CD
T ₅	20.43 B	24.54 BC	23.98 C
T ₆	19.44 B	22.03 C	22.19 D
T ₇	18.14 C	23.33 C	23.19 C
LSD	1.285	2.380	1.545
CV (%)	1.77	2.79	1.32

T₁ = Control

T₂ =10 ppm arsenic solution

T₃=10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₃ =100 ppm arsenic solution+ mycorrhiza

T₆ =500 ppm arsenic solution

T₇ =500 ppm arsenic solution+ mycorrhiza

➤ **Nutrient uptake:**

The inoculation of arbuscular mycorrhizal fungi in response to nutrient uptake (N, P, K and S) by brinjal shoots at 60 DAS is presented in Table 8. It is revealed from the study that mycorrhizal fungi have a positive role in response to nutrient uptake and mycorrhizal fungi inoculated treatments significantly enhanced nutrient uptake by brinjal shoot in comparison to other treatment. The highest nutrient uptake was recorded in case of treatment T₃ (10 ppm arsenic solution + mycorrhiza) and those were 2.7 % total N, 0.77 % P, 4.56 % K and 0.77 % S and the lowest result was found in case of treatment T₆ (500 ppm arsenic solution) and those were 1.53 % total N, 0.30 % P, 1.41 % K and 0.27 % S. It was clearly showed from the table that due to toxicity of arsenic the percent nutrient uptake by shoots of brinjal was the lowest but when mycorrhiza was inoculated percentage of nutrient uptake was increased.

Arsenic uptake

Influence of AMF inoculation in response to arsenic uptake by shoots of brinjal which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 8. The amount of arsenic uptake by shoots of brinjal at 60 DAS differed significantly due to inoculation of AMF and different concentrations of arsenic solution. Due to inoculation of AMF, lower amount of arsenic was found in AMF inoculated pots than non-inoculated ones. The amount of arsenic increased with the increase rate of arsenic concentrations but in all the cases inoculation of AMF the

rate of arsenic decreased. The lowest amount of arsenic was found in treatment T₃ (10 ppm arsenic solution + mycorrhiza) and that was 59 ppm on the other hand the highest amount was found in treatment T₆ (500 ppm arsenic solution) and that was 159 ppm. Between the treatment T₂ and T₃, the amount of arsenic was higher in treatment T₂ and that was 129.3 ppm but inoculation of mycorrhiza on that treatment the amount decreased significantly to 59.0 ppm. Same kind of result was found from other treatments. It was observed from the table that inoculation of mycorrhizal fungi helps to reduce arsenic uptake up to 50%.

➤ Chlorophyll analysis

Influence of AMF inoculation in response to the content of chlorophyll in leaves of brinjal, which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 8. The amount of chlorophyll in leaves of brinjal at 60 DAS differed significantly due to inoculation of AMF and different concentrations of arsenic solution. Due to inoculation of AMF, higher amount of chlorophyll was found in AMF inoculated pots than non-inoculated ones. The amount of chlorophyll increased with the decrease rate of arsenic concentrations and in all the cases inoculation of AMF the rate of chlorophyll increased. The lowest amount of chlorophyll was found in treatment T₇ (500 ppm arsenic solution + mycorrhiza) and that was 0.1177 ppm on the other hand the highest amount was found in treatment T₃ (10 ppm arsenic solution + mycorrhiza) and that was 0.2243 ppm. Among the treatment T₂, T₄ and T₆, the amount of chlorophyll was higher in treatment T₂ and that was 0.1437 ppm and lower in treatment T₆. It was observed from the table that when lower amount of arsenic concentration used, inoculation of mycorrhizal fungi helps to increase chlorophyll content in all the treatments than non inoculated ones.

Table 8. Influence of AMF inoculation on nutrient uptake and arsenic uptake by shoot and chlorophyll content by leaves of brinjal at 60 DAS in soil amended with different concentrations of arsenic solution

Treatments	Nutrient uptake				Arsenic (As) (ppm)	Chlorophyll content
	Total N %	P %	K %	S %		
T ₁	2.10 C	0.63 AB	2.40 BC	0.40 BC	0.00 D	0.1260 B
T ₂	1.56 D	0.48 AB	2.64 BC	0.76 A	129.3 A	0.1437 B
T ₃	2.70 A	0.76 AB	4.56 A	0.77 A	59.00 B	0.2243 A
T ₄	2.56 A	0.43 AB	4.13 A	0.46 B	51.00 C	0.1340 B
T ₅	2.33 B	0.56 AB	3.40 AB	0.40 B	86.00 B	0.1617 B
T ₆	1.53 D	0.30 B	1.41 CD	0.27 C	159.00 A	0.1320 B
T ₇	2.13 C	0.45 A	1.41 CD	0.64 A	80.33 A	0.1173 B
LSD	0.1949	0.3375	1.226	0.1378	32.18	2.18
CV (%)	5.18	4.78	6.46	4.64	8.79	9.98

T₁ = Control

T₂ =10 ppm arsenic solution

T₃=10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ =100 ppm arsenic solution+ mycorrhiza

T₆ =500 ppm arsenic solution

T₇=500 ppm arsenic solution+ mycorrhiza

➤ **Root colonization:**

Root Colonization was demonstrated by the presence of vesicles, arbuscules and/or hyphae in root tissue. The highest percent root colonization 43.00% of 10 ppm + mycorrhiza inoculated plants were recorded at 60 DAS and lowest 9.36% of 500 ppm + mycorrhiza inoculated plants were recorded at 30DAS. On the other hand no root colonization was found in non-inoculated poly bags.

Table 9. Root colonization (%) in brinjal by the influence of AMF inoculation at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Root colonization (%)		
	30 DAS	45 DAS	60 DAS
T ₂	13.45	13.56	23.23
T ₃	16.66	17.89	43.00
T ₄	15.11	14.21	17.23
T ₅	11.23	11.10	21.21
T ₆	9.99	12	11.27
T ₇	9.36	11.45	10.25

T₁ = Control

T₂ = 10 ppm arsenic solution

T₃ = 10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ = 100 ppm arsenic solution+ mycorrhiza

T₆ = 500 ppm arsenic solution

T₇ = 500 ppm arsenic solution+ mycorrhiza





A

B



C

D



E

F



Figure 8. Role of mycorrhiza on germination and seedling growth of tomato in arsenic treated soil.

A= 10 ppm arsenic solution, B= Mycorrhiza +10 ppm arsenic solution
C= 100 ppm arsenic solution, D= Mycorrhiza +100 ppm arsenic solution
E= 500 ppm arsenic solution, F= Mycorrhiza +500 ppm arsenic solution

B. Tomato

➤ Seedling emergence

The influence of AMF inoculation on seedling emergence of tomato at different growth periods in soil amended with different concentrations of arsenic solution is shown in table 10. The seed germination was recorded after 7, 10 and 15 days of sowing. The seedling emergence was varied at different concentrations but with increase of incubation period the emergence was increased. Higher seed germination was recorded in treatment T₃ in all the three recorded time (Fig.8). Among the treatment T₂, T₄ and T₆ higher germination was recorded in treatment T₂. In the same way among the treatment T₃, T₅ and T₇ higher germination was recorded in treatment T₃, where mycorrhiza was added with 10 ppm arsenic solution. In comparison to 10 ppm arsenic + mycorrhiza gave better germination than 100 ppm arsenic + mycorrhiza treatment.

➤ Number of leaves:

The influence of AMF inoculation on number of leaves of tomato at different growth periods in soil amended with different concentrations of Arsenic solution is presented in Table 10. The number of leaves of tomato was recorded after 10, 20 and 30 days of sowing. The highest number of leaves of tomato was recorded in treatment T₃ and the lowest number of leaves of tomato was recorded in treatment T₄ at 20 DAS. Similar results were also obtained at 30 DAS. It was observed that the number of leaves per plant of mycorrhizal treatment is more than the other treatment. Among the treatments it was revealed that with the increase of arsenic concentration the number of leaves of tomato decreasing.

➤ **Shoot height**

The influence of AMF inoculation on shoot height of Tomato, seeds sown in soil amended with different concentrations of arsenic solution are shown in Table 10. The shoot height was varied significantly at different concentrations. The shoot height was recorded after 30, 45 and 60 days after sowing. The highest shoot height was recorded in T₃ treatment after 60DAS. In all the three recorded periods it was observed that the shoot height of tomato decreased with the increase of arsenic concentration and shoot height was higher in mycorrhiza inoculated poly bags than non-inoculated.

➤ **Root length**

Root length of tomato influenced by AMF inoculation in soil amended with different concentrations of arsenic solution is presented in Table 10. Data were recorded after 30, 45 and 60 days of sowing. Root length of tomato ranges from 15.67cm-19.37cm, 12.60cm-17.47cm and 15.0cm-25.27cm at 30 DAS, 45DAS and 60DAS respectively. In comparison to all the treatments mycorrhizal treatment T₃ (10 ppm arsenic solution + mycorrhiza) gave the highest result and those were 17.47cm and 28.07cm at 45DAS and 60DAS respectively and the lowest root length of tomato was recorded in case of treatment T₆ (500 ppm arsenic solution) and those were 13.13cm, 13.87cm and 15.30cm at 30 DAS, 45DAS and 60DAS respectively. It was revealed from the table the root length of Tomato was the lowest in only arsenic treated poly bags but when we added mycorrhiza with those treatments the root length of tomato increased. Among the treatment T₃, T₅ and T₇ treatment T₇ gave the lowest result so it was clearly

exposed that with the increase of arsenic concentration the root length of tomato decreasing.

B. Tomato

Table 10. Influence of AMF inoculation on germination percentage, number of leaves, shoot height and root length of tomato at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Germination percentage			Number of leaves			Shoot height (Cm)			Root length (Cm)		
	7 DAS	10 DAS	15 DAS	10 DAS	20 DAS	30 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
T ₁	13.40 A B	13.40 AB	14.40 A	2.66 A	6.00 ABC	5.00 B	17.43 A	18.30 AB	19.07 AB	5.19 a	5.63 a	5.83 ab
T ₂	14.40 A	14.20 A	15.00 A	2.00 A	5.66 ABC	6.00 AB	17.83 A	16.97 AB	18.27 AB	4.74 a	5.20 a	6.13 ab
T ₃	14.60 A	14.78 AB	17.00 A	2.66 A	7.00 ABC	7.00 AB	18.30 A	18.67 A	19.77 A	5.77 a	6.63 a	7.86 a
T ₄	9.40 A B	8.60 B	14.80 A	2.00 A	4.66 C	5.00 B	17.17 A	17.60 AB	18.73 AB	4.96 a	5.10 a	5.99 ab
T ₅	13.20 A B	13.29 AB	15.00 A	2.00 A	5.66 A	5.66 AB	16.63 A	16.97 AB	18.13 AB	4.00 a	5.90 a	6.64 ab
T ₆	7.20 C	13.37 AB	12.60 A	2.33 A	6.33 AB	6.33 AB	15.33 A	16.27 B	17.87 B	4.13 a	4.12 a	4.87 b
T ₇	8.60 BC	9.80 AB	12.40 A	2.00 A	5.33 BC	6.66 AB	17.10 A	17.27 AB	18.13 AB	4.22 a	4.44 a	4.50 b
LSD	5.01	6.56	NS	NS	1.381	1.569	NS	2.067	1.605	2.121	NS	2.238
CV (%)	8.44	9.60	5.18	7.80	3.37	4.82	9.41	6.66	4.86	5.39	4.83	6.25

T₁ = Control

T₂ =10 ppm arsenic solution

T₃=10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ =100 ppm arsenic solution+ mycorrhiza

T₆ =500 ppm arsenic solution

T₇=500 ppm arsenic solution+ mycorrhiza

➤ Fresh weight of shoot

The influence of AMF inoculation on fresh weight of shoot of tomato at different growth periods in soil amended with different concentrations of arsenic solution is presented in Table 11. Fresh weight of shoot of tomato ranges from 10.30-19.50, 11.46-20.66 and 12.07-22.82 at 30 DAS, 45 DAS and 60 DAS respectively. The highest fresh weight of shoot of Tomato was recorded in treatment T₃ followed by treatment T₂, T₅ and the lowest fresh weight of shoot of tomato was recorded in treatment T₄ at 30 DAS. Similar results were also obtained at 45 DAS and 60 DAS. Incase of treatment T₂ and T₄ it was clearly showed that with the increase of arsenic concentration the fresh weight of shoot of tomato decreased. Incase of only arsenic treatment T₂ (10 ppm arsenic solution) the fresh weight of shoot of tomato at 60DAS was 18.20 gm but it increased to 22.82 gm when we inoculated mycorrhiza with that 10 ppm arsenic solution.

➤ Fresh weight of root

The role of AMF inoculation on fresh weight of root of tomato, which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 11. The variation of fresh weight of root of tomato was recorded due to the effect of mycorrhiza inoculation against arsenic solution. Result revealed that treatment T₃ (10 ppm arsenic solution + mycorrhiza) gave the highest results at 60DAS and at 30 DAS, 45 DAS the amount was 5.787gm and 6.630gm respectively which is significantly better in comparison to each of the other treatments. Treatment T₁ (Control) showed the second highest fresh weight of root of tomato at 60DAS. The lowest fresh weight of root of tomato was recorded from the only arsenic treated treatments (T₂, T₄ and T₆) and Fresh weight of root of tomato decreased when the rate of arsenic concentration increased. The variation of fresh weight of root of tomato was recorded due to the effect of mycorrhiza inoculation against arsenic solution.

➤ Dry weight of shoot

The role of AMF inoculation on dry weight of shoot of tomato, which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 11. There was a remarkable variation of dry weight of shoot among the 7 different treatments. The variation of dry weight of shoot of tomato was recorded due to the effect of mycorrhiza inoculation against arsenic solution. Result revealed that treatment T₃(10 PPM arsenic solution + mycorrhiza) gave the highest 3.050gm 3.257gm 3.913gm at 30 DAS, 45 DAS and 60 DAS respectively which is significantly better in comparison to each of the other treatments. Treatment T₂ (10 PPM arsenic



solution) showed the second highest dry weight of shoot of tomato. The dry weight of shoot of tomato decreased when the rate of arsenic concentration increased.

➤ **Dry weight of root**

The role of AMF inoculation on dry weight of root of tomato, which was sown in soil, amended with different concentrations of arsenic solution is shown in Table 11. The variation of dry weight of root of tomato was recorded due to the effect of mycorrhiza inoculation against arsenic solution. Result revealed that treatment T₃ (10 ppm arsenic solution + mycorrhiza) gave the highest dry weight of root of tomato and those were 0.62gm 0.91gm 2.40 gm at 30 DAS, 45 DAS and 60 DAS respectively which is significantly better in comparison to each of the other treatments. Treatment T₄(100PPm arsenic solution) showed the second highest dry weight of root of tomato. Dry weight of root of tomato decreased when the rate of arsenic concentration increased.

Table 11. Influence of AMF inoculation on fresh and dry weight of shoot and root of tomato at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Fresh weight of shoot (g)			Fresh weight of root (g)			Dry weight of shoot (g)			Dry weight of root (g)		
	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
T ₁	12.6 9 C	14.7 5 BC	16.7 9 B	5.19 a	5.63 a	5.83 ab	2.17 B	2.30 B	2.51 BC	0.56 A	0.69 AB	1.16 BC
T ₂	16.2 7 B	17.1 9 B	18.2 0 B	4.74 a	5.20 a	6.13 ab	2.30 B	2.79 AB	3.71 A	0.53 A	0.78 AB	1.61 B
T ₃	19.5 0 A	20.6 6 A	22.8 2 A	5.77 a	6.63 a	7.86 a	3.05 A	3.27 A	3.91 A	0.62 A	0.91 A	2.40 A
T ₄	10.3 0 D	11.4 6 D	12.0 7 C	4.96 a	5.10 a	5.99 ab	1.70 C	1.57 C	1.89 C	0.62 A	0.76 AB	0.98 C
T ₅	14.2 0 BC	17.0 9 B	18.1 6 B	4.00 a	5.90 a	6.64 ab	1.86 C	2.26 B	3.16 B	0.45 A	0.69 AB	0.91 C
T ₆	13.6 7 C	16.6 0 B	17.0 2 B	4.13 a	4.12 a	4.87 b	2.32 B	2.31 B	2.22 C	0.34 A	0.47 AB	0.74 C
T ₇	12.7 6 C	13.5 3 CD	17.5 8 B	4.22 a	4.44 a	4.50 b	1.66 C	1.66 C	2.06 C	0.34 A	0.36 B	0.69 C
LSD	1.60 5	2.60 2	3.47 6	NS	NS	2.23 8	0.29 77	0.611 1	0.70 26	NS	0.46 73	0.58
CV (%)	6.35	9.20	9.23	5.39	4.83	6.25	5.50	6.90	7.77	5.67		5.55

T₁ = Control

T₂ = 10 ppm arsenic solution

T₃ = 10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ = 100 ppm arsenic solution + mycorrhiza

T₆ = 500 ppm arsenic solution

T₇ = 500 ppm arsenic solution + mycorrhiza

➤ **Percent vigourity**

The role of AMF inoculation on vigourity percentage of tomato, which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 12. There was a remarkable variation of vigourity percentage of tomato among the 7 different treatments. Result revealed that treatment T₃ (10 ppm Arsenic solution + mycorrhiza) gave the highest percent vigourity of tomato and those were 24.11%, 27.75% and 29.37% at 30 DAS, 45 DAS and 60 DAS respectively which is significantly better in comparison to each of the other treatments. Treatment T₁ (Control) showed the second highest percent vigourity. The lowest vigourity percentage of tomato was recorded from the 500 ppm arsenic treated treatment (T₆) and percent vigourity of tomato decreased when the rate of arsenic concentration increased. The variation of vigourity percentage of tomato was recorded due to the effect of mycorrhizal inoculation against arsenic concentration.

Table 12. Influence of AMF inoculation on percent vigourity of Tomato at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Vigourity (%)		
	30 DAS	45 DAS	60 DAS
T ₁	21.99 B	23.00 B	24.66 BC
T ₂	19.22 D	22.68 B	23.31 BC
T ₃	24.11 A	27.75 A	29.37 A
T ₄	20.19 CD	22.17 BC	24.26 BC
T ₅	19.22 D	22.62 B	24.63 B
T ₆	19.01 D	21.11 C	21.83 E
T ₇	21.20 BC	22.21 B	22.91 D
LSD	1.169	1.318	0.7693
CV (%)	3.17	3.20	1.77

T₁ = Control

T₂ =10 ppm arsenic solution

T₃=10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ =100 ppm arsenic solution+ mycorrhiza

T₆ =500 ppm arsenic solution

T₇ =500 ppm arsenic solution+ mycorrhiza

➤ Nutrient uptake

The inoculation of arbuscular mycorrhizal fungi in response to nutrient uptake (N, P, K and S) by tomato shoots at 60 DAS is represented in Table 13. It is revealed from the study that mycorrhizal fungi have a positive role in response to nutrient

uptake and mycorrhizal fungi inoculated treatments significantly enhanced nutrient uptake by tomato shoot in comparison to other treatment. The highest nutrient uptake was recorded in case of treatment T₃ (10 ppm arsenic solution + mycorrhiza) and those were 2.6 % total N, 0.62 % P, 2.68 % K and 0.58 % S and the lowest result was found in case of treatment T₆ (500 ppm arsenic solution) and those were 1.49 % total N, 0.23 % P, 0.97 % K and 0.21 % S. It was clearly showed from the table that due to toxicity of arsenic the percent nutrient uptake by shoots of tomato was the lowest. Mycorrhizal inoculation increased nutrient uptake when the soil was amended with arsenic.

Arsenic uptake

Influence of AMF inoculation in response to arsenic uptake by shoots of tomato which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 13. The amount of arsenic uptake by shoots of tomato at 60 DAS differed significantly due to inoculation of AMF and different concentrations of arsenic solution. Due to inoculation of AMF, lower amount of arsenic was found in AMF inoculated pots than non-inoculated ones. The amount of arsenic increased with the increase rate of arsenic concentrations but in all the cases inoculation of AMF the rate of arsenic decreased. The lowest amount of arsenic was found in treatment T₃ (10 ppm arsenic solution + mycorrhiza) and that was 77.67 ppm on the other hand the highest amount was found in treatment T₆ (500 ppm arsenic solution) and that was 435.3ppm. Between the treatment T₂ and T₃, the amount of arsenic was higher in treatment T₂ and that was 150.5 ppm but inoculation of mycorrhiza on that treatment the amount decreased significantly to 77.67 ppm. Same kind of result was found from other treatments. It was observed from the table that inoculation of mycorrhizal fungi helps to reduce arsenic uptake up to 50%.

➤ Chlorophyll analysis

Influence of AMF inoculation in response to the content of chlorophyll in leaves of tomato, which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 13. The amount of chlorophyll in leaves of tomato at 60 DAS differed significantly due to inoculation of AMF and different concentrations of arsenic solution. Due to inoculation of AMF, higher amount of chlorophyll was found in AMF inoculated pots than non-inoculated ones. The amount of chlorophyll increased with the decrease rate of arsenic concentrations and in all the cases inoculation of AMF the rate of chlorophyll increased. The lowest amount of chlorophyll was found in treatment T₇ (500 ppm arsenic solution + mycorrhiza) and that was 0.1033 ppm on the other hand the highest amount was found in treatment T₃ (10 ppm arsenic solution + mycorrhiza) and that was 0.2673 ppm. Among the treatment T₂, T₄ and T₆, the amount of chlorophyll was higher in treatment T₂ and that was 0.1850 ppm and lower in treatment T₆. It was observed from the table that when lower amount of arsenic concentration used, inoculation of mycorrhizal fungi helps to increase chlorophyll content in all the treatments than non inoculated ones.



Table 13. Influence of AMF inoculation on nutrient uptake and arsenic uptake by shoot and chlorophyll content by leaves of tomato at 60 DAS in soil amended with different concentrations of arsenic solution

Treatments	Nutrient uptake				Arsenic (As) (ppm)	Chlorophyll content
	Total N %	P %	K %	S %		
T ₁	1.70 A	0.44 ABC	2.397 A	0.55 A	150.5 C	0.1630 BC
T ₂	1.66 A	0.50 AB	1.153 BC	0.32 AB	77.67 C	0.1850 B
T ₃	2.60 A	0.62 A	2.687 A	0.58 A	334.0 B	0.2673 A
T ₄	2.43 A	0.52 AB	1.703 B	0.55 A	314.4 B	0.1767 B
T ₅	1.79 A	0.27 BC	1.153 BC	0.35 AB	442.3 A	0.2037 B
T ₆	1.43 A	0.236 C	0.9767 C	0.21 B	435.3 A	0.1170 CD
T ₇	1.51 A	0.39 ABC	1.340 BC	0.33 AB	40.05	0.1033 D
LSD	NS	0.2387	0.6561	0.2516	7.81	0.05626
CV (%)	1.80	2.62	8.21	4.56	150.5 C	9.56

T₁ = Control

T₂ = 10 ppm arsenic solution

T₃ = 10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ = 100 ppm arsenic solution + mycorrhiza

T₆ = 500 ppm arsenic solution

T₇ = 500 ppm arsenic solution + mycorrhiza



➤ Root colonization

Root colonization was demonstrated by the presence of vesicles, arbuscules and/or hyphae in root tissue. The highest percent root colonization 34.00% of 10 ppm + mycorrhiza inoculated plants were recorded at 60 DAS and lowest 13.67% of 500 ppm + mycorrhiza inoculated plants were recorded at 30DAS. On the other hand no root colonization was found in non-inoculated poly bags.

Table 14. Root colonization (%) in tomato by the influence of AMF inoculation at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Root colonization (%)		
	30 DAS	45 DAS	60 DAS
T ₂	14.23	14.34	20.23
T ₃	17.66	29.89	34.00
T ₄	14.11	17.21	17.23
T ₅	16.23	18.10	25.21
T ₆	14.09	16.67	17.27
T ₇	13.67	15.45	17.25

T₂ = 10 ppm arsenic solution

T₃ = 10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ = 100 ppm arsenic solution + mycorrhiza

T₆ = 500 ppm arsenic solution

T₇ = 500 ppm arsenic solution + mycorrhiza



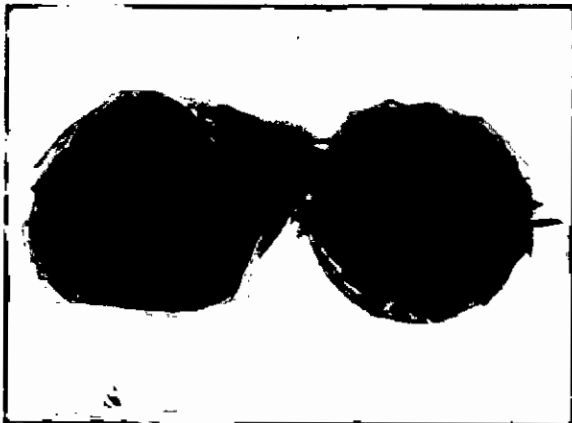
A

B



C

D



E

F



Figure 9. Role of mycorrhiza on germination and seedling growth of chili in arsenic treated soil.

A= 10 ppm arsenic solution, B= Mycorrhiza +10 ppm arsenic solution
C= 100 ppm arsenic solution, D= Mycorrhiza +100 ppm arsenic solution
E= 500 ppm arsenic solution, F= Mycorrhiza +500 ppm arsenic solution

C. Chili

➤ Seedling emergence

The influence of AMF inoculation on seedling emergence of chili at different growth periods in soil amended with different concentrations of arsenic solution is shown in table 15. The seed germination was recorded after 7, 10 and 15 days of sowing. The seedling emergence was varied at different concentrations but with increase of incubation period the emergence was increased. Higher seed germination was recorded in treatment T₃ in all the three recorded time (Fig.9). Among the treatment T₂, T₄ and T₆ higher germination was recorded in treatment T₂. In the same way among the treatment T₃, T₅ and T₇ higher germination was recorded in treatment T₃, where mycorrhiza was added with 10 ppm arsenic solution. In comparison to 10 ppm arsenic + mycorrhiza gave better germination than 100 ppm arsenic + mycorrhiza treatment.

➤ Number of leaves

The influence of AMF inoculation on number of leaves of chili at different growth periods in soil amended with different concentrations of Arsenic solution is presented in Table 15. The number of leaves of chili was recorded after 10, 20 and 30 days of sowing. The highest number of leaves of Chili was recorded in treatment T₃ and the lowest number of leaves of chili was recorded in treatment T₄ at 20 DAS. Similar results were also obtained at 30 DAS. It was observed that the number of leaves per plant of mycorrhizal treatment is more than the other treatment. Among the treatments it was revealed that with the increase of arsenic concentration the number of leaves of chili decreasing.

➤ Shoot height

The influence of AMF inoculation on shoot height of chili, seeds sown in soil amended with different concentrations of arsenic solution are shown in Table 15. The shoot height was varied significantly at different concentrations. The shoot height was recorded after 30, 45 and 60 days after sowing. The highest shoot height was recorded in T₃ treatment after 60DAS. In all the three recorded periods it was observed that the shoot height of chili decreased with the increase of arsenic concentration and shoot height was higher in mycorrhiza inoculated poly bags than non-inoculated.

Root length

Root length of chili influenced by AMF inoculation in soil amended with different concentrations of arsenic solution is presented in Table 15. Data were recorded after 30, 45 and 60 days of sowing. Root length of chili ranges from 11.50cm-15.70cm, 12.33cm-16.73cm and 14.40cm-17.83cm at 30 DAS, 45DAS and 60DAS respectively. In comparison to all the treatments mycorrhizal treatment T₃ (10 ppm arsenic solution + mycorrhiza) gave the highest result and those were 16.73cm and 17.83cm at 45DAS and 60DAS respectively and the lowest root length of chili was recorded in case of treatment T₆ (500 ppm arsenic solution) and those were 11.50cm, 12.33cm and 14.40cm at 30 DAS, 45DAS and 60DAS respectively. . It was revealed from the table the root length of chili was the lowest in only arsenic treated poly bags but when we added mycorrhiza with those treatments the root length of chili increased. Among the treatment T₃, T₅ and T₇ treatment T₇ gave the lowest result so it was clearly exposed that with the increase of arsenic concentration the root length of Tomato decreasing.



Table 15. Influence of AMF inoculation on germination percentage, number of leaves, shoot height and root length of chili at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Germination percentage			Number of leaves			Shoot height (Cm)			Root length (Cm)		
	7 DAS	10 DAS	15 DAS	10 DAS	20 DAS	30 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
T ₁	13.4 0 AB	13.4 0 AB	14.4 0 A	2.00 A	5.66 AB	7.00 BC	18.9 7 B	18.7 3 C	20.73 AB	13.00 BC	13.9 0 BC	14.4 3 B
T ₂	14.4 0 A	14.2 0 A	15.0 0 A	2.00 A	5.66 AB	10.0 A	17.6 0 BC	17.3 7 C	17.83 C	12.40 CD	14.4 0 BC	15.0 7 B
T ₃	14.6 0 A	14.7 8 AB	17.0 0 A	2.00 A	6.33 A	9.00 AB	13.7 3 D	18.9 7 BC	22.20 A	15.70 A	16.7 3 A	17.8 3 A
T ₄	9.40 0 AB	8.60 0 B	14.8 0 A	1.00 B	4.66 B	5.66 C	16.2 3 C	18.0 3 C	18.03 C	10.87 D	12.9 0 BC	17.6 7 A
T ₅	13.2 0 AB	13.2 9 AB	15.0 0 A	1.66 A	6.00 A	9.00 AB	22.3 7 A	20.7 7 AB	19.60 BC	14.33 AB	14.9 0 AB	15.1 3 B
T ₆	7.20 C	13.3 7 AB	12.6 0 A	2.00 A	5.33 AB	6.00 C	22.7 0 A	21.4 3 A	19.80 ABC	11.50 CD	12.3 3 C	14.4 0 B
T ₇	8.60 BC	9.80 AB	12.4 0 A	1.00 B	6.00 A	7.33 BC	19.4 3 B	19.0 0 BC	18.57 BC	12.23 CD	13.3 3 BC	14.4 0 B
LSD	NS	NS	11.7	0.38 9	1.11 0	1.91 1	1.83 8	1.82 7	2.325	1.822	2.06 7	2.22 7
CV (%)	8.44	9.60	5.18	4.32	6.23	3.45	5.55	6.67	3.44	5.84	3.34	6.34

T₁ = Control

T₂ = 10 ppm arsenic solution

T₃ = 10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ = 100 ppm arsenic solution + mycorrhiza

T₆ = 500 ppm arsenic solution

T₇ = 500 ppm arsenic solution + mycorrhiza

➤ **Fresh weight of shoot**

The influence of AMF inoculation on fresh weight of shoot of chili at different growth periods in soil amended with different concentrations of arsenic solution is presented in Table 16. Fresh weight of shoot of chili ranges from 6.713-10.77, 7.030-11.24 and 8.900-19.18 at 30 DAS, 45 DAS and 60 DAS respectively. The highest fresh weight of shoot of chili was recorded in treatment T₃ followed by treatment T₂, T₅ and the lowest fresh weight of shoot of chili was recorded in treatment T₇ at 30 DAS. Similar results were also obtained at 45 DAS and 60 DAS. In case of treatment T₂ and T₄ it was clearly showed that with the increase of arsenic concentration the fresh weight of shoot of chili decreased. In case of only arsenic treatment T₂ (10 ppm arsenic solution) the fresh weight of shoot of chili at 60DAS was 13.75gm but it increased to 19.18 gm when we inoculated mycorrhiza with that 10 ppm arsenic solution.

Fresh weight of root

The role of AMF inoculation on fresh weight of root of chili, which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 16. There was a remarkable variation of fresh weight of root of chili among the 7 different treatments. The variation of fresh weight of root of chili was recorded due to the effect of mycorrhiza inoculation against arsenic solution. Result revealed that treatment T₃ (10 ppm arsenic solution + mycorrhiza) gave the highest results and those were 3.77gm, 4.16 gm and 6.17gm at 30 DAS, 45 DAS and 60 DAS respectively which is significantly better in comparison to each of the other treatments. Treatment

T₁ (Control) showed the second highest fresh weight of root of chili. The lowest fresh weight of root of chili was recorded from the only arsenic treated treatments (T₂, T₄ and T₆) and fresh weight of root of chili decreased when the rate of arsenic concentration increased.

➤ **Dry weight of shoot**

The role of AMF inoculation on dry weight of shoot of chili, which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 16. The variation of dry weight of shoot of Chili was recorded due to the effect of mycorrhiza inoculation against arsenic solution. Result revealed that treatment T₃(10 PPM arsenic solution + mycorrhiza) gave the highest 3.28gm 3.62gm 4.35gm at 30 DAS, 45 DAS and 60 DAS respectively which is significantly better in comparison to each of the other treatments. Treatment T₁ (Control) showed the second highest dry weight of shoot of chili. The dry weight of shoot decreased when the rate of arsenic concentration increased.

➤ **Dry weight of root**

The role of AMF inoculation on dry weight of root of chili, as affected by different concentrations of arsenic solution is shown in Table 16. There was no significant variation of dry weight of root among the 7 different treatments. The variation of dry weight of root was recorded due to the effect of mycorrhiza inoculation against arsenic solution. Result revealed that treatment T₃ (10 ppm arsenic solution + mycorrhiza) gave the highest dry weight of root of chili and those were

1.07gm 1.29gm 2.34gm at 30 DAS, 45 DAS and 60 DAS respectively which is significantly better in comparison to each of the other treatments. Treatment T₁ (Control) showed the second highest dry weight. Dry weight of root decreased when the rate of arsenic concentration increased.

Table 16. Influence of AMF inoculation on fresh and dry weight of shoot and root of chili at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Fresh weight of shoot (g)			Fresh weight of root (g)			Dry weight of shoot (g)			Dry weight of root (g)		
	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
T ₁	9.67 7 D	10.5 4 CD	11.3 3 C	2.96 A	3.26 ABC	4.39 B	2.53 AB	3.18 A	3.70 ABC	0.89 AB	0.97 AB	1.96 AB
T ₂	8.56 7 D	9.36 3 D	11.1 4 C	2.88 0 A	4.02 AB	3.47 BC	2.32 ABC	2.38 B	2.53 D	0.58 B	0.85 AB	1.63 B
T ₃	25.6 0 A	29.3 3 A	31.8 3 A	3.77 A	4.16 A	6.17 A	3.28 A	3.62 A	4.35 A	1.07 A	1.29 A	2.34 A
T ₄	12.9 6 C	14.6 0 BC	15.3 7 BC	2.55 A	2.60 C	2.50 C	2.38 ABC	2.74 B	3.56 BC	0.53 B	0.91 AB	1.45 B
T ₅	18.2 9 B	16.6 1 B	20.4 5 B	2.83 A	2.95 BC	3.66 BC	2.71 AB	2.97 AB	3.22 CD	0.59 B	0.77 B	1.34 B
T ₆	8.52 3 D	9.49 0 D	15.1 9 BC	2.59 A	3.26 ABC	3.62 BC	1.38 C	2.45 B	4.11 AB	0.85 AB	0.88 B	1.50 B
T ₇	4.78 0 E	7.39 0 D	10.9 6 C	2.76 A	2.95 BC	3.06 C	1.96 BC	2.99 AB	2.70 D	0.89 AB	0.78 B	1.50 B
LSD	1.46 5	4.49 6	5.73 3	NS	1.07 2	1.21 4	0.94 64	0.79 96	0.702 6	0.328 0	0.45 01	0.59 27
CV (%)	6.52	8.82	4.45	9.10	8.80	7.59				5.04	4.45	3.98



T₁ = Control

T₂ = 10 ppm arsenic solution

T₃ = 10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ = 100 ppm arsenic solution + mycorrhiza

T₆ = 500 ppm arsenic solution

T₇ = 500 ppm arsenic solution + mycorrhiza

➤ **Percent vigourity:**

The role of AMF inoculation on vigourity percentage of chili as affected by different concentrations of arsenic solution is presented in Table 17. There was a remarkable variation of vigourity percentage of chili among the 7 different treatments. Result revealed that treatment T₃ (10 ppm Arsenic solution + mycorrhiza) gave the highest percent vigourity of chili and those were 42.26%, 46.88% and 49.14 % at 30 DAS, 45 DAS and 60 DAS respectively which is significantly different from other treatments. Treatment T₁ (Control) showed the second highest percent vigourity. The lowest vigourity percentage of chili was recorded from the 500 ppm arsenic treated treatment (T₆) and percent vigourity of chili decreased when the rate of arsenic concentration increased.

Table 17. Influence of AMF inoculation on percent vigourity of chili at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Vigourity (%)		
	30 DAS	45 DAS	60 DAS
T ₁	29.11 B	35.09 B	45.35 B
T ₂	28.25 B	32.48 CD	44.77 B
T ₃	42.26 A	46.88 A	49.14 A
T ₄	26.34 B	34.49 BC	44.02 BC
T ₅	26.64 B	32.51 CD	42.35 CD
T ₆	22.25 C	32.03 D	41.70 D
T ₇	28.21 B	34.08 BCD	42.35 CD
LSD	3.192	2.032	2.013
CV (%)	3.28	2.55	3.88

T₁ = Control

T₂ =10 ppm arsenic solution

T₃=10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ =100 ppm arsenic solution+ mycorrhiza

T₆ =500 ppm arsenic solution

T₇=500 ppm arsenic solution+ mycorrhiza

➤ **Nutrient uptake:**



The inoculation of arbuscular mycorrhizal fungi in response to nutrient uptake (N, P, K and S) by Chili shoots at 60 DAS is represented in Table 18. It is revealed from the study that mycorrhizal fungi have a positive role in response to nutrient uptake and mycorrhizal fungi inoculated treatments significantly enhanced nutrient

uptake by chili shoot in comparison to other treatment. The highest nutrient uptake was recorded in case of treatment T₃ (10 ppm arsenic solution + mycorrhiza) and those were 2.33 % total N, 2.03 % P, 1.38 % K and 1.22 % S and the lowest result was found in case of treatment T₆ (500 ppm arsenic solution) and those were 1.41 % total N, 0.99 % P, 0.693 % K and 0.143 % S. It was clearly showed from the table that due to toxicity of arsenic the percent nutrient uptake by shoots of chili was the lowest but inoculation of mycorrhiza increased nutrient uptake whe soil was treated with arsenic.

Arsenic uptake

Influence of AMF inoculation in response to arsenic uptake by shoots of chili as affected by different concentrations of arsenic solution is presented in Table 18. The amount of arsenic uptake by shoots of chili at 60 DAS differed significantly due to inoculation of AMF and different concentrations of arsenic solution. Due to inoculation of AMF, lower amount of arsenic was found in AMF inoculated pots than non-inoculated ones. The amount of arsenic increased with the increase rate of arsenic concentrations but in all the cases inoculation of AMF the rate of arsenic decreased. The lowest amount of arsenic was found in treatment T₃ (10 ppm arsenic solution + mycorrhiza) and that was 89.3 ppm on the other hand the highest amount was found in treatment T₆ (500 ppm arsenic solution) and that was 289.3 ppm. Between the treatment T₂ and T₃, the amount of arsenic was higher in treatment T₂ and that was 161.7 ppm but inoculation of mycorrhiza on that treatment the amount decreased significantly to 89.67 ppm. Similar result was found from other treatments. It was observed from the table that inoculation of mycorrhizal fungi helps to reduce arsenic uptake up to 50%.

➤ Chlorophyll analysis

Influence of AMF inoculation in response to the content of chlorophyll in leaves of chili as affected by different concentrations of arsenic solution is presented in Table

18. The amount of chlorophyll in leaves of chili at 60 DAS differed significantly due to inoculation of AMF and different concentrations of arsenic solution. Due to inoculation of AMF, higher amount of chlorophyll was found in AMF inoculated pots than non-inoculated ones. The amount of chlorophyll increased with the decrease rate of arsenic concentrations and in all the cases inoculation of AMF the rate of chlorophyll increased. The lowest amount of chlorophyll was found in treatment T₇ (500 ppm arsenic solution + mycorrhiza) and that was 0.1340 ppm on the other hand the highest amount was found in treatment T₃ (10 ppm arsenic solution + mycorrhiza) and that was 0.4197 ppm. Among the treatment T₂, T₄ and T₆, the amount of chlorophyll was higher in treatment T₂ and that was 0.4063 ppm and lower in treatment T₆. It was observed from the table that when lower amount of arsenic concentration used, inoculation of mycorrhizal fungi helps to increase chlorophyll content in all the treatments than non inoculated ones.

Table 18. Influence of AMF inoculation on nutrient uptake and arsenic uptake by shoot and chlorophyll content by leaves of chili at 60 DAS in soil amended with different concentrations of arsenic solution

Treatments	Nutrient uptake				Arsenic (As) (ppm)	Chlorophyll content
	Total N %	P %	K %	S %		
T ₁	1.86 BC	1.13 B	0.91 AB	1.04 B	161.7 A	0.1570 A
T ₂	1.54 CD	1.16 B	1.35 A	0.29 AB	89.67 B	0.4063 A
T ₃	2.33 A	2.03 A	1.38 A	1.21 A	126.7 B	0.4197 A
T ₄	2.06 AB	1.43 B	1.06 AB	0.67 AB	251.3 A	0.2680 A
T ₅	1.80 BC	1.16 B	0.70 B	0.58 AB	289.3 A	0.1550 A
T ₆	1.41 D	0.99 B	0.69 B	0.14 B	116.0 A	0.1460 A
T ₇	1.66 CD	1.40 B	0.78 B	0.45 AB	61.52	0.1340 A
LSD	0.3468	0.4605	0.4394	0.9414		NS
CV (%)	8.81	6.21	2.89	7.76	161.7 A	8.20

T₁ = Control

T₂ = 10 ppm arsenic solution

T₃ = 10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ = 100 ppm arsenic solution + mycorrhiza

T₆ = 500 ppm arsenic solution

T₇ = 500 ppm arsenic solution + mycorrhiza

➤

Root colonization:

Root colonization was confirmed by the presence of vesicles, arbuscules and/or hyphae in root tissue. The highest percent root colonization 38.00% of 10 ppm + mycorrhiza inoculated plants were recorded at 60 DAS and lowest 13.36% of 500 ppm inoculated plants were recorded at 30DAS. On the other hand no root colonization was found in non-inoculated poly bags.

Table 19. Root colonization (%) in chili by the influence of AMF inoculation at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Root colonization (%)		
	30 DAS	45 DAS	60 DAS
T ₂	16.22	16.74	22.23
T ₃	19.66	30.84	38.00
T ₄	17.11	15.21	17.23
T ₅	15.67	19.10	27.21
T ₆	13.36	17.67	18.27
T ₇	13.77	16.45	18.25

T₂ = 10 ppm arsenic solution

T₃ = 10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ = 100 ppm arsenic solution + mycorrhiza

T₆ = 500 ppm arsenic solution

T₇ = 500 ppm arsenic solution + mycorrhiza



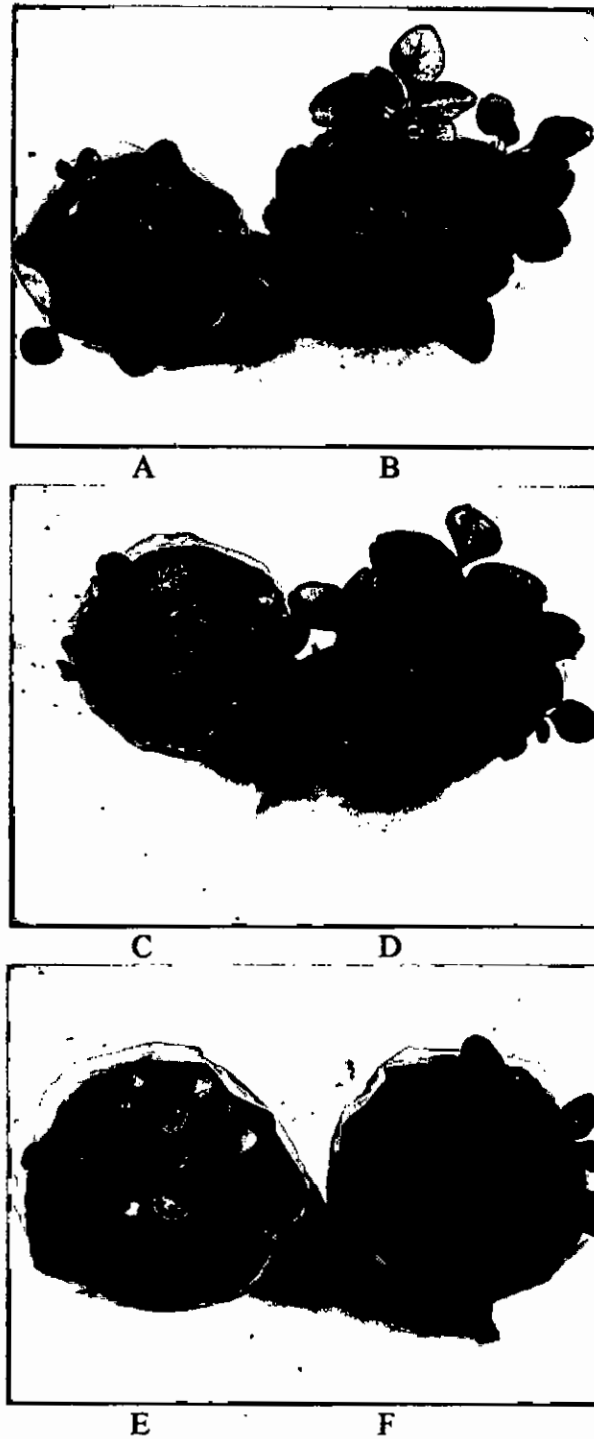


Figure 10. Role of mycorrhiza on germination and seedling growth of danta in arsenic treated soil.

A= 10 ppm arsenic solution, B= Mycorrhiza +10 ppm arsenic solution
 C= 100 ppm arsenic solution, D= Mycorrhiza +100 ppm arsenic solution
 E= 500 ppm arsenic solution, F= Mycorrhiza +500 ppm arsenic solution

➤ **Seedling emergence**

The influence of AMF inoculation on seedling emergence of danta seeds sown in soil amended with different concentrations of arsenic is shown in Table 20. The seed germination was recorded after 7, 10 and 15 days of sowing. The seedling emergence was varied at different concentrations but with increase of incubation period the emergence was increased. Higher seed germination was recorded in treatment T₃ in all the three recorded time (Fig.10). Among the treatment T₂, T₄ and T₆ higher germination was recorded in treatment T₂. In the same way among the treatment T₃, T₅ and T₇ higher germination was recorded in treatment T₃, where mycorrhiza was added with 10 ppm arsenic solution. In comparison to 10 ppm arsenic + mycorrhiza gave better germination than 100 ppm arsenic + mycorrhiza treatment.

➤ **Number of leaves**

The influence of AMF inoculation on number of leaves of danta at different growth periods in soil amended with different concentrations of Arsenic solution is presented in Table 20. The number of leaves of danta was recorded after 10, 20 and 30 days of sowing. The highest number of leaves of danta was recorded in treatment T₃ and the lowest number of leaves of danta was recorded in treatment T₆ at 10 DAS. Similar results were also obtained at 20 DAS and 30 DAS. It was observed that the number of leaves per plant of mycorrhizal treatment is more than the other treatment. Among the treatments it was revealed that with the increase of arsenic concentration the number of leaves of danta decreasing.

➤ Shoot height

The influence of AMF inoculation on shoot height of danta, seeds sown in soil amended with different concentrations of arsenic solution are shown in Table 20. The shoot height was varied significantly at different concentrations. The shoot height was recorded after 30, 45 and 60 days after sowing. The highest shoot height was recorded in T₃ treatment after 60DAS. In all the three recorded periods it was observed that the shoot height of danta decreased with the increase of arsenic concentration and shoot height was higher in mycorrhiza inoculated poly bags than non-inoculated.

Root length

Root length of danta influenced by AMF inoculation in soil amended with different concentrations of arsenic solution is presented in Table 20. Data were recorded after 30, 45 and 60 days of sowing. Root length of Danta ranges from 17.30 cm-27.17cm, 22.10 cm-27.60 cm and 24.33 cm-32.73 cm at 30 DAS, 45DAS and 60 DAS respectively. In comparison to all the treatments mycorrhizal treatment T₃ (10 ppm arsenic solution + mycorrhiza) gave the highest result and those were 27.17cm and 27.60 cm at 45DAS and 60DAS respectively and the lowest root length of Danta was recorded in case of treatment T₆ (100 ppm arsenic solution) and those were 17.30 cm, 22.10 cm and 28.53 cm at 30 DAS, 45DAS and 60DAS respectively. It was revealed from the table the root length of danta was the lowest in only arsenic treated poly bags but when we added mycorrhiza with those treatments the root length of danta increased. Among the treatment T₂, T₄ and T₆ treatment T₆ gave the lowest result so it was clearly exposed that with the increase of arsenic concentration the root length of danta decreasing.



Table 20. Influence of AMF inoculation on germination percentage, number of leaves, shoot height and root length of danta at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Germination percentage			Number of leaves			Shoot height (Cm)			Root length (Cm)		
	7 DAS	10 DAS	15 DAS	10 DAS	20 DAS	30 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
T ₁	13.80 C	15.80 AB	17.00 A	4.66 A	7.33 AB	7.66 AB	19.00 AB	19.00 AB	23.70 AB	25.37 AB	27.13 A	29.93 B
T ₂	16.00 A BC	17.40 A	18.60 A	4.00 A	7.00 AB	8.00 AB	15.73 CD	17.87 BC	23.03 B	25.00 AB	25.30 A	29.20 BC
T ₃	18.80 A	18.80 A	18.80 A	5.00 A	7.11 AB	8.66 A	20.27 A	20.60 A	25.60 A	27.17 A	27.60 A	32.73 A
T ₄	15.00 BC	17.00 AB	16.80 A	4.33 A	6.78 B	7.86 AB	15.23 CD	16.03 CD	21.33 BC	20.83 D	22.70 B	24.33 D
T ₅	17.20 A B	18.60 A	18.60 A	4.66 A	7.66 A	8.66 A	14.13 DE	14.20 D	20.30 C	24.17 C	27.53 A	28.33 BC
T ₆	10.80 D	14.20 AB	16.40 A	4.33 A	6.33 B	7.33 AB	17.40 BC	11.87 E	17.33 D	17.30 E	22.10 B	28.53 B
T ₇	10.00 D	14.20 B	17.00 A	4.66 A	6.33 B	7.66 AB	18.00 BC	15.67 CD	22.37 BC	21.67 CD	22.40 B	26.37 CD
LSD	2.928	3.006	NS	NS	0.9380	1.121	2.439	2.307	2.388	2.383	2.402	2.361
CV (%)	5.45	3.67	3.32	3.79	7.84	7.83	2.34	4.67	7.45	2.56	8.89	2.96

T₁ = Control

T₂ = 10 ppm arsenic solution

T₃ = 10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ = 100 ppm arsenic solution + mycorrhiza

T₆ = 500 ppm arsenic solution

T₇ = 500 ppm arsenic solution + mycorrhiza

➤ Fresh weight of shoot

The influence of AMF inoculation on fresh weight of shoot of danta at different growth periods in soil amended with different concentrations of arsenic solution is presented in Table 21. Fresh weight of shoot of Danta ranges from 15.47-24.20, 17.56-25.97 and 20.73-27.56 at 30 DAS, 45 DAS and 60 DAS respectively. The highest fresh weight of shoot of danta was recorded in treatment T₃ followed by treatment T₅, T₁ and the lowest fresh weight of shoot of Danta was recorded in treatment T₇ at 30 DAS. Similar results were also obtained at 45 DAS and 60 DAS. In case of treatment T₂ and T₄ it was clearly showed that with the increase of arsenic concentration the fresh weight of shoot of danta decreased. In case of only arsenic treatment T₂ (10 ppm arsenic solution) the fresh weight of shoot of danta at 60DAS was 13.75gm but it increased to 19.18 gm when we inoculated mycorrhiza with that 10 ppm arsenic solution.

➤ **Fresh weight of root**

The role of AMF inoculation on fresh weight of root of danta, which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 21. There was a remarkable variation of fresh weight of root of danta among the 7 different treatments. The variation of Fresh weight of root of Brinjal was recorded due to the effect of mycorrhiza inoculation against arsenic solution. Result revealed that treatment T₃ (10 ppm arsenic solution + mycorrhiza) gave the highest results and those were 7.75gm, 24.03gm and 29.03gm at 30 DAS, 45 DAS and 60 DAS respectively which is significantly better in comparison to each of the other treatments. Treatment T₁ (Control) showed the second highest fresh weight of root of Brinjal. The lowest Fresh weight of root of Danta was recorded from the only arsenic treated treatments (T₂, T₄ and T₆) and fresh weight decreased when the rate of arsenic concentration increased.

➤ **Dry weight of shoot**

The role of AMF inoculation on dry weight of shoot of danta, which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 21. There was a remarkable variation of dry weight of shoot among the 7 different treatments. The variation of dry weight of shoot of danta was recorded due to the effect of mycorrhiza inoculation against arsenic solution. Result revealed that treatment T₃(10 PPM arsenic solution + mycorrhiza) gave the highest 3.28gm 3.62gm 4.35gm at 30 DAS, 45 DAS and 60 DAS respectively which is significantly better in

comparison to each of the other treatments. Treatment T₁ (Control) showed the second highest dry weight of shoot. The dry weight of shoot of danta decreased when the rate of arsenic concentration increased.

➤ **Dry weight of root**

The role of AMF inoculation on dry weight of root of danta, which was sown in soil, amended with different concentrations of arsenic solution is shown in Table 21. The variation of dry weight of root of Danta was recorded due to the effect of mycorrhiza inoculation against arsenic solution. Result revealed that treatment T₃ (10 ppm arsenic solution + mycorrhiza) gave the highest dry weight of root of danta and those were 0.89gm 1.29gm 2.34gm at 30 DAS, 45 DAS and 60 DAS respectively which is significantly better in comparison to each of the other treatments. Treatment T₁ (Control) showed the second highest dry weight. Dry weight of root of danta decreased when the rate of arsenic concentration increased.

Table 21. Influence of AMF inoculation on fresh and dry weight of shoot and root of danta at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Fresh weight of shoot (g)			Fresh weight of root (g)			Dry weight of shoot (g)			Dry weight of root (g)		
	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
T ₁	22.47 B	24.56 AB	25.59 ABC	7.25 A	22.62 AB	23.61 B	2.38 ABC	3.12 AB	4.11 AB	0.85 AB	1.10 AB	2.26 A
T ₂	22.78 AB	17.56 C	20.73 D	7.20 A	20.92 BCD	21.73 BC	2.32 ABC	2.90 AB	3.56 BC	0.58 B	1.06 AB	1.63 B
T ₃	24.0 A	25.97 A	27.56 A	7.75 A	24.03 A	29.03 A	3.28 A	3.62 A	4.35 A	0.87 AB	1.29 A	2.34 A
T ₄	22.78 AB	24.57 AB	24.05 BC	5.25 C	18.61 CDE	20.50 C	2.53 AB	2.74 B	2.72 AB	0.57 B	0.97 AB	1.45 B
T ₅	24.06 AB	25.07 A	26.04 AB	6.68 AB	21.32 ABC	23.54 B	2.71 AB	2.97 AB	3.70 ABC	0.82 AB	0.77 B	1.94 AB
T ₆	20.43 C	24.14 AB	23.40 BC	3.70 D	16.90 DE	16.65 D	1.96 BC	2.45 B	2.53 D	1.07 A	0.85 AB	1.56 AB
T ₇	15.47 E	22.20 AB	22.09 CD	5.86 BC	15.57 E	19.16 CD	1.38 C	2.38 B	3.22 CD	0.59 B	0.91 AB	1.50 B
LSD	1.59 0	2.31 7	2.43 9	1.05 2	3.24 5	2.53 5	0.94 64	0.79 96	0.702 6	0.328 0	0.45 01	0.59 27
CV (%)	4.39	8.43	7.49	9.98	3.45	5.67	2.46	5.46	3.56	3.45	6.45	5.34

T₁ = Control

T₂ = 10 ppm arsenic solution

T₃ = 10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ = 100 ppm arsenic solution + mycorrhiza

T₆ = 500 ppm arsenic solution

T₇ = 500 ppm arsenic solution + mycorrhiza



➤ **Percent vigourity:**

The role of AMF inoculation on vigourity percentage of danta as affected by different concentrations of arsenic solution is presented in Table 22. There was a remarkable variation of vigourity percentage of danta among the 7 different treatments. Result revealed that treatment T₃ (10 ppm Arsenic solution + mycorrhiza) gave the highest percent vigourity of Danta and those were 42.88%, 46.26% and 49.14% at 30 DAS, 45 DAS and 60 DAS respectively which is significantly better in comparison to each of the other treatments. Treatment T₁ (Control) showed the second highest percent vigourity. The lowest vigourity percentage of danta was recorded from the 500 ppm arsenic treated treatment (T₆) and percent vigourity of danta decreased when the rate of arsenic concentration increased.

Table 22. Influence of AMF inoculation on percent vigourity of danta at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Vigourity (%)		
	30 DAS	45 DAS	60 DAS
T ₁	29.11 B	34.40 BC	45.35 B
T ₂	28.25 B	34.03 BCD	44.77 B
T ₃	42.88 A	46.26 A	49.14 A
T ₄	26.34 B	35.09 B	42.61 CD
T ₅	26.64 B	32.51 CD	42.53 CD
T ₆	22.26 C	32.08 D	41.70 D
T ₇	28.21 B	32.47 CD	44.02 BC
LSD	3.192	2.032	2.013
CV (%)	6.07	3.28	2.55

T₁ = Control

T₂ = 10 ppm arsenic solution

T₃ = 10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ = 100 ppm arsenic solution + mycorrhiza

T₆ = 500 ppm arsenic solution

T₇ = 500 ppm arsenic solution + mycorrhiza



➤ Nutrient uptake

The inoculation of arbuscular mycorrhizal fungi in response to nutrient uptake (N, P, K and S) by danta shoots at 60 DAS is represented in Table 23. It is revealed from the study that mycorrhizal fungi have a positive role in response to nutrient uptake and mycorrhizal fungi inoculated treatments significantly enhanced nutrient uptake by danta shoot in comparison to other treatment. The highest nutrient uptake was recorded in case of treatment T₃ (10 ppm arsenic solution + mycorrhiza) and those were 2.2 % total N, 0.94 % P, 2.53 % K and 0.70 % S and the lowest result was found in case of treatment T₆ (500 ppm arsenic solution) and those were 0.50 % total N, 0.34 % P, 1.48 % K and 0.24 % S. It was clearly showed from the table that due to toxicity of arsenic the percent nutrient uptakes by shoots of danta was the lowest but when mycorrhiza was inoculated nutrient uptake in shoot was increased.

Arsenic uptake

Influence of AMF inoculation in response to arsenic uptake by shoots of danta which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 23. The amount of arsenic uptake by shoots of danta at 60 DAS

differed significantly due to inoculation of AMF and different concentrations of arsenic solution. Due to inoculation of AMF, lower amount of arsenic was found in AMF inoculated pots than non-inoculated ones. The amount of arsenic increased with the increase rate of arsenic concentrations but in all the cases inoculation of AMF the rate of arsenic decreased. The lowest amount of arsenic was found in treatment T₃ (10 ppm arsenic solution + mycorrhiza) and that was 92 ppm on the other hand the highest amount was found in treatment T₆ (500 ppm arsenic solution) and that was 634.7 ppm. Between the treatment T₂ and T₃, the amount of arsenic was higher in treatment T₂ and that was 176.3 ppm but inoculation of mycorrhiza on that treatment the amount decreased significantly to 92.0 ppm. Same kind of result was found from other treatments. It was observed from the table that inoculation of mycorrhizal fungi helps to reduce arsenic uptake up to 50%.

➤ Chlorophyll Analysis

Influence of AMF inoculation in response to the content of chlorophyll in leaves of danta which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 23. The amount of chlorophyll in leaves of danta at 60 DAS differed significantly due to inoculation of AMF and different concentrations of arsenic solution. Due to inoculation of AMF, higher amount of chlorophyll was found in AMF inoculated pots than non-inoculated ones. The amount of chlorophyll increased with the decrease rate of arsenic concentrations and in all the cases inoculation of AMF the rate of chlorophyll increased. The lowest amount of chlorophyll was found in treatment T₇ (500 ppm arsenic solution + mycorrhiza) and that was 0.1090 ppm on the other hand the highest amount was found in treatment T₃ (10 ppm arsenic solution + mycorrhiza) and that was 0.2330 ppm. Among the treatment T₂, T₄ and T₆, the amount of chlorophyll was higher in treatment T₂ and that was 0.1267 ppm and lower in treatment T₆. It was observed from the table that when lower amount of arsenic concentration used, inoculation of mycorrhizal fungi helps to increase chlorophyll content in all the treatments than non inoculated ones.

Table 23. Influence of AMF inoculation on nutrient uptake and arsenic uptake by shoot and chlorophyll content by leaves of danta at 60 DAS in soil amended with different concentrations of arsenic solution

Treatments	Nutrient uptake				Arsenic (As) (ppm)	Chlorophyll content
	Total N %	P %	K %	S %		
T ₁	1.96 A	0.62 B	2.36 AB	0.70 A	176.3 E	0.1817 AB
T ₂	1.42 A	0.33 B	1.56 D	0.30 B	92.00 F	0.1267 B
T ₃	2.20 A	0.94 A	2.53 A	0.70 A	516.7 C	0.2330 A
T ₄	1.79 A	0.54 B	1.97 C	0.33 B	427.0 D	0.1127 B
T ₅	1.80 A	0.37 B	2.12 BC	0.28 B	634.7 A	0.1857 AB
T ₆	0.503 B	0.34 B	1.48 D	0.24 B	548.0 B	0.1050 B
T ₇	1.66 A	0.37 B	1.51 D	0.27 B	6.996	0.1090 B
LSD	0.8966	0.2869	0.3280	0.1488	4.53	0.09744
CV (%)	2.56	6.78	8.78	9.23	176.3 E	5.01

T₁ = Control

T₂ = 10 ppm arsenic solution

T₃ = 10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ = 100 ppm arsenic solution + mycorrhiza

T₆ = 500 ppm arsenic solution

T₇ = 500 ppm arsenic solution + mycorrhiza

➤ **Root colonization**

Root colonization was demonstrated by the presence of vesicles, arbuscules and/or hyphae in root tissue. The highest percent root colonization 40.00% of 10 ppm + mycorrhiza inoculated plants were recorded at 60 DAS and lowest 16.36% of 500 ppm + mycorrhiza inoculated plants were recorded at 30DAS. On the other hand no root clonization was found in non-inoculated poly bags.

Table 24. Root colonization (%) in danta by the influence of AMF inoculation at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Root colonization (%)		
	30 DAS	45 DAS	60 DAS
T ₂	14.23	14.34	20.23
T ₃	17.66	29.89	40.00
T ₄	14.11	17.21	17.23
T ₅	16.23	18.10	25.21
T ₆	14.09	16.67	17.27
T ₇	16.36	15.45	17.25

T₂ =10 ppm arsenic solution

T₃=10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ =100 ppm arsenic solution+ mycorrhiza

T₆ =500 ppm arsenic solution

T₇ =500 ppm arsenic solution+ mycorrhiza

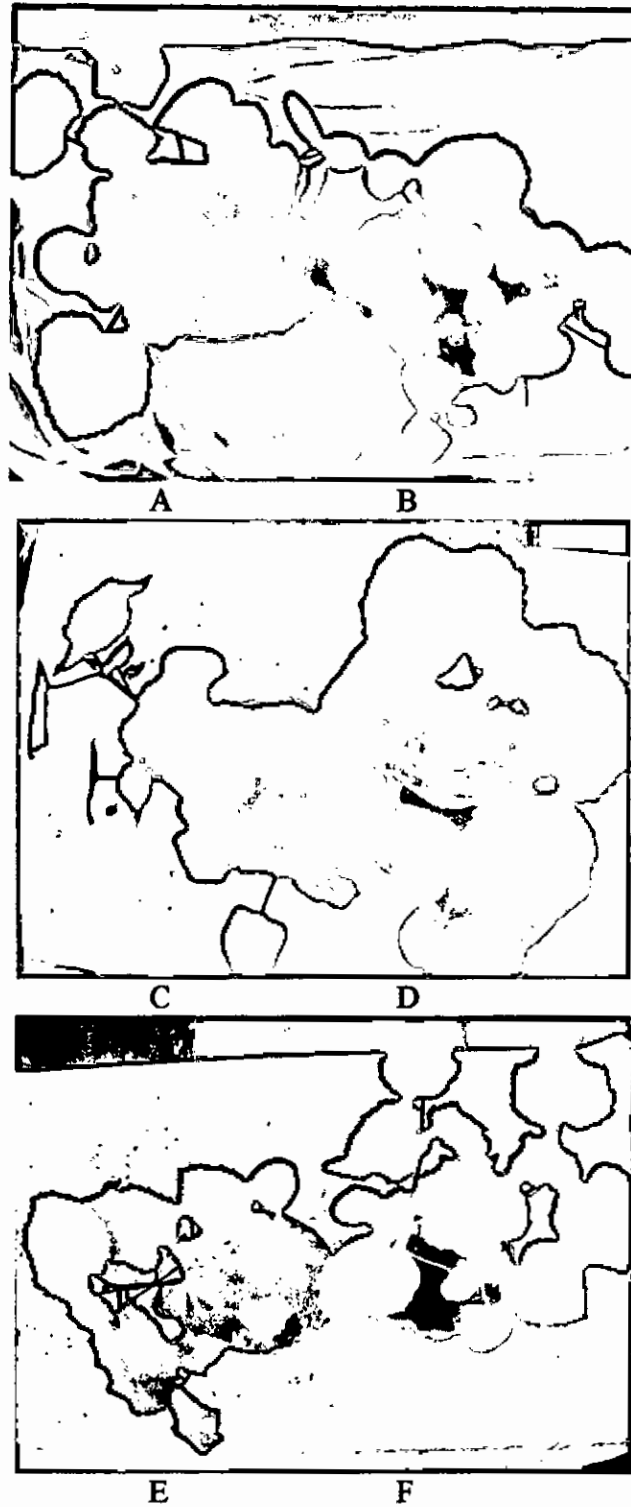


Figure 11. Role of mycorrhiza on germination and seedling growth of okra in arsenic treated soil.
 A= 10 ppm arsenic solution, B= Mycorrhiza +10 ppm arsenic solution
 C= 100 ppm arsenic solution, D= Mycorrhiza +100 ppm arsenic solution
 E= 500 ppm arsenic solution, F= Mycorrhiza +500 ppm arsenic solution

E. okra

➤ Seedling emergence

The influence of AMF inoculation on seedling emergence of okra seeds sown in soil amended with different concentrations of arsenic is shown in Table 25. The seed germination was recorded after 7, 10 and 15 days of sowing. The seedling emergence was varied at different concentrations but with increase of incubation period the emergence was increased. Higher seed germination was recorded in treatment T₃ in all the three recorded time (Fig.11). Among the treatment T₂, T₄ and T₆ higher germination was recorded in treatment T₂. In the same way among the treatment T₃, T₅ and T₇ higher germination was recorded in treatment T₃, where mycorrhiza was added with 10 ppm arsenic solution. In comparison to 10 ppm arsenic + mycorrhiza gave better germination than 100 ppm arsenic + mycorrhiza treatment.

➤ Number of leaves

The influence of AMF inoculation on number of leaves of okra at different growth periods in soil amended with different concentrations of Arsenic solution is presented in Table 25. The number of leaves of okra was recorded after 10, 20 and 30 days of sowing. The highest number of leaves of okra was recorded in treatment T₃ and the lowest number of leaves was recorded in treatment T₇ at 10 DAS. Similar results were also obtained at 20 DAS and 30 DAS. It was observed that the number of leaves per plant of mycorrhizal treatment is more than the other treatment. Among the treatments it was revealed that with the increase of arsenic concentration the number of leaves of okra decreasing.



➤ Shoot height

The influence of AMF inoculation on shoot height of okra, seeds sown in soil amended with different concentrations of arsenic solution are shown in Table 25. The shoot height was varied significantly at different concentrations. The shoot height was recorded after 30, 45 and 60 days after sowing. The highest shoot height was recorded in T₃ treatment after 60DAS. In all the three recorded periods it was observed that the shoot height of okra decreased with the increase of arsenic concentration and shoot height was higher in mycorrhiza inoculated poly bags than non-inoculated.

Root length

Root length of okra influenced by AMF inoculation in soil amended with different concentrations of arsenic solution is presented in Table 25. Data were recorded after 30, 45 and 60 days of sowing. Root length of okra ranges from 19.57 cm-27.33 cm, 21.17 cm-29.23 cm and 23.03 cm-30.27 cm at 30 DAS, 45DAS and 60DAS respectively. In comparison to all the treatments mycorrhizal treatment T₃ (10 ppm arsenic solution + mycorrhiza) gave the highest result and those were 29.23 cm and 30.27 cm at 45DAS and 60DAS respectively and the lowest root length of okra was recorded in case of treatment T₇ (100 ppm arsenic solution) and those were 19.57 cm, 21.17 cm and 23.03 cm at 30 DAS, 45DAS and 60DAS respectively. It was revealed from the table the root length of okra was the lowest in only arsenic treated poly bags but when mycorrhiza was added with those treatments the root length of okra increased. Among the treatment T₂, T₄ and T₆ treatment T₆ gave the lowest result so it was clearly exposed that with the increase of arsenic concentration the root length of okra decreasing.

Table 25. Influence of AMF inoculation on germination percentage, number of leaves, shoot height and root length of okra at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Germination percentage			Number of leaves			Shoot height (Cm)			Root length (Cm)		
	7 DAS	10 DAS	15 DAS	10 DAS	20 DAS	30 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
T ₁	6.80 A	6.80 A	6.80 A	4.00 A	5.33 A	5.56 A	25.3 0 a	27.4 0 ab	31.40	25.0 0 ab	26.4 3 b	28.7 7 ab
T ₂	7.40 A	7.40 A	7.20 A	3.66 A	4.66 A	5.00 A	24.1 7 ab	26.4 7 abc	30.10	20.8 3 cd	25.2 3 bc	27.0 0 ab
T ₃	7.60 A	7.60 A	7.80 A	4.66 A	5.66 A	5.66 A	26.2 3 a	28.0 7 a	32.37	27.3 3 a	29.2 3 a	30.2 7 a
T ₄	7.40 A	7.00 A	7.00 A	3.66 A	5.00 A	5.33 A	24.37 ab	25.40 cd	30.77	22.7 0 bc	24.3 0 c	26.4 3 b
T ₅	7.20 A	7.20 A	7.20 A	3.33 A	5.33 A	5.34 A	21.9 3 b	24.2 3 d	30.10	22.9 7 bc	26.3 0 b	28.1 3 ab
T ₆	7.00 A	7.00 A	7.00 A	3.23 A	5.00 A	5.33 A	24.4 7 ab	26.4 0 abc	30.97	21.0 7 cd	22.3 3 d	25.9 3 bc
T ₇	7.00 A	7.00 A	7.00 A	3.33 A	5.00 A	5.00 A	23.7 3 ab	25.7 0 bcd	30.40	19.5 7 d	21.1 7 d	23.0 3 c
LSD	NS	NS	NS	NS	NS	NS	2.40 4	1.80 5	NS	2.64 9	1.81 0	3.06 6
CV (%)	5.45	3.4 5	3.32	3.04	7.45	6.74	5.56	3.87	6.79	25.0 0 ab	26.4 3 b	28.7 7 ab

T₁ = Control

T₂ =10 ppm arsenic solution

T₃=10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ =100 ppm arsenic solution+ mycorrhiza

T₆ =500 ppm arsenic solution

T₇ =500 ppm arsenic solution+ mycorrhiza

➤ **Fresh weight of shoot**

The influence of AMF inoculation on fresh weight of shoot of okra at different growth periods in soil amended with different concentrations of arsenic solution is presented in Table 26. Fresh weight of shoot of okra ranges from 6.71-10.77, 7.03-11.24 and 8.90-19.18 at 30 DAS, 45 DAS and 60 DAS respectively. The highest fresh weight of shoot of okra was recorded in treatment T₃ followed by treatment T₁, T₂ and the lowest fresh weight of shoot of okra was recorded in treatment T₅ at 30 DAS. Similar results were also obtained at 45 DAS and 60 DAS. Incase of treatment T₂ and T₄ it was clearly showed that with the increase of arsenic concentration the fresh weight of shoot of okra decreased. Incase of only arsenic treatment T₂ (10 ppm arsenic solution) the fresh weight of shoot of okra at 60DAS was 34.83gm but it increased to 37.50 gm when we inoculated mycorrhiza with that 10 ppm arsenic solution.

➤ **Fresh weight of root**

The role of AMF inoculation on fresh weight of root of okra, which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 26 There was a remarkable variation of fresh weight of root of okra among the 7



different treatments. The variation of fresh weight of root of okra was recorded due to the effect of mycorrhiza inoculation against arsenic solution. Result revealed that treatment T₃ (10 ppm arsenic solution + mycorrhiza) gave the highest results and those were 6.56gm, 6.9gm and 7.67gm at 30 DAS, 45 DAS and 60 DAS respectively which is significantly better in comparison to each of the other treatments. Treatment T₁ (Control) showed the second highest fresh weight of root of okra. The lowest fresh weight of root was recorded from the only arsenic treated treatments (T₂, T₄ and T₆) and Fresh weight of root of Okra decreased when the rate of arsenic concentration increased.

➤ Dry weight of shoot

The role of AMF inoculation on dry weight of shoot of okra, which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 26. There was a remarkable variation of dry weight of shoot among the 7 different treatments. The variation of dry weight of shoot of okra was recorded due to the effect of mycorrhiza inoculation against arsenic solution. Result revealed that treatment T₃(10 PPM arsenic solution + mycorrhiza) gave the highest 6.86 gm 7.5 gm 7.83 gm at 30 DAS, 45 DAS and 60 DAS respectively which is significantly better in comparison to each of the other treatments. Treatment T₁ (Control) showed the second highest dry weight of shoot of okra. The dry weight of shoot of okra decreased when the rate of arsenic concentration increased. The variation of dry weight of shoot of okra was recorded due to the effect of mycorrhiza inoculation against arsenic solution.

➤ Dry weight of root

The role of AMF inoculation on dry weight of root of okra, which was sown in soil, amended with different concentrations of arsenic solution is shown in Table 26. There was a remarkable variation of dry weight of root among the 7 different treatments. The variation of dry weight of root of okra was recorded due to the effect of mycorrhiza inoculation against arsenic solution. Result revealed that treatment T₃ (10 ppm arsenic solution + mycorrhiza) gave the highest dry weight of root of okra and those were 1.51gm 1.57gm 1.84 gm at 30 DAS, 45 DAS and 60 DAS respectively which is significantly better in comparison to each of the other treatments. Treatment T₂ (10 ppm arsenic solution) showed the second highest dry weight of root of okra. Dry weight of root of okra decreased when the rate of arsenic concentration increased. The variation of dry weight of root of okra was recorded due to the effect of mycorrhiza inoculation against arsenic solution.

Table 26. Influence of AMF inoculation on fresh and dry weight of shoot and root of okra at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Fresh weight of shoot (g)			Fresh weight of root (g)			Dry weight of shoot (g)			Dry weight of root (g)		
	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
T ₁	31.6 8 b	33.3 5 ab	34.4 4 ab	6.26 0 ab	6.88 7 ab	7.56 7 a	6.26 0 ab	6.88 7 ab	7.56 a	0.70 B	0.95 BC	1.24 B
T ₂	31.3 0 b	33.2 9 ab	34.8 3 ab	5.66 7 ab	6.38 7 abc	7.40 0 a	5.66 7 ab	6.38 7 abc	7.40 a	1.10 B	1.36 AB	1.43 AB
T ₃	34.9 3 a	36.0 6 a	37.5 0 a	6.56 7 ab	6.90 0 a	7.67 0 a	6.56 7 ab	6.90 0 a	7.67 a	1.51 A	1.57 A	1.84 A
T ₄	29.4 0 b	30.4 3 b	32.5 2 b	5.55 7 ab	6.23 0 bc	6.02 0 b	5.55 7 ab	6.23 0 bc	6.02 b	1.07 B	1.09 BC	1.28 AB
T ₅	24.9 6 c	30.4 0 b	31.2 7 b	5.66 7 ab	6.47 7 abc	5.79 7 b	5.66 7 ab	6.47 7 abc	5.79 b	0.71 B	0.82 C	0.96 B
T ₆	28.9 4 b	31.2 3 b	32.1 9 b	5.41 7 b	6.14 3 bc	6.49 3 b	5.41 7 b	6.14 3 bc	6.49 b	0.73 B	1.15 ABC	1.36 AB
T ₇	29.6 8 b	32.3 7 b	33.2 7 b	5.47 3 ab	5.77 3 c	6.46 0 b	5.47 3 ab	5.77 3 c	6.46 b	1.01 B	1.10 BC	1.26 B
LSD	2.71 4	3.35 0	3.40 9	0.97 76	0.69 13	0.82 68	0.97 76	0.69 13	0.828	0.386	0.40 96	0.53 37
CV (%)	5.07	5.80	5.68	6.78	5.45	9.87	6.78	5.45	9.87	4.56	2.56	7.89

T₁ = Control

T₂ =10 ppm arsenic solution

T₃=10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ =100 ppm arsenic solution+ mycorrhiza

T₆ =500 ppm arsenic solution

T₇=500 ppm arsenic solution+ mycorrhiza

➤ **Percent vigourity**

The role of AMF inoculation on vigourity percentage of okra, which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 27. There was a remarkable variation of vigourity percentage of okra among the 7 different treatments. Result revealed that treatment T₃ (10 ppm Arsenic solution + mycorrhiza) gave the highest percent vigourity of okra and those were 42.17%, 56.85% and 58.92% at 30 DAS, 45 DAS and 60 DAS respectively which is significantly better in comparison to each of the other treatments. Treatment T₁ (Control) showed the second highest percent vigourity. The lowest vigourity percentage of okra was recorded from the 500 ppm arsenic treated treatment (T₆) and percent vigourity of okra decreased when the rate of arsenic concentration increased.

Table 27. Influence of AMF inoculation on percent vigourity of okra at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Vigourity (%)		
	30 DAS	45 DAS	60 DAS
T ₁	38.66 B	43.98 B	48.91 B
T ₂	36.92 C	43.83 B	47.25 CD
T ₃	42.17 A	56.85 A	58.92 A
T ₄	37.06 C	40.25 CD	46.62 D
T ₅	38.48 B	41.52 C	46.18 DE
T ₆	23.32 E	38.85 D	45.29 E
T ₇	35.66 D	39.70 CD	47.96 BC
LSD	1.135	2.160	1.142
CV (%)	1.77	2.47	1.32

T₁ = Control

T₂ =10 ppm arsenic solution

T₃=10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ =100 ppm arsenic solution+ mycorrhiza

T₆ =500 ppm arsenic solution

T₇=500 ppm arsenic solution+ mycorrhiza

➤ **Nutrient uptake**

The inoculation of arbuscular mycorrhizal fungi in response to nutrient uptake (N, P, K and S) by okra shoots at 60 DAS is represented in Table 28. It is revealed from the study that mycorrhizal fungi have a positive role in response to nutrient uptake and mycorrhizal fungi inoculated treatments significantly enhanced nutrient uptake by okra shoot in comparison to other treatment. The highest nutrient uptake was recorded in case of treatment T₃ (10 ppm arsenic solution + mycorrhiza) and those were 3.9 % total N, 0.60 % P, 3.33 % K and 0.73 % S and the lowest result was found in case of treatment T₆ (500 ppm arsenic solution) and those were 1.46% total N, 0.29 % P, 1.56 % K and 0.23 % S. It was clearly showed from the table that due to toxicity of arsenic the percent nutrient uptake by shoots of okra was the lowest but mycorrhiza inoculation increased nutrient uptake of the crops.

Arsenic uptake

Influence of AMF inoculation in response to arsenic uptake by shoots of okra as affected by different concentrations of arsenic solution is presented in Table 28. The amount of arsenic uptake by shoots of Brinjal at 60 DAS differed significantly due to inoculation of AMF and different concentrations of arsenic solution. Due to inoculation of AMF, lower amount of arsenic was found in AMF inoculated pots than non-inoculated ones. The amount of arsenic increased with the increase rate of arsenic concentrations but in all the cases inoculation of AMF the rate of arsenic decreased. The lowest amount of arsenic was found in treatment T₃ (10 ppm arsenic solution + mycorrhiza) and that was 59 ppm on the other hand the highest amount was found in treatment T₆ (500 ppm arsenic solution) and that was 159 ppm. Between the treatment T₂ and T₃, the amount of arsenic was higher in treatment T₂ and that was 129.3 ppm but inoculation of mycorrhiza on that treatment the amount decreased significantly to

59.0 ppm. Similar result was found from other treatments. It was observed from the table that inoculation of mycorrhizal fungi helps to reduce arsenic uptake up to 50%.

➤ Chlorophyll Analysis

Influence of AMF inoculation in response to the content of chlorophyll in leaves of okra, which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 28. The amount of chlorophyll in leaves of okra at 60 DAS differed significantly due to inoculation of AMF and different concentrations of arsenic solution. Due to inoculation of AMF, higher amount of chlorophyll was found in AMF inoculated pots than non-inoculated ones. The amount of chlorophyll increased with the decrease rate of arsenic concentrations and in all the cases inoculation of AMF the rate of chlorophyll increased. The lowest amount of chlorophyll was found in treatment T₇ (500 ppm arsenic solution + mycorrhiza) and that was 0.1003 ppm on the other hand the highest amount was found in treatment T₃ (10 ppm arsenic solution + mycorrhiza) and that was 0.2683 ppm. Among the treatment T₂, T₄ and T₆, the amount of chlorophyll was higher in treatment T₄ and that was 0.1867 ppm and lower in treatment T₆. It was observed from the table that when lower amount of arsenic concentration used, inoculation of mycorrhizal fungi helps to increase chlorophyll content in all the treatments than non inoculated ones.

Table 28. Influence of AMF inoculation on nutrient uptake and arsenic uptake by shoot and chlorophyll content by leaves of okra at 60 DAS in soil amended with different concentrations of arsenic solution

Treatments	Nutrient uptake				Arsenic (As) (ppm)	Chlorophyll content mg(g)
	Total N %	P %	K %	S %		
T ₁	2.33 C	0.55 A	2.44 B	0.50 AB	259.0 AB	0.1630 BC
T ₂	2.66 C	0.57 A	2.58 AB	0.39 B	301.7 A	0.1840 B
T ₃	3.90 A	0.60 A	3.33 A	0.73 A	222.0 B	0.2683 A
T ₄	3.30 B	0.50 A	2.56 AB	0.52 AB	230.3 B	0.1867 B
T ₅	2.43 C	0.37 A	1.65 C	0.28 B	110.3 C	0.2047 B
T ₆	1.46 D	0.29 A	1.57 C	0.23 B	99.33 C	0.1170 CD
T ₇	2.46 C	0.40 A	1.63 C	0.36 B	50.09	0.1003 D
LSD	0.4247	NS	0.7672	0.3081	3.53	0.0546
CV (%)	9.10	4.56	6.87	6.78	259.0 AB	8.20

T₁ = Control

T₂ =10 ppm arsenic solution

T₃=10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ =100 ppm arsenic solution+ mycorrhiza

T₆ =500 ppm arsenic solution

T₇=500 ppm arsenic solution+ mycorrhiza

➤ **Root colonization**

Root colonization was demonstrated by the presence of vesicles, arbuscules and/or hyphae in root tissue. The highest percent root colonization 63.00% of 10 ppm + mycorrhiza inoculated plants were recorded at 60 DAS and lowest 18.36% of 500 ppm arsenic solution was recorded at 30DAS. On the other hand no root colonization was found in non-inoculated poly bags.

Table 29. Root colonization (%) in okra by the influence of AMF inoculation at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Root colonization (%)		
	30 DAS	45 DAS	60 DAS
T ₂	19.23	21.34	36.23
T ₃	23.68	29.29	63.00
T ₄	21.14	35.21	37.23
T ₅	26.23	28.90	40.21
T ₆	18.36	27.67	29.27
T ₇	19.31	26.45	24.25

T₂ = 10 ppm arsenic solution

T₃ = 10 ppm arsenic solution + mycorrhiza

T₄ = 100 ppm arsenic solution

T₅ = 100 ppm arsenic solution + mycorrhiza

T₆ = 500 ppm arsenic solution

T₇ = 500 ppm arsenic solution + mycorrhiza



Chapter 5

Discussion

CHAPTER 5

DISCUSSION

The roots of different agricultural crops associated with AM mycorrhizal fungi collected from different arsenic affected areas of Pabna are reported here. Preliminary studies were done to know the occurrence of AM fungal association in those agricultural crops root. The infection percentage and intensity of infection of AM fungi were observed in the present study. It was found from the study that the infection percentage and intensity of infection differed from crop to crop and location to location. Among the four villages, the highest percentage of infection was found in vatikoa village and that was 6.5% and the lowest infection percentage was recorded from Saiyadpur village and that was 4.16%. Among the selected plant species, Chili roots showed the highest percentage of infection and intensity of infection of AM fungi.

The percent root infection in different plant species recorded in the present study is in agreement with the previous report. There was a wide range of variation in percent infection of AMF in different location and among the plant species of a particular area. A wide range of variation was also recorded in different crops grown in different locations. Our results are in agreement with Saif (1977). There was a lack of definite correlation between percentage of infection and intensity of infections. These differences in the colonization pattern of the crops studied might explain the generally held view that crops with course root gained more VAM compared to those with fine roots or these differences might be due to presence of diverse type of VAM in the rhizosphere soil of individual crop plant species.

In the present study most of the species showed lower infection. The plant with low mycorrhizal infection has low intensity of infection suggesting that arbuscules are either absent or low in number. Arbuscules are the nutrient exchanging organ of VAM for effecting association (Smith and Gianinazzi-Pearson, 1990); absence of this structure may influence the further growth of the VAM for more infection. The high percentage of root infection in different agricultural crops recorded in the present study is in agreement with the results of (Saif, 1977; Abbott and Robson, 1977). In this study varied percentages of AMF infection of agricultural crops root collected from different arsenic affected location of Pabna area was observed. Dehne (1987) and Sieverding (1991) reported that these type of variation in percentage of infection and intensity of infection of VAM under natural condition is depended on the indigenous VAM fungi , presence of host plant and different edaphic factors (e.g. soil texture, pH, conductivity, organic matter, phosphorus etc.). The result of the present study corroborating with the findings of Islam (2006) and Ali (2008). Islam (2006) found that mycorrhizal root colonization differed among the crops ranging from 20.00 to 49.75%. Ali (2006) reported that mycorrhizal colonization differed among the selected crops; tomato, okra, chili, danta and brinjal ranging from 17.65 to 50.64 %.

The results of this experiment indicate that, shoot height and root length of crops were higher in all the mycorrhiza inoculated treatments than non-inoculated treatments. Shoot height and root length of all crops differed significantly due to the relevance of different concentrations of arsenic solution and inoculation of mycorrhiza. Among the 7 treatments, treatment T₃ (10 ppm arsenic +mycorrhiza) gave the best result whereas,

treatment T₆ (500 ppm arsenic solution) gave the lowest result in all the three recorded periods. In all the three recorded periods mycorrhizal inoculation significantly enhanced shoot height and root length of all crops in comparison to non-inoculated. This was probably due to uptake of nutrient, which increased vegetative growth. Shoot height and root length of crops was decreased with the increase of arsenic concentrations. Ultra *et al.* (2007) reported the AM inoculation as well as P application reduced As toxicity symptoms, most clearly so in the +AM–P treatment. They also reported that plant growth was highest in the +AM + P treatment. Xia *et al.* (2007) while conducted an experiment under glasshouse condition in an As-contaminated soil, reported arbuscular mycorrhizal (AM) fungus (*Glomus mosseae*) increased both root length markedly under the zero-P treatments. Ahmed *et al.* (2006) reported that plant height, plant biomass and shoot and root P concentration/off take increased significantly due to mycorrhizal infection. Plant height, plant biomass, root length and mycorrhizal infection decreased significantly with increasing As concentration.

Both fresh and dry weight of shoots and roots were observed in this experiment. In this study, A significant reduction of fresh and dry weight of shoot and root of all crops was observed due to arsenic concentrations. The fresh and dry weight of shoot and root of five crops were recorded after 30, 45 and 60 days of sowing. In comparison to all the treatments better result was obtained where mycorrhiza was inoculated than non-inoculated. The results indicated a positive effect of mycorrhizal inoculation on fresh and dry weight of shoot and root when soil amended with different concentrations of arsenic solution. This was probably due to the uptake of nutrient, which increased vegetative growth and hence greater translocation of photosynthesis

from leaf to shoot and thereby enhanced shoot growth and weight. Tarafdar and Parveen (1996) reported that shoot biomass was significantly improved in all cases of inoculated plants. The results of the present study is more or less similar with Carling and Brown, (1980) who reported that colonization by most *Glomus* isolates significantly increased plant shoot dry weight in low fertility soil. Root, shoot and total plant dry weight were significantly greater in mycorrhizal plants than in non-mycorrhizal controls in *Abelmoscus esculentus* (Krishna and Bagyaraj, 1982). Fresh weights of root and shoot increased when the plants were inoculated with AMF (Matsubara *et al.*, 1994). Root and shoot dry weights were higher in mycorrhizal than non-mycorrhizal plants (Giri *et al.*, 2005).

The findings of the present study are similar with the findings of Xia *et al.* (2007). They reported that dry weight and root biomass increased markedly when maize plants are inoculated with arbuscular mycorrhizal (AM) fungus (*Glomus mosseae*) under glasshouse condition in an arsenic amended soil. Plant height, leaf/ pod number, plant biomass, root length and mycorrhizal infection decreased significantly with increasing As concentration (Ahmed *et al.*, 2006). Mycorrhizal colonization increased plant biomass at As application rates Of 25, 50 and 75 mg kg⁻¹ (Liu *et al.*, 2005). Agely *et al.* (2005) found that the AM fungi not only tolerate As amendment, but their presence increased frond dry mass at the highest As application rate.

In this study, significant reduction in mycorrhizal root colonization was observed due to arsenic toxicity. Ahmed *et al.*, (2006) reported that mycorrhizal infection decreased significantly with increasing As concentration. Liu *et al.* (2005) conducted a glasshouse pot experiment on tomato plants to study the effect of arbuscular

mycorrhizal (AM) colonization by *Glomus mosseae* BEG167 on the yield and arsenate uptake of in soil experimentally contaminated with five As levels. Mycorrhizal colonization was little affected by As application and declined only in soil amended with 150 mg As kg⁻¹. Saha (2008) reported that a remarkable variation was observed in the plants treated with mycorrhiza. In his experiment he used 10 treatments and in all the cases 10 ppm arsenic solution + mycorrhiza showed the better root colonization performance than the other treatments.

It is revealed from the study that the arbuscular mycorrhizal fungi have a positive role in response to nutrient uptake (N, P, K and S). Mycorrhiza inoculated treatments significantly enhanced nutrient uptake by crops shoots in comparison to other treatments. Among the 7 different treatments the highest result was found in case of treatment T₃, on the other hand T₆ gave the lowest result. Results of this experiment also showed that the percentage of nutrient uptake decreased with the increase of arsenic concentration. Arbuscular mycorrhizal (AM) fungus has their most significant effect on P uptake. Shoot P concentration/off take, root P off take and mycorrhizal infection decreased significantly with increase of As concentration, which is supported by Saha (2008) and Akhtar (2008). In their experiments P uptake by shoots of Red Amaranthus and Radish was the highest in 10 ppm arsenic solution + mycorrhiza and with the increase of arsenic concentration the amount of P was significantly reduced.

Dong *et al.* (2007) reported the influence of AM inoculation on plant growth and phosphorus (P) nutrition. Mycorrhizal inoculation substantially improved plant P nutrition and in contrast markedly decreased root to shoot As translocation and shoot As concentrations. Khan *et al.* (1995) reported that nitrogen fixation as well as N and



P contents in groundnut increased only by dual inoculation with AM fungi and *Bradyrhizobium*. Nutrient uptake was enhanced significantly in soybean shoot by inoculation of AM fungi. The VAM fungi promote phosphorus uptake in low phosphate soil during the early stages of plant growth (Sasai, 1991). Shnyreva and Kulaev (1994) identified the effect of VAM mycorrhization on maize plants by *Glomus* spp., phosphorus content in the VA-mycorrhizal root tissues increased by 35 % for the species *G. mosseae* and by 98 % for *G. fasciculatum*. Phosphorus uptake was influenced significantly by the inoculation of AM fungi over control by many selected crops. Nutrient uptake was enhanced significantly in Pigeonpea shoot by inoculation of AM fungi.

Chapter 6

Summary and Conclusion

CHAPTER 6

SUMMARY AND CONCLUSION

Experiment was conducted in the laboratory of the Department of plant Pathology, Sher-e-bangla Agricultural University, Dhaka, Bangladesh during the period from March 2008 to May 2008 to evaluate the role of mycorrhizal fungi on plant nutrition, Plant growth and arsenic uptake of some selected agricultural crops. The selected experimental crops were danta (*Amaranthus oleraceus*), Tomato (*Lycopersicon esculentum*), chili (*Capsicum frutescens*), brinjal (*Solanum melongena*) and okra (*Abelmoschus esculentus*). Seven treatments such as T₁ : Control, T₂ :10 ppm arsenic solution, T₃:10 ppm arsenic solution + mycorrhiza, T₄ : 100 ppm arsenic solution, T₅ :100 ppm arsenic solution+ mycorrhiza, T₆ :500 ppm arsenic solution, T₇ :500 ppm arsenic solution+ mycorrhiza were used in this study. Each treatment was replicated five times. Root and rhizospheric soil of *Leucas aspera* used as natural inoculums and seeds were collected from BADC (Bangladesh Agricultural Development Corporation). Data on shoot fresh weight, shoot dry weight, root dry weight were recorded. The variation of arbuscular mycorrhizal root infection and intensity of infection of different agricultural crops root collected from different arsenic affected villages of Pabna district were investigated. The percentage root infection and intensity of infection were varied from location to location and crop to crop. Among the five villages, the highest infection percentage was recorded from Vatikoa village and that was 6.5%. The lowest infection percentage was recorded from Saiyadpur village and

that was 4.16 Shoot and root growth of the crops were increased with mycorrhizal inoculation to the crops.

In this experiment we also determine different chemical parameters like Chlorophyll analysis, arsenic analysis and nutrient analysis were also performed. The nutrient uptake (N, P, K and S) was highly influenced by AMF inoculation in arsenic amended soil. The highest amount of nutrient uptake was recorded when 10ppm arsenic solution with mycorrhiza was used.

A positive effect of mycorrhizal inoculation and infection on danta, chili, brinjal, okra and tomato growth was observed when arsenic was applied to the crop. Importantly, reduced translocation of As to aerial plant parts was found with the inoculation of AM fungi. When crops were grown in arsenic amended soil, then arsenic translocated within the shoots of those crops but mycorrhiza inoculation significantly reduced the translocation of arsenic.

Even in a high level of arsenic concentration, mycorrhiza fungi improved plant growth of the crops tested. It means physical and chemical growth of crops increased by the influence of AMF or plant can tolerate in a high concentrations of arsenic solution.

Bangladesh is one of the most densely populated countries in the world. The food demand of our increasing population increased day by day. It is essential to improve our crop production for burgeoning population but technologies to meet up those problems are limited. Crop production need to be increased through a low imputes method and the point of view mycorrhizal technology will be a model technology for

our country. It is the least expensive, simple and nature farming technology. By using this technology not only crop production can be improved but also arsenic toxicity of the crops can be reduced. Decreased uptake of As by shoots of danta, tomato, brinjal, chili and okra has particularly important implications for human health, and suggests mycorrhizal inoculation may contribute to strategies to minimize As intake through consumption of crops in arsenic contaminated areas.

REFERENCES

- Abbott, L. K. and Robson, A. D. (1977). The distribution and abundance of vesicular-arbuscular endophytes in some Western Australian soils. *Aus. J. Bot.* **25**: 515 - 522.
- Abdul, B.A. and Anderson, J.D. (1972). Physiological and biochemical deterioration of seeds. *In*: Kozlowsky, T.T. (ed) *Seed Biology*. Academic Press, New York. pp. 283 - 315.
- Agely, A. A., Sylvia, D.M. and Ma, L.Q. (2005). Mycorrhizae increase arsenic uptake by the hyperaccumulator Chinese brake fern (*Pteris vittata* L.). *J. Environ. Qual.* **34**(6): 2181-2186.
- Ahmed, F.R.S., Killham, K. and Alexander, I. (2006). Influences of arbuscular mycorrhizal fungus *Glomus mosseae* on growth and nutrition of lentil irrigated with arsenic contaminated water. *Plant Soil.* **283**(1-2): 33-41.
- Akhtar, B. (2008). Mycorrhizal status of crops grown in arsenic affected areas of manikganj district and effect of mycorrhiza on growth of some crops in arsenic amended soil. M.S. Thesis. Dept. of Plant Path. Sher-e-Bangla Agricultural University, Bangladesh.
- Alam, M.G., Allinson, G., Stagnitti, F., Tanaka, A., Westbrooke, M. (2002). Arsenic contamination in Bangladesh groundwater: a major environmental and social disaster. *Inter. J. Environ. Health Res.* **12**: 235-253.
- Ali, M. (2008). Influence of Arbuscular Mycorrhizal Fungi on growth and nutrient uptake of some vegetable crops. M.S. Thesis. Dept. of Pl. Path. Sher-e-Bangla Agricultural University, Bangladesh.
- Barea, J. M. (1991). Vesicular-arbuscular mycorrhizae as modifiers of soil fertility. *In*: Stewart, B. A. (ed.), *Advan. Soil Science*. Springer-verlag, New York, Inc. pp. 1-40.



- BBS (Bangladesh Bureau of Statistics). 2007. Monthly Statistical Bulletin, June. Ministry of Planning, Government of the People's Republic of Bangladesh, Dhaka, pp. 66-67.
- Bonifacio, E., Nicolotti, G., Zanini, E. and Cellerino, G.P. (1999). Heavy metal uptake by mycorrhizae of beech in contaminated and uncontaminated soils. *Fresenius Environ. Bull.* **7**: 408-413.
- Bradley, R., Burt, A. J. and Read, D. J. (1981). Mycorrhizal infection and resistance to heavy metal toxicity in *Calluna vulgaris*. *Nature*, **292**: 335-337.
- Bradley, R., Burt, A.J. and Read, D.J. (1982). The biology of mycorrhiza in the Ericaceae.VIII. The role of mycorrhizal infection in heavy metal resistance. *New Phytol.* **91**: 197-209.
- Bremner, J.M. and C.S. Mulvaney. 1982. Total Nitrogen. In: Methods of Soil Analysis. Miller, R.H. and D.R. Xeeny. American. Soc. Agron. Inc. Madison, Wisconsin, USA. pp. 595-622.
- Brown, M. T. and Wilkins, D. A. (1985). Zinc tolerance of mycorrhizal Betula. *New Phytol.* **99**: 101-106.
- Cao, X., Ma, L.Q. and Shiralipour, A. (2003). Effects of compost and phosphate amendments on arsenic mobility in soils and arsenic uptake by the hyper accumulator *Pteris vittata* L. *Environ Polluion.* **126**: 157-167.
- Carling, D.E. and Brown, M.F. 1980. Relative effect of vesicular mycorrhizal fungi on growth and yield of soyabeans. *Soil. Sci. Soi. America. J.* **44**(3): 528-592.
- Chen, B. D., Xiao, X. Y. and Zhu, Y. G. (2007). The arbuscular mycorrhizal fungus *Glomus mosseae* gives contradictory effects on phosphorus and arsenic acquisition by *Medicago sativa* Linn. *J. Sci. Total Environ.* **379**(2/3): 226-234.
- Das, H.K., Mitra, A.K., Sengupta, P.K., Hossain, A., Islam, F. and Rabbani, G.H. (2004). Arsenic concentrations in rice, vegetables, and fish in Bangladesh: a preliminary study. *Environ. Inert.* **30**: 383-387.

- Dehne, H. W. (1987). Improvement of the VA-mycorrhizal status in agriculture and horticulture. *In: Trans. XIIIth Inter. Cong. Soil. Sci.* Hamburg. pp. 817 – 825
- Dong, Y., Zhu, Y.G., Smith, F.A., Wang, Y., Chen, B. (2007). Arbuscular mycorrhiza enhanced arsenic resistance of both white clover (*Trifolium repens* Linn.) and ryegrass (*Lolium perenne* L.) plants in an arsenic-contaminated soil. Department of Soil Environmental Science, Research Center for Eco-Environmental Sciences, the Chinese Academy of Sciences, 18 Shuangqing Road, Beijing 100085, China.
- Edathii, T.T., Manian, S. and Udaiyan, K. (1994). Early establishment of native vesicular arbuscular mycorrhizas in three vegetable crops of South India – a comparative study. *Pertanika J. Trop. Agril. Sci.* 17(3): 157-161.
- Eltrop, L. and Marschner, H. (1996). Growth and mineral nutrition of non-mycorrhizal and mycorrhizal Norway spruce (*Picea abies*) seedlings grown in semi-hydroponic sand culture. Growth and mineral nutrient uptake in plants supplied with different forms of nitrogen. *New Phytol.* 133(3): 469-478.
- Gaur, A. and Adholeya, A. (2000). Response of three Vegetable crops to AM fungal inoculum in nutrient deficient soils amended with organic matter. *Symbiosis.* 29: 19-31.
- George, E. (2000). Nutrient uptake. *In: Arbuscular Mycorrhizas: Physiology and function.* Kluwer Academic Press. pp. 307-344
- Giri, B., Kapoor, R. and Mukerji, K.G. (2005). Effect of the Arbuscular Mycorrhizae *Glomus fasciculatum* and *G. macrocarpum* on the growth and nutrient content of *Cassia siamea* in a semi-arid Indian wasteland soil. *New Forests.* 29(1): 63-73.
- Gonzalez, C.C., Harris, P. J. and Dodd, J. (2002). Arbuscular mycorrhizal fungi confer enhanced arsenate resistance on *Holcus lanatus*. *New Phytol.* 155: 163–171.

- Harley, J.L. (1989). The significance of mycorrhiza. *Mycol. Res.* **92**: 129–139.
- Harrison, M.J., Dewbre, G.R. and Liu, J. (2002). A phosphate transporter from *Medicago trunculata* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. *Plant Cell.* **14**: 1-17.
- Hayman, D.S. (1983). The physiology of vesicular-arbuscular endomycorrhizal symbiosis. *Canadian. J. Bot.* **61**(3). p. 944-963.
- Howeler, R.H., Sieverding, E. and Saif, S. (1987). Practical aspects of mycorrhizal technology in some tropical crops and pastures. *Plant Soil.* **100**: 249-283.
- <http://www.atsdr.Cdc.gov/cercla/05list.html>
- Hunt, J. 1980. Determination of total sulphur in small amount of plant material *Analyst.* **105**: 83-85.
- Islam, S. (2006). The role of Arbuscular Mycorrhizal (AM) fungi on growth and nutrient uptake of some legumes. M.S. Thesis. Dept. of Plant Path. Sher-e-Bangla Agricultural University, Bangladesh.
- Jones, M. D. and Hutchinson, T. C. (1988). The effects of nickel and copper on the axenic growth of ectomycorrhizal fungi. *Canadian J. Bot.* **66**: 119–124.
- Jain, M. and Grade, R. (1997). Effect of As on chlorophyll and protein contents and enzymic activities in greening tissues. *J. Water, Air and Soil pollution.* **93**:109-115.
- Jalali, B.L. and Chand, H. (1988). Role of VAM in biological control of plant diseases. *In: Mohadevan, A., Raman, N. and Natarajan, K. (eds.), Mycorrhizae for Green Asia. Proceedings of the First Asian Conference on Mycorrhizae, Madras, India. pp. 209-215.*
- Karagiannidis, N., Bletsos, F. and Stavropoulos, N. (2002). Effect of *Verticillium* wilt (*Verticillium dahliae* Kleb.) and Mycorrhiza (*Glomus mosseae*) on root colonization, growth and nutrient uptake in tomato and eggplant seedlings. *Scientia Horticulturae.* **94**(1-2): 145-156.

- Khan, M.K., Sakamoto, K. and Yoshida, T. (1995). Dual inoculation of groundnut with *Glomus* sp. and *Bradyrhizobium* sp. enhanced the symbiotic nitrogen fixation as assessed by ^{15}N -technique. *Soil Sci. Plant Nutrition* 41(4): 769-779.
- Kim, D.Y., Lee, Y.J., Goo, N.I., Jung, J. and Kim, J.G. (2006). Effects of Arbuscular mycorrhizal fungi inoculation on Arsenic and Phosphorus Uptake by *Trifolium repens* and *Oenothera odorata* Jacq. in Arsenic Contaminated Soil. International Union of Soil Science. 158-163.
- Koske, R. E. and Gemma, J. N. (1989). A modified procedure for staining roots to detect VA-mycorrhizas. *Mycol. Res.* 92: 486-488.
- Krishna, K.R. and Bagyaraj, D.J. (1982). Effect of vesicular arbuscular mycorrhiza and soluble phosphate on *Abelmoscus esculentus* (L.) Moench, *Plant Soil.* 64: 209-213.
- Leung, H.M., Ye Z.H. and Wong M.H. (2006). Interactions of mycorrhizal fungi with *Pteris vittata* (As hyperaccumulator) in As-contaminated soils. *Environ. Pollution.* 139: 1-8.
- Leyval, C., Turnau, K. and Haselwandter, K. (1997). Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhzia.* 7: 139-153.
- Liu, Y., Zhu, Y.G. and Chen, B.D. (2005). Yield and arsenate uptake of arbuscular mycorrhizal tomato colonized by *Glomus mosseae* BEG167 in As spiked soil under glasshouse conditions. *Environ Inter.* 31: 867-873.
- Mahmud, R., Mridha, M.A.U., Sultana, A., Sultana, N., Xie, H.L. and Umemura, H. (1999). Biodiversity of VAM fungi in soils of crop field. *Nat. Farm. Sustain. Environ.* 2:43-50.
- Marschner, H. and Dell, B. (1994). Nutrient uptake in mycorrhizal symbiosis. *Plant soil.* 159: 89-102.
- Masri, B. M. (1997). Mycorrhizal inoculation for growth enhancement and improvement of the water relations in mungosteen (*Garcinia mangostana* L.) seedlings. *Ph. D. Thesis. University putra Malaysia. Serdung. Malaysia.*



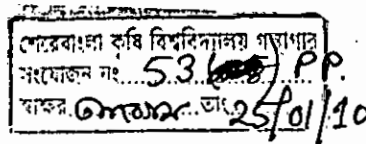
- Matsubara, V. I., Haraba, T. and Yakuwa, T. (1994). Effect of vesicular-arbuscular mycorrhizal fungi inoculation on seedling growth in several species of vegetable crops. *J. Japanese. Soc. Hort. Sci.* **63** (3): 619-628.
- Meharg, A.A. and Cairney, J.W.G. (1999). Co-evolution of mycorrhizal symbionts and their hosts to metal contaminated environments. *Adv. Ecol. Res.* **30**: 70-112.
- Meharg, A.A. and Macnair, M.R. (1992). Suppression of the high affinity phosphate-uptake system: a mechanism of arsenate tolerance in *Holcus lanatus* L. *J. Exp. Bot.* **43**: 519-524.
- Melamed, R., Jurinak, J.J. and Dudley, L.M. (1995). Effect of adsorbed phosphate on transport of arsenate through an oxisol. *Soil Sci. Soc. American. J.* **59**: 1289-1294.
- Milivojevic, D., Nikolic, B., Dremic, G. (2006). Effects of Arsenic on phosphorus content in different organs and chlorophyll fluorescence in primary leaves of soybean. **50** (3):149-151.
- Milivojevic, D., Nikolic, B., Dremic, G. (2006). Effects of Arsenic on phosphorus content in different organs and chlorophyll fluorescence in primary leaves of soybean. **50** (3):149-151.
- Miller, R.M. and Jastrow, J.D. (1994). Vesicular Arbuscular Mycorrhizae and biogeochemical cycling. In: F.L. Pfleger and R.G. Linderman (eds.). *Mycorrhizae and Plant Health. American. Phytopathol. Soc. St. Paul, MN.* 189-212.
- Mitevae. (2002). Accumulation and effect of Arsenic on tomatoes. *J. of Communications in Soil Science and Plant Analysis.* **33**: 1917-1926.
- Mosse, B. (1973). Advances in the study of vesicular-arbuscular mycorrhiza. *Annual Rev. Phytopathol.* **11**: 171-196.
- Mridha, M. A. U. and Xu, H. L. 2001. Nature Farming with arbuscular mycorrhizae in Bangladesh. *J. Crop Prod.* **3**: 303-312.
- Mridha, M. A. U., Sultana, A., Sultana, N., Xu, H. L. and Umemura, H. (1999). Biodiversity of VA mycorrhizal fungi of some vegetable crops in Bangladesh. *Proc. International Symposium on World Food Security and Crop Production*



- Technologies for Tomorrow, October 8-9, 1998. Kyoto, Japan 330-331.
- Mridha, M.A.U and Dhar, P.P. (2007). Biodiversity of arbuscular mycorrhizal colonization and spore population in different agro forestry trees and crop species growing in Dinajpur, Bangladesh. *J. Forest Res.* **18**: 91-96.
- Murphy, J. and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta.* **27**: 31-36.
- Nedumpara and Mercy, J. (1996). Interactions of Vesicular-Arbuscular Mycorrhizal fungi, herbicides and crops. *Iowa State University.* **57-06(b)**, p. 3557, 97.
- Peryea, F.J. and Kammereck, R. (1997). Phosphate-enhanced movement of arsenic out of lead arsenate-contaminated topsoil and through uncontaminated subsoil. *Water Air Soil Pollution.* **93**: 243-254.
- Rahman, A., Hiroshi, H.G., Rahman, M., Islam, Nazrul., Tasmen, M. M. (2007). Effect of arsenic on photosynthesis, growth and yield of five widely cultivated rice. (*Oryza sativa* L.) varieties in Bangladesh. *J. of Dynamic characterization of intensively fluctuations in semi conductor lasers under digital modulation.* **66** (6):1072-1079.
- Read, D.J., Koucheiki, H.K. and Hodgson, J. (1976). Arbuscular mycorrhizal in natural vegetation systems. I. The occurrence of infection. *New Phytol.* **77**: 641.
- Saha, N.K. (2008). Mycorrhizal status of crops grown in arsenic affected areas of Sonargaon district and effect of mycorrhiza on growth of some crops in arsenic amended soil. M.S. Thesis. Dept. of Plant Path. Sher-e-Bangla Agricultural University, Bangladesh.
- Saif, S.R. (1977). The influence of stage of host development on vesicular-arbuscular mycorrhizae and endogonaceous spore population in field-grown vegetable crops. 1. Summer-grown crops. *New Phytol.* **79**: 341 - 348.
- Sasai, K. (1991). Effect of phosphate application on infection of vesicular arbuscular mycorrhizal fungi in some horticultural crops. *Scientific Rep. Miyagi Agric. Coll.* **39**:1-9.
- Semra, D. (2002). Influence of arbuscular mycorrhiza on some physiological growth parameters of pepper. Turkey *J. of Biology.* **28**: 85-90.

- Sharples, J.M., Meharg, A.A. and Chambers, S.M. (2000). Symbiotic solution to arsenic contamination. *Nature*, **404**: 951–952.
- Shnyreva, A.V. and Kulaev, I.S. (1994). Effect of vesicular arbuscular mycorrhiza on phosphorus metabolism in agricultural plants. *Microb. Res.* **149**(2): 139-143.
- Sieverding, E. (1991). Vesicular-arbuscular mycorrhizal management in tropical agrosystem. Germany: GTZ publishers.
- Smith, E., Naidu, R. and Alston, A.M. (1998). Arsenic in the soil environment: A review. *Adv. Agron.* **64**: 149–195.
- Smith, S. E. and Gianinazzi-Pearson, V. (1990). Phosphate uptake and arbuscular activity in mycorrhizal *Allium cepa* L.: effect of photon irradiance and phosphate nutrition. *Aus. J. Plant Physiol.* **17**: 177- 188.
- Smith, S.E. and Read, D.J. (1997). Mycorrhizal Symbiosis, 2nd edn. Academic Press, London, U.K.
- Sreeramulu, K.R., Shetty, Y.V. and shetty, T.K.P. (1996). Effect of VA mycorrhiza on the growth of two important leafy vegetables. *Madras Agril. J.* **83**(6): 362-364.
- Sylvia, D.M. and Neal, L. H. (1990). Nitrogen affects the phosphorus response of VA-mycorrhiza. *New Phytol.* **115**: 303 - 310.
- Syvertsen, J.P. and Graham, J.J. (1999). Phosphorus supply and arbuscular mycorrhizas fungi increase growth and net gas exchange response in two citrus spp. grown at elevated CO₂. *Plant Soil* **208**: 209-219.
- Tarafdar, J.C. and Praveen, K. (1996). The role of Vesicular Arbuscular Mycorrhizal fungi on crop, tree and grasses grown in an arid Environ. *J. of Arid Environ.* **34**: 197-203.
- Trimble, M.R. and Knowles, N.R. (1995). Influence of Vesicular-Arbuscular Mycorrhizal fungi and Phosphorus on growth, Carbohydrate partitioning and mineral-nutrition of greenhouse Cucumber (*Cucumis-sativus* L) plants during establishment. *Canadian J. Plant Sci.* **75**: 239-250.

- Trotta, A., Falaschi, P., Cornara, L., Minganti, V., Fusconi, A., Drava, G. and Berta, G. (2006). Arbuscular mycorrhizae increase the arsenic translocation factor in the As hyperaccumulating fern *Pteris vittata* L. *Chemosphere*, **65**(1):74-81.
- Ultra, V.U., Tanaka, S., Sakurai, K. and Iwasaki, K. (2007). Effects of arbuscular mycorrhiza and phosphorus application on arsenic toxicity in sunflower (*Helianthus annuus* L.) and on the transformation of arsenic in the rhizosphere. *Plant Soil*. **290**(1-2): 29-41.
- Wassermann, J.L., Mineo, L., Majumdar, S.K. and Vantyne, C. (1987). Detection of heavy metals in oak mycorrhizae of northeastern Pennsylvania forests, using X-ray microanalysis. *Canadian J. Bot.* **65**: 2622-2627.
- Xia, Y.S., Chen, B.D., Christie, P., Smith, A., Wang, Y.S. and Li, X.L. (2007). Arsenic uptake by arbuscular mycorrhizal maize (*Zea mays* L.) grown in an arsenic -contaminated soil with added phosphorus. *J. Environ. Sci.* **19**(10): 1245-1251.



Sher-e-Bangla Agricultural University
Library
Accession No. 37382
Sign: [Signature] Date: 12-12-13