MYCORRHIZAL STATUS OF CROPS GROWN IN ARSENIC AFFECTED AREAS OF SONARGAON AND INFLUENCE OF MYCORRHIZAE ON GROWTH OF SELECTED CROPS IN ARSENIC AMENDED SOIL

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CERTIFICATE

This is to certify that the thesis entitled " **MYCORRHIZAL STATUS OF CROPS GROWN IN ARSENIC AFFECTED AREAS OF SONARGAON AND INFLUENCE OF MYCORRHIZAE ON GROWTH OF SELECTED CROPS IN ARSENIC AMENDED SOIL**" submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfilment 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 **BONYA AKHTER, REGISTRATION NO. 00903,** under my supervision and guidance. No part of this thesis has been submitted for any other degree in any other institutions.

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

Dated: 30.04.2009
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Supervisor

Dedicated to
My
Beloved parents who laid the foundation of my success

LIST OF ABBREVIATIONS

ABBREVIATION	FULL WORD
%	Percentage
°C	Degree Celsius
AMF	Arbuscular Mycorrhizal Fungi
As	Arsenic
BARI	Bangladesh Agricultural Research Institute
C	Carbon
cm	Centimeter
DAS	Days after sowing
et al.	And others
g/gm	Gram
K	Potassium
Kg	Kilogram
L	Liter
N	Nitrogen
N	Normal
P	Phosphorus
ppb	Parts per billion
ppm	Parts per million
S	Sulphur
VAM	Vesicular Arbuscular Mycorrhiza
µg	Microgram

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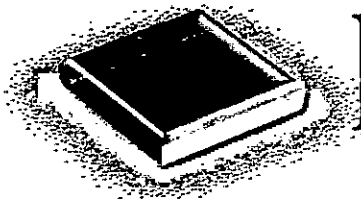
ABSTRACT

The contamination of crops because of irrigation with arsenic contaminated water causing problem in human health in Bangladesh. Mycorrhizal fungi can reduce the contamination of arsenic toxicity to the crops. This study were conducted the mycorrhizal status of different crop roots collected from different arsenic (As) affected villages of Sonargaon area and also examined the role of arbuscular mycorrhizal fungi on some selected crops (wheat, spinach and red amaranthus) grown in arsenic amended soil. Plants were grown with or without arbuscular mycorrhizal fungi (AMF) inoculation in soil amended with three levels of arsenic solution (10ppm.100ppm and 500ppm). The higher concentration of arsenic contaminated soil affected the seed germination. At 500ppm, treated soil completely inhibited seed germination of red amaranthus but little germination was found in wheat and spinach. A positive germination response to AMF was observed in all the selected crops. Root length, shoot height, leaf number, fresh and dry weight of shoot and root, mycorrhizal root colonization, per cent vigority, nutrient (N, P, K and S) uptake increased significantly due to mycorrhizal infection and decreased significantly with increasing arsenic concentrations. Mycorrhizal inoculation reduced As concentration in shoots of red amaranthus. Thus, crops with compatible AMF inoculation can minimize arsenic toxicity and increase plants growth and nutrient uptake.

CHAPTER 1



INTRODUCTION

CHAPTER 1

INTRODUCTION

Arsenic ranks first on the US Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) Priority List of Hazardous Substances (http://www.atsdr.Cdc.gov/cercla/05list.html), and the US Environmental Protection Agency (EPA) has classified arsenic as a group a human carcinogen. 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). Arsenic is released in to the environment in both inorganic and organic form. Arsenate [As (V)] and arsenite [As (III)] are the inorganic, phytoavailable form in soil solution. Inorganic species of As, arsenate (AsO_4^{-3}, referred to as As V) and arsenite (AsO_3^{-3}, referred to as As III), are carcinogenic. Organic arsenic species are generally considered less toxic than inorganic species to a wide range of organisms, including plants, animals and humans (Tamaki and Frankenberger, 1992).

Bangladesh occupies a territory in the north-western part of the Indian subcontinent above the Bay of Bengal. Arsenic in groundwater is a severe problem in West Bengal and in Bangladesh, where it is estimated to cause 200,000 to 270,000 deaths per year. It has an area of 147,570 km^2 and a population of 125

million with 75 to 80% living in the rural areas. Currently 97% of the population of Bangladesh use tube well water for drinking, cooking and irrigation purposes, and surface water is also used for agricultural purposes.

"Arsenic in irrigation water poses a potential threat to soils and crops, the food chain generally, and consequently to human health," says CIMMYT agronomist Craig Meisner. "On average, a Bangladeshi adult drinks about 4 to 5 liters of water a day and consumes about 450 grams of rice. Assuming 200 ppb arsenic in the drinking water and about 0.5 milligrams per kilogram in rice grain, the total daily intake of arsenic would be around 1.2 milligrams, which may not be safe."

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. The story of the arsenic contamination of the groundwater in Bangladesh is a tragic one. Arsenic is a carcinogen which causes arsenicosis which causes skin problems including skin cancer, melanosis, hyperkeratosis, bladder, kidney and lung cancer, disease to the blood vessels of the legs and feet which can lead to gangrene, and is suspected to contribute to diabetic, high blood pressure, and reproductive disorders. [WHO].

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 μg 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 μg L^{-1} and 43 had concentrations >50 μg L^{-1}. Contaminated groundwater is also used for irrigation of paddy, and other crops. Rice is the main staple food for the population. This practice enhances the level of As in the soils rendering them unsuitable for agriculture. This situation poses a serious threat on human and livestock health and highlights the need for scientific studies that would better describes the fate of As in the natural environment and identifies all potential routes of exposure.

Red amaranthus (*Amaranthus tricolor* L.) belongs to the Amaranthaceae family and is commonly used as vegetable in Bangladesh. Amaranth leaves contain 17.4-38.3 % dry matter as crude protein, averaging 5% lysine and thus having potential as a protein supplement (Oliveira and Carvalho, 1975). In Bangladesh, about 18,285 acre of land was under red amaranthus cultivation and total production was about 32710 metric tons (BBS, 2007).

Wheat (*Triticum aestivum* L.), an important cereal crop is produced widely and extensively all over the world. Wheat is the second most important cereal crop next to rice in Bangladesh. In Bangladesh, about 9,88,000 acre of land was under wheat cultivation and total production was about 7,37,000 metric tons (BBS, 2007). It is rich in carbohydrate.

Spinach (*Spinacia oleracea*), belongs to the family *chenopodiaceae* and is commonly used vegetable in Bangladesh. About 14,790 acre of land was under spinach cultivation in Bangladesh and total production was about 29,330 metric tons (BBS, 2007).

Arbuscular mycorrhiza (AM) fungi are the outcome of 450 million years of evolution, which has led to adaptations in both plants and fungi that underpin their symbiotic development and function. Arbuscular mycorrhiza (AM) 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. The word *mycorrhiza* was first used by German researcher A.B. Frank in 1885, and originates from the Greek *mycos*, meaning 'fungus' and *rhiza*, meaning 'root'. Mycorrhiza is a symbiotic mutualistic relationship between special soil fungi and fine plant roots; it is neither the fungus nor the root, but rather the structure formed from these two partners. Since the association is mutualistic, both organisms gain 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. They help increase vegetable production in several ways thorough improvement in nutrient uptake, plant resistance to diseases.

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-fining species (Barea, 1991).

The AM association can help in higher production of growth regulating substances (Danneberg *et al.*, 1992) and increase plant resistance against pest and diseases (Bethlenfalvay and Linderman, 1992). Moreover, it 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). Many reports have indicated that VAM (Vesicular Arbuscular Mycorrhiza) can decrease the severity of diseases caused by root pathogenic fungi, bacteria and nematodes. VAM fungi suppress the incidence of wilt caused by *Fusarium oxysporum* (Jalali and Thareja, 1981).

After entering the plant, arsenic can disturb plant metabolism, as arsenate decouples phosphorylation in mitochondria and arsenite inactivates many enzymes by reacting with sulphydryl groups of proteins (Dixon, 1997). Arsenic a chemical analogue of phosphate, arsenic 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 arsenic speciation in soil and may enhance arsenic 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 adapted to As-polluted soils are generally associated with mycorrhizal fungi (Meharg and Cairney, 1999; Sharples *et al.*, 2000a, b; 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 mycorrhizal (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 1988a, b). 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).

On view of the above facts, the present study was undertaken to achieve the following objectives-

Objectives:

- To assess the mycorrhizal status of some standing crops grown in different arsenic affected area of Sonargaon in Bangladesh.
- To assess the effect of arsenic on seed germination, seedling growth of some selected crops.
- To assess the interaction of arsenic and mycorrhiza on different physical and chemical growth parameters of some selected crops.

CHAPTER 2
REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

To know the role of AMF inoculation on selected crops growth in many areas of the world a number of studies were done. The symbiotic association between a fungus and the roots of a higher plant is termed as "Mycorrhiza". 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:

The effect of VA mycorrhizal and soluble phosphorus on *Abelmoscus esculentus* (L.) was studied by Krishna and Bagyaraj (1982). 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.

Baath and Hayman (1984) studied on the effect of soil volume and plant density on mycorrhizal infection and growth response was studied with onion. There was a significant negative correlation between percentage vesicular-arbuscular mycorrhizal infection and root density.

The growth response due to mycorrhiza decreased when less soil was available for the plant. The root shoot ration decreased with increasing plant density in both mycorrhizal and non-mycorrhizal plants. Pot size did not effect root and shoot ratio.

Sylvia (1990) reported that the flow of carbon to the soil mediated by mycorrhizae serves several important functions. It can increase plant tolerance to salinity (Pond *et al.*, 1984) and it can decrease plant susceptibility to diseases (Jalali and Chand, 1988). Arbuscular mycorrhizal fungi colonize or infect the roots of most species of vascular plants (Morton and Benny, 1990) except for a few belonging to the families Chenopodiecea, Crucifereae, Cyperaceae, Juncaceae and Caryo-phyllaceae (Richardson *et al.*, 2000; Sramek *et al.*, 2000).

Sasai (1991) investigated in field tests on Maize, Soybean, Tomato, Carrot and *Arctium lappa* for the application of phosphorus fertilizers increased after shoot dry weight, increased shoot phosphorus content after the second cropping (86 days after sowing) and decreased mycorrhizal infection rate to varying degrees. Mycorrhizal spore number in rhizosphere soil (Soybean, Tomato and Maize) was much higher in soil without added phosphorus. It is concluded that AM fungi promote phosphate uptake in low phosphate soils during the early stages of plant growth.

Brown *et al.* (1992) reported that Soybean (*Glycine max* cv. clark) plants associated with Maize (*Zea mays*) by AM hyphae had greater nodule activity (C_2H_2 reduction) than plants of the nonassociated comparison treatment. In associated Maize plants, Cob dry mass and VAM colonization were significantly smaller than in nonassociated plants. Conc. of N in associated Soybeans and P in nonassociated ones, were significantly greater than in their respective nonassociated or associated counterparts. Nutrient balance was better in the associated than in the nonassociated plants. Transport of products of photosynthesis was investigated by exposing maize plants to $^{13}CO_2$ and later evaluating the distribution of the C among plants and soil. All the data suggest that nutrient distribution is modified in plant associations that include AM hyphae. Implications of this phenomenon for agro-ecosystem management are discussed.

Matsubara *et al.*, (1994) reported the effects of vesicular-arbuscular mycorrhizal fungus (VAMF) inoculation on seedling growth in 17 species of vegetable crops. Growth was noticeably enhanced by VAMF inoculation to roots in Welsh onion, asparagus, pea, celery, and cucumber. 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 60 th 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) studied the Vesicular Arbuscular Mycorrhizal (VAM) association with many vesicular 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 Kumar (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 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.

Wani and Konde (1996) investigated AM spores with root zones association in garlic. They recorded AM spore ranging from 62 to 242 per 50 g of rhizosphere soil.

Sreeramulu *et al.*, (1996) noted a greater number of AM spores with root zones of inoculated *Amaranthus viridis* and *Trigonella foenuni* than that of un-inoculated 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.

Arriola (1997) reported that Arbuscular mycorrhizal root colonization in all the Amaranthaceae species, positively correlated with maximum border cell production. Commercially available forms of the arbuscular mycorrhizal fungus *Glomus intraradices* and *Trichoderma harzianum* investigated as biocontrol agents of *Fusarium oxysporum f. sp. asparagi* inoculated (at high and low concentrations) asparagus. Death rates of biocontrol treated plants were less than half those of plants inoculated only with *F. oxysporum*. Shoot height, weight and number of shoots produced were greater in biocontrol treated plants than in plants inoculated only with *F. oxysporum*.

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, Chenopodiaeae 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.

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.

George (2000) studied on the colonization of plant roots by 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. The evidence for distinct differences between nonmycorrhizal and mycorrhizal plants in the use of non-soluble nutrient sources in soil is contradictory.

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. The beneficial effect

of the AMF supersedes the pathogenic effect of *V dahliae*; P and N uptake were higher in mycorrhizal treatments than in controls

Phiri *et al.*, (2003) reported that AM root infection in both coarse and fine roots was significantly greater in plants established from plantlets than those established from stakes with differences of 21 and 31%, respectively. Nutrient uptake efficiency (mug of shoot nutrient uptake per in of root length) and use efficiency (g of shoot biomass produced per g of shoot nutrient uptake) for N, P, K, Ca, and Mg were also greater with plants established from plantlets than those established from stakes. Improved nutrient acquisition could be attributed to relief from P stress and possibly uptake of some essential micronutrients resulting from AM association.

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%). AM inoculation improved the growth performance

of seedlings in terms of height and stem diameter. Among the two AM fungi used, the efficiency of *Glomus macrocarpum* was higher than that of *G. fasciculatum* under both nursery and field conditions.

Srivastava *et al.*, (2007) examined the effect of arbuscular mycorrhizal fungi (AMF) and pseudomonads as the microbial inoculants in vegetable based cropping systems under organic farming practices. A significant increase in yield was observed in the inoculated plots over the control. The mycorrhizal inoculation followed by 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.

2.2. 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 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). 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) said 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.

Trotta *et al.* (2006) studied the effects of arbuscular mycorrhizae on growth and As hyperaccumulation in the Chinese brake fern *Pteris vittata*. The 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) were investigated the effects of arbuscular mycorrhizal fungi (*Glomus mosseae*) inoculation on arsenic and phosphorus uptake by

Trifolium repensin and *Oenothera odorata*. These results indicate that inoculation of AM fungi to host plants obtained 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.

Ahmed *et al.* (2006) reported that Arsenic contamination of irrigation water represents a major constraint to Bangladesh agriculture. This study 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.

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.

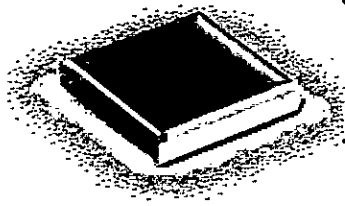
Dong *et al.* (2007) reported that, in a compartmented cultivation system, white clover and ryegrass were grown together in arsenic (As) contaminated soil. The influence of AM inoculation on plant growth, As uptake, phosphorus nutrition, and plant competitions were investigated. Results showed that both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

Chen *et al.* (2007) observed that mycorrhizal fungi may play an important role in protecting plants against arsenic contamination. They 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.

CHAPTER 3



MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

3.1. Study of root colonization

Root samples of some standing selected crops were collected from different arsenic affected locations of Sonargaon area for the detection of AMF infection and intensity of infection.

a. Selection of location:

Eight different arsenic affected locations of Sonargaon 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 during January-07 to May-07 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 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) and it was conducted in the Plant Pathology laboratory of Sher-e-Bangla Agricultural University. 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% H_2O_2 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:

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

At least 100 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 *et al.*, 2007)

3.2. Study of spore population in soil:

After confirming mycorrhizal association in root system, we identify the spore population in soils and then isolated. However, we obtained a little amount of mycorrhizal spore so that by using that data we cannot find any variation to make any table. The identification was done in the mycorrhiza laboratory, Department of Botany, Chittagong University.

3.3. Experiments were conducted in blotter method, plastic tray and poly bag:

Three experiments (blotter method, plastic tray and poly bag) were performed in the seed pathology lab and in the net house of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from August 2007 to December 2007 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 1% cow dung, 10% sand and 89% soil.

c. Mycorrhizal assessment:

Conducting the experiment necessitated the collection of natural inoculums. For this reason, a survey programme was conducted in the Agronomy farm field of Sher-e-Bangla Agricultural University during the August 2007. 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.

e. Preparation of inoculums:

Cassia tora roots were collected from Agronomy field along with rhizosphere soil. The presence of AM fungi within the root sample was confirmed 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 1liter arsenic solution at first 4gm Sodium hydroxide was taken in a 100ml measuring cylinder. Sodium hydroxide was diluted with distilled water and the volume of the cylinder rose up to the 100ml mark. Then 1.32gm arsenic powder was taken in another 1000ml measuring cylinder and dilute with Sodium hydroxide. 10% HCL was taken in a beaker. Then HCL was added into the 1000ml measuring cylinder to make it acidic. At lasts the volume of the flask rise up to 1000ml mark with distilled water.

g. Selection of crops:

Three different agricultural crops (red amaranthus, wheat and spinach) were selected for these experiments.

h. Collection of seeds:

Seeds of different agricultural plants namely red amaranthus, wheat and spinach were collected from BADC (Bangladesh Agricultural Development Corporation).

3.4. Effect of arsenic on seed germination

3.4.1. Blotter method

a. Treatments :

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 100 ppm arsenic solution

T_4 = 500 ppm arsenic solution

b. Preparation of blotter plate:

Nine cm size Petri plates were taken from the Seed Pathology Lab of SAU. Petri plates were autoclaved at 121^0C for 15 minutes for sterilization. Then 9cm three layer blotting paper were placed on it. For this study, three replications and four treatments were used for each crop. Out of 30 Petri plate, 9 Petri plates were soaked with 10 ppm arsenic solution, 9 Petri plates were soaked with 100 ppm arsenic solution, 9 Petri plates were soaked with 500 ppm arsenic solution and rest of 3 Petri plates (1 Petri plate for each crop) were soaked with distilled water.

c. Application of arsenic solution:

Arsenic solution and water was applied for two to three times with the interval of a day in arsenic treated and control Petri plates, respectively.

d. Data recording:

Data was recorded on seedling emergence (%) at 3 DAS, 5 DAS and 7 DAS.

3.5. Role of mycorrhiza on plant growth in arsenic amended soil

3.5.1. In Plastic tray:

a. Treatments:

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution+ mycorrhiza

T_4 = 100 ppm arsenic solution

T_5 = 100 ppm arsenic solution + mycorrhiza

T_6 = 500 ppm arsenic solution

T_7 = 500 ppm arsenic solution+ mycorrhiza

b. Preparation of plastic tray:

Plastic trays of 18″ × 12″ size were bought from the market, which has the capacity to fill with 8 kg soil. At first polythene sheet was placed on it. Before preparation of substratum, soil was sterilized by formaldehyde (0.05%) and used it as base soil. Seven plastic trays were taken for seven treatments and each of them

contains two replications of the three crops. 2/3rd portion of all the plastic tray were filled with substratum. Then a layer of both root inoculums (150 gm) and soil inoculums (600 gm) were placed in 3 plastic trays. Then six trays were amended for 2 to 3 times with 10 ppm, 100ppm and 500ppm arsenic solution. The rest of one tray was remaining untreated control.

c. Sowing of seeds:

Seeds of each crop sown in every tray maintaining two lines as two replications. For that, 20 seeds required for each crop and seed-to-seed distance was 1 cm.

d. Intercultural operation:

The trays were irrigated whenever necessary to maintain field moisture condition. Intercultural operation (weeding, thinning) were done when necessary to ensure the normal growth of the crops. The trays were carefully observed regularly to record any change of plant growth.

e. Harvesting:

When the seedlings were 20 days then one line of each crop harvested. Next harvest was done at 30 days after sowing.

f. Data recording:

Data on different variables were recorded. Data were recorded on seedling emergence (%) (3 DAS, 5 DAS and 7 DAS), shoot height and root length (cm) (20 DAS and 30 DAS), shoot fresh weight (g) (20 DAS and 30 DAS), root fresh

weight (g) (20 DAS and 30 DAS). The amount of dry weight of shoots and roots were too little to record.

3.5.2. In Poly bags:

a. Treatments:

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 =10 ppm arsenic solution + sterilized mycorrhiza

T_4 =10 ppm arsenic solution + mycorrhiza

T_5 = 100 ppm arsenic solution

T_6 = 100 ppm arsenic solution+ sterilized mycorrhiza

T_7 = 100 ppm arsenic solution+ mycorrhiza

T_8 = 500 ppm arsenic solution

T_9 = 500 ppm arsenic solution+ sterilized mycorrhiza

T_{10} = 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. Before preparation of substratum, soil was sterilized by formaldehyde (0.05%) and used it as base soil. Then base soil and cow dung were mixed properly with a ratio of 19:1. Substratum was taken into the perforated seedling bags. At first $\frac{2}{3}$ rd portion of the seedling

bags were filled with substratum. Then a layer of both inoculums i.e. root inoculums 25 gm and soil inoculums 100 gm, were placed in each treated bag. For red amaranthus, 3 replications and 10 treatments were prepared. Both 25 g roots and 100 gm soil (rhizosphere) of sterilized inoculums were used in 10 ppm arsenic solution + sterilized mycorrhiza, 100 ppm arsenic solution+ sterilized mycorrhiza and 500 ppm arsenic solution+ sterilized mycorrhiza treated poly bags to maintain the same nutrient status between the mycorrhizal and non-mycorrhizal bags. The inoculums layer (both mycorrhizal and non-mycorrhizal) of each bag was covered with a thin soil (substratum) layer of 2 cm below the surface in which seed were sown. 90 poly bags were prepared for red amaranthus for the present study.

Then 27 poly bags were amended for 2 to 3 times with 10 ppm arsenic solution, another 27 poly bags were amended with 100 ppm arsenic solution and the next 27 poly bags were amended with 500 ppm arsenic solution. The rest of nine poly bags were remaining untreated control.

c. Sowing of seeds:

For red amaranthus, nine poly bags of each treatment contain 10 seeds. . Then each poly bag was covered with a thin layer of sand to protect the seeds from adverse condition. To avoid the chance of contamination a space of 30 cm was maintained between the inoculated and non-inoculated poly bags.

d. Intercultural operation:

The pots were irrigated whenever necessary to maintain field moisture condition. Intercultural operation (weeding and thinning) were done when necessary to ensure the normal growth of the crops. The pots 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 three seedlings bags were harvested randomly for red amaranthus. Shoots and roots of red amaranthus were collected. 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. Then shoots and roots were dried in an oven for 72 hours at 70°C until the samples gave constant weight.

f. Data recording:

Data on different plant variables were recorded. Data were recorded on seedling emergence (%) (3 DAS, 5 DAS and 7das), fresh and dry weight of shoot (g) (30 DAS, 45 DAS, 60 DAS), fresh and dry weight of root (g) (30 DAS, 45 DAS, 60 DAS), shoot and root length (cm) (30 DAS, 45 DAS, 60 DAS). percent vigority

➢ **Seedling emergence:**

The seedling bags were observed carefully regularly after seed sowing and the seedlings emergences were measured by percentage at 3 DAS, 5 DAS and 7 DAS.

➢ **Growth and biomass:**

Shoot length: Shoot length was measured by a meter scale (cm) and data was recorded.

Root length: After washing, root length was measured by a meter scale.

Shoot fresh weight: Shoots were taken in another polythene bag and marked. Then, they were brought to the laboratory and weight of shoots was recorded.

Root fresh weight: Roots were taken in polythene bag and marked. There after, it was brought to the laboratory, weight of roots was measured with an electric balance and data was recorded. Some roots were taken from each seedling and preserved in a vial with 5% formalin for future mycorrhizal study.

Shoot dry weight: Shoots were taken in brown envelope and dried in the oven at 70°C for 70 hours. Then weight was measured and recorded.

Root dry weight: After taking the roots for mycorrhizal study, the rest were weighted again and recorded. Then those were taken separately in brown envelop for drying. Then roots were dried in the oven at 70°C for 70 hours and weight was recorded.

➢ **Vigor index**: Vigor index was calculated by using the fowling formula

Vigor index = (Mean root length + Mean shoot length) × 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 0.5g 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, 6ml 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% H_2O_2, conc. H_2SO_4 and a catalyst mixture (K_2SO_4: $CuSO_4.5H_2O$: 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 H_3BO_3 with 0.01 N H_2SO_4 (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 4^0C in a refrigerator until tested for arsenic.

➢ **Detection of arsenic in plant samples**

Arsenic was detected in the ADM Lab, Department of Pharmacology, Bangladesh Agricultural University, Mymensingh with Hydride Generation Atomic Absorption Spectrophotometer (HG-AAS; PG-990, PG Instruments Ltd. UK; Photograph 1). Arsenic was detected by forming AsH_3 at below p^H 1.0 after

the reaction of As with a solution of potassium borohydride (KBH_4 = 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, 100ml of concentrated HCl was taken in a 1000ml calibrated volumetric flask and then deionized water was added up to the mark of 1000ml 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, 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 μg/L in 10% HCl in separate 25ml calibrated volumetric flasks following proper acid washing and drying from 1000 μg As/L working solution of As_2O_5 (arsenic pentaoxide).

➢ Operation of HG-AAS 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.

i. Statistical analysis:

All data were analyzed in the computer using SPSS Program for F-test and mean separations were done by DMRT at 5% level of significance.

CHAPTER 4
RESULTS

CHAPTER 4

ESULTS

4.1. Study of root colonization:

Different crops root were collected from different villages of Sonargaon area to know the percent root infection and intensity of infection of arbuscular mycorrhizal fungi.

a. Noail village:

The percentage of AM-fungal association in different crops root collected from Noail village of Sonargaon area is presented in Figure 1. It was observed that the percentage of root infection of *Amaranthus gangeticus* and *Zea mays* varied from field to field. The percent root infection was ranged from 0-36 %. Among the five plant species, the highest root infection was observed in *Zea mays* (36%) (Field- b) and the lowest was observed in *Amaranthus gangeticus*. No infection was recorded in *Cucumis sativus* and *Capsicum frutescens*.

Percentage of intensity of infection of AMF on different crops root collected from Noail village of Sonargaon area is presented in Table 1. Intensity of infection was also varied at different scale. *Amaranthus gangeticus* root collected from the "field-a" showed 8% intensity of infection in scale 1, 5% in scale 2 and 2% in scale 3 whereas, the root sample collected from "field-b" showed 14% intensity of infection in scale 1 and 9% in scale 2 and no intensity of infection was found in scale-3. *Zea mays* root collected from the "field-a" showed 19% intensity of infection in scale 1 and 8% in scale 2 whereas, the root sample collected from "field-b" showed 22% intensity of infection in scale 1 and 9% in scale 2and 5% in scale 3.

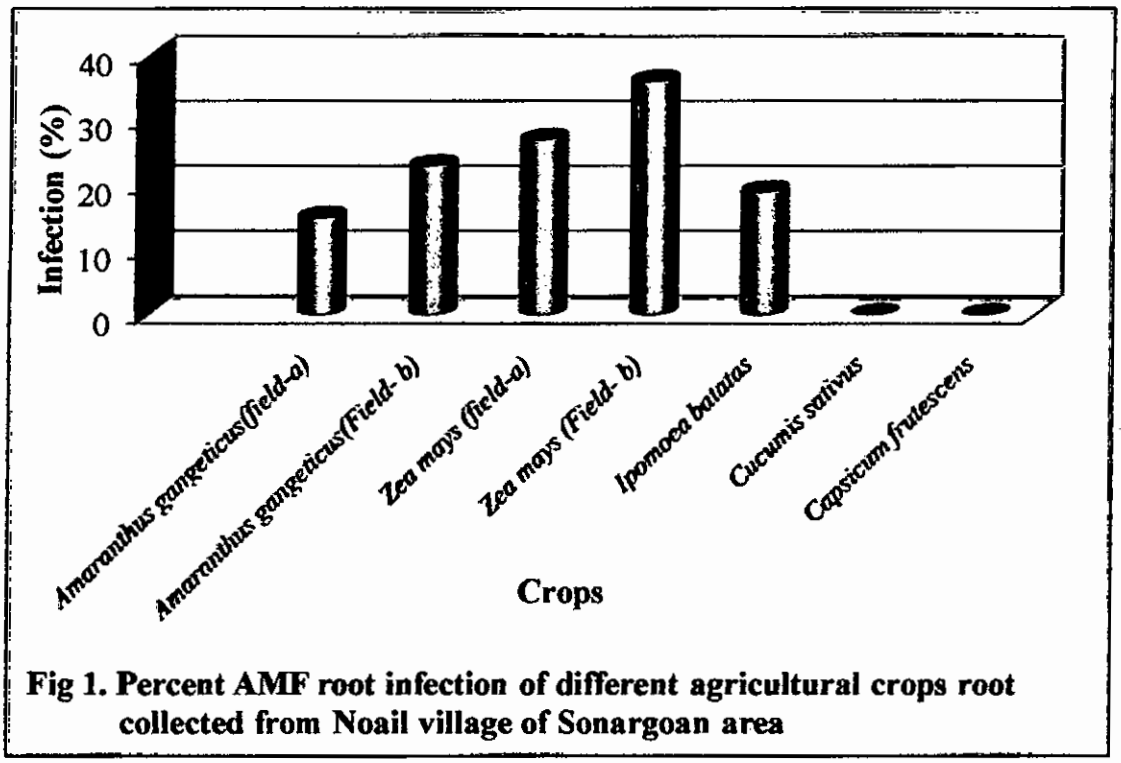


Fig 1. Percent AMF root infection of different agricultural crops root collected from Noail village of Sonargoan area

Table 1. Percent intensity of infection of AMF on different crops root collected from Noail village of Sonargaon area

Name of the plant species	Intensity of infection (%)			
	Scale			
	0	1	2	3
Amaranthus gangeticus(field-a)	85	8	5	2
Amaranthus gangeticus(Field- b)	77	14	9	0
Zea mays (field-a)	73	19	8	0
Zea mays (Field- b)	64	22	9	5
Ipomoea batatas	81	17	2	0
Cucumis sativus	100	0	0	0
Capsicum frutescens	100	0	0	0

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

b. Balua dighir par village:

The data of percent AM-fungal root infection of different weeds root collected from Balua dighir par village of Sonargaon area are presented in Figure 2. Six different crops were collected from Balua dighir par village. It was observed that the percentage root infection varied from location to location and field to field. The highest percent root infection was recorded in *Zea mays* from field-a and that was 36% and the lowest root infection was recorded in *Capsicum frutescens* from field-a and that was 7%. No infection was recorded *Corcorus capsularis* and *Allium cepa*.

Percentage of intensity of infection of AMF on different crops root collection from Balua dighir par village of Sonargaon area is presented in Table 2. To determine the intensity of infection 0-3 scale was used. In scale 1 intensity of infection was recorded in 7 cases. Among them the lowest percentage was found in *Capsicum frutescens* and that was 6% and the highest was found in *Zea mays* and that was 23%. In scale 2 intensity of infection was also recorded in 6 cases. In scale 3 *Ipomoea batatas* (field-a) and *Zea mays* collected from both field showed 2%, 3%, and 2% intensity of infection, respectively.

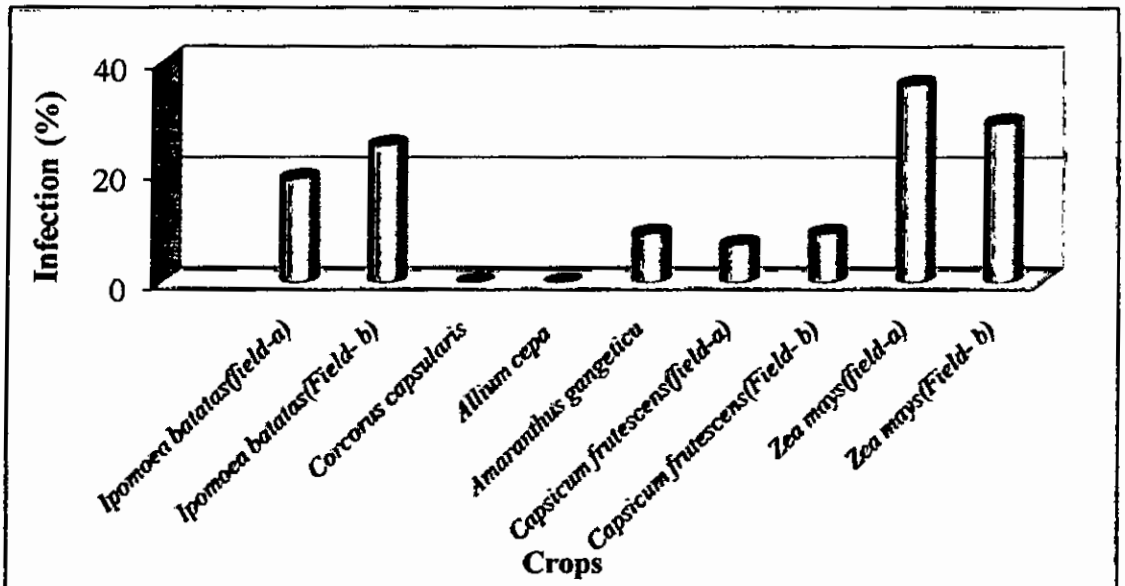


Fig 2. Percent AMF root infection of different agricultural crops root collected from Balua dighirpar village of Sonargoan area

Table 2. Percent intensity of infection of AMF on different crops root collected from Balua dighir par village of Sonargaon area

Name of the plant species	Intensity of infection (%)			
	Scale			
	0	1	2	3
Ipomoea batatas(field-a)	81	11	6	2
Ipomoea batatas(Field- b)	75	17	8	0
Corcorus capsularis	100	0	0	0
Allium cepa	100	0	0	0
Amaranthus gangeticu	91	9	0	0
Capsicum frutescens(field-a)	93	6	1	0
Capsicum frutescens(Field- b)	91	7	2	0
Zea mays(field-a)	64	23	11	3
Zea mays(Field- b)	71	21	6	2

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

c. Kattukali village:

Percent AMF root infection of different plants root collected from Kattukali village of Sonargaon area are shown in Figure 3. Seven plants were collected from Kattukali village. Present investigation reveals that the percent root infection ranged from 0% to 33%. The highest percentage of root infection was found in *Zea mays* (33%). No infection was recorded in case of *Lagenaria vulgaris*, *Solanum melongena*, *Oryza sativa* (field-a) and *Oryza sativa* (field-b).

Percentage of intensity of infection of AMF on different crops root collection from Kattukali village of Sonargaon area is presented in Table 3. To determine the intensity of infection 0-3 scale was used. Intensity of infection was also varied at different scale. The highest percentage of intensity was found in *Zea mays* (29%) in scale 1 and 4% in scale 2. There were no intensity of infection was found in scale 3.

d. Raisdia village:

Percent AMF root infection of different crops root collected from Raisdia village of Sonargaon area are shown in Figure 4. Seven crops were collected from Raisdia village. Present investigation reveals that the percent root infection ranged from 0% to 42%. The highest percentage of root infection was found in *Zea mays* (42%) followed by *Corcorus capsularis* (32%), *Cucerbita mosch*ata (29%) which was collected from field-b and *Cucerbita moschata* (23%) from field-a. There was no infection found in *Allium cepa* and *Solanum melongena.*

Percentage of intensity of infection of AMF on different crops root collection from Raisdia village of Sonargaon area is presented in Table 4. To determine the intensity of infection 0-3 scale was used. Intensity of infection was also varied at different scale. The highest percentage of intensity was found in *Zea mays* and that was 33 in scale 1 and 9 in scale 2. There was no intensity of infection was found in 'scale 3'.

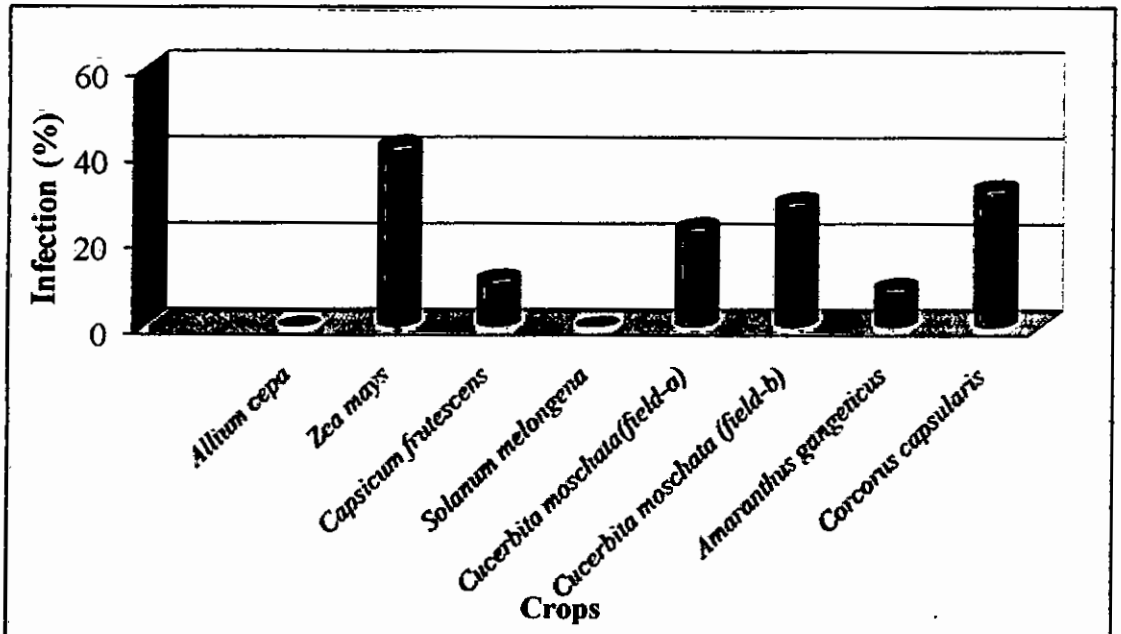


Fig 4. Percent AMF root infection of different agricultural crops root collected from Raisdia village of Sonargoan area

Table 4. Percent intensity of infection of AMF on different crops root collected from Raisdia village of Sonargaon area

Name of the plant species	Intensity of infection (%)			
	Scale			
	0	1	2	3
Allium cepa	100	0	0	0
Zea mays	58	33	9	0
Capsicum frutescens	89	9	2	0
Solanum melongena	100	0	0	0
Cucerbita moschata(field-a)	77	19	4	0
Cucerbita moschata (field-b)	71	21	8	0
Amaranthus gangeticus	91	6	3	0
Corcorus capsularis	68	29	3	0

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

e. Fotekandi village:

Root samples collected from Fotekandi village of Sonargaon area were to observe the VAM association. The percent AMF root infection of different agricultural crops root collected from Fotekandi village of Sonargaon area are presented in Figure 5. It was observed that the percent root infection was varied from location to location and one species to another species. The maximum percent root infection was recorded in *Zea mays* (field-b) and that was 51% whereas 45% in field-a. The lowest percent root infection was recorded in *Corcorus capsularis* and that was 5%.

Percentage of intensity of infection of AMF on different crops root collection from Kattukali village of Sonargaon area is presented in Table 5. Intensity of infection was also varied at different scale. The highest intensity of infection was recorded in *Zea mays* (field-b) and it was32% in scale 1, 9% in scale 2 and 4% in scale 3. Intensity of infection was zero in case of *Allium cepa, Oryza sativa, Abelmoscus esculentus* and *Vigna unguiculata*.

f. Humchadi village:

Percent AMF root infection of different plants root collected from Humchadi village of Sonargaon area are shown in Figure 6. Seven plants were collected from Humchadi village. Present investigation reveals that the percent root infection ranged from 0% to 31%. The highest percentage of root infection was found in *Ipomoea batatas* (31%). No infection was recorded in case of Cicer *arietinum* and *Lagenaria vulgaris*.

Percentage of intensity of infection of AMF on different crops root collection from Humchadi village of Sonargaon area is presented in Table 6. 0-3 scale was used to determine the intensity of infection. Intensity of infection was also varied at different scale. The highest percentage of intensity was found in *Ipomoea batatas* (23%) in scale 1 and 8% in scale 2. There was no intensity of infection was found in scale 3.

g. Boroikandi village:

The results of the percentage of AM-fungal association in different crops root collected from Boroikandi village of Sonargaon area is presented in Figure 7. Six different plant species were collected from Boroikandi village of Sonargaon area to determine the infection percentage and intensity of infection (%). Percent root infection ranged from 0% to 41%. The highest percentage root infection was found in *Zea mays* and that was 41% which was collected from field-b followed by *Zea mays* (field-a) (33%) and *Ipomoea batatas* (29%). There was no infection found in *Oryza sativa* (field-a), *Oryza sativa* (field-b) and *Solanum melongena*.

Percentage of intensity of infection of AMF on different crops root collection from Boroikandi village of Sonargaon area is presented in Table 7. Intensity of infection was also varied at different scale. The highest intensity of infection was recorded in *Zea mays* and it was 29% in scale 1, 11% in scale 2 and 1% in scale 3. No intensity of infection was recorded in scale 3.

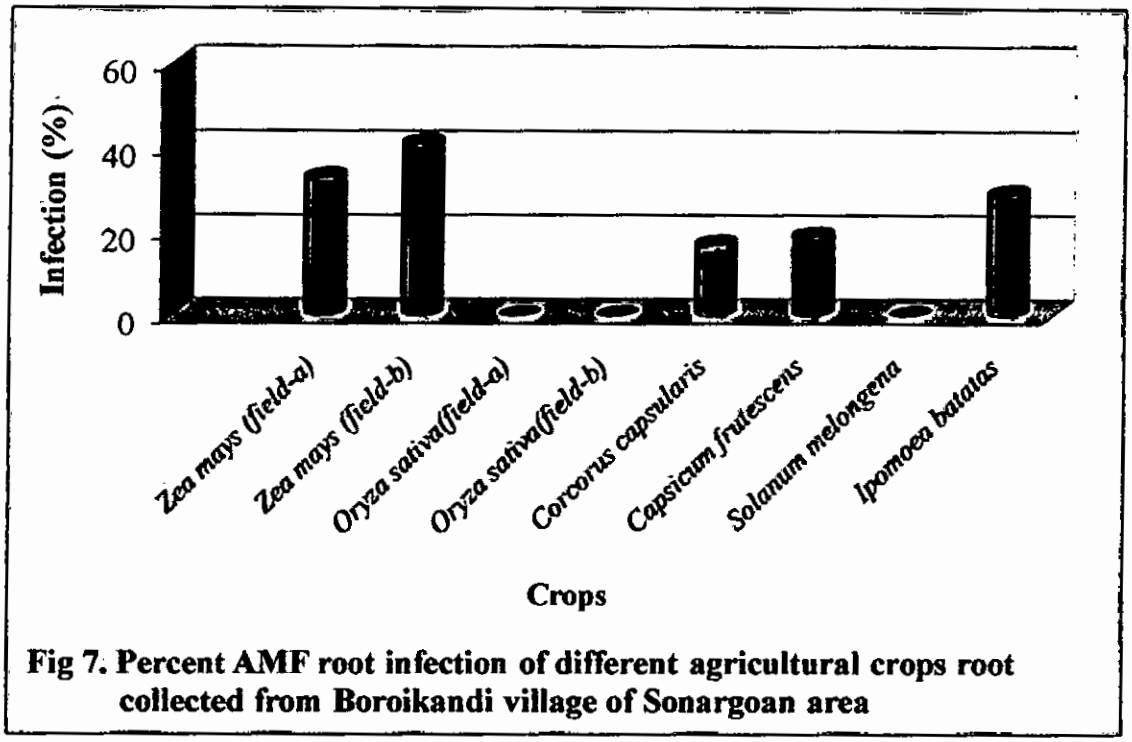


Fig 7. Percent AMF root infection of different agricultural crops root collected from Boroikandi village of Sonargoan area

Table7. Percent intensity of infection of AMF on different crops root collected from Boroikandi village of Sonargaon area

Name of the plant species	Intensity of infection (%)			
	Scale			
	0	1	2	3
Zea mays (field-a)	67	28	5	0
Zea mays (field-b)	59	29	11	1
Oryza sativa(field-a)	100	0	0	0
Oryza sativa(field-b)	100	0	0	0
Corcorus capsularis	83	14	3	0
Capsicum frutescens	81	11	8	0
Solanum melongena	100	0	0	0
Ipomoea batatas	79	19	8	2

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

h. Darpat village:

Root samples collected from Darpat village of Sonargaon area were to observe the VAM association. The percent AMF root infection of different crops root collected from Darpat village of Sonargaon area are presented in Figure 8. It was observed that the percent root infection was varied from location to location and one species to another species. The maximum percent root infection was recorded in *Lycopersicon esculentum* (field-a) and that was 15% whereas 10% from field-b. The lowest root infection was recorded in *Momordica charantea* 5%.

Percentage of intensity of infection of AMF on different crops root collection from Darpat village of Sonargaon area is presented in Table 8. Intensity of infection was also varied at different scale. The highest intensity of infection was recorded in *Lycopersicon esculentum* (field-a) and it was12% in scale 1 and 3% in scale 2. Intensity of infection was zero in case of *Allium cepa, Allium sativus, Brassica oleracea* and *Rhaphnus sativus.*

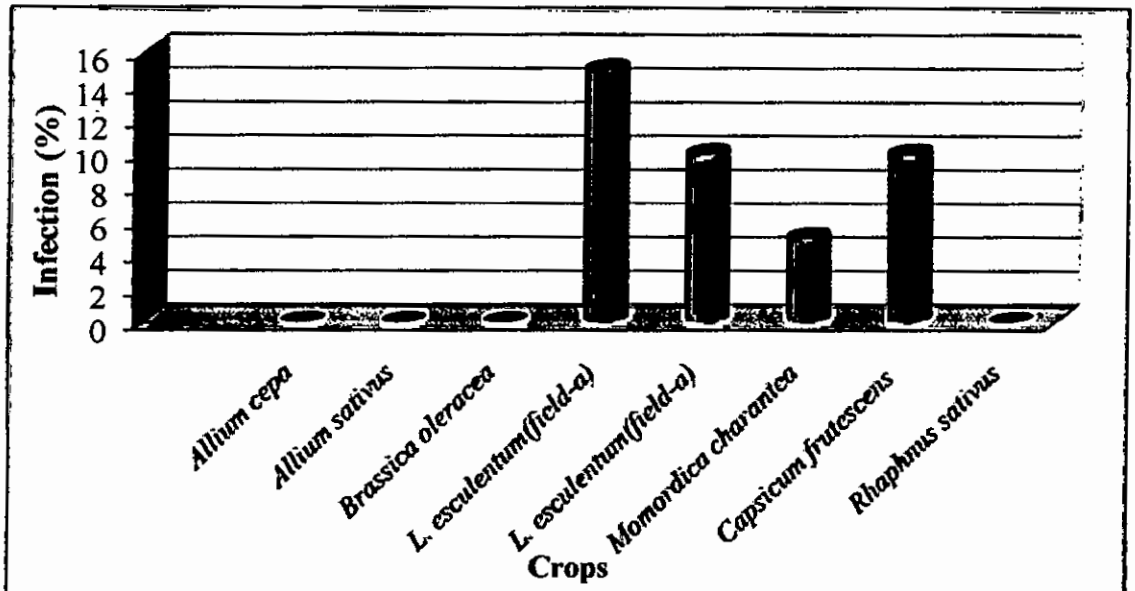


Fig 8. Percent AMF root infection of different agricultural crops root collected from Darpat village of Sonargoan area

Table 8. Percent intensity of infection of AMF on different crops root collected from Darpat village of Sonargaon area

Name of the plant species	Intensity of infection (%)			
	Scale			
	0	1	2	3
Allium cepa	100	0	0	0
Allium sativus	100	0	0	0
Brassica oleracea	100	0	0	0
Lycopersicon esculentum(field-a)	85	12	3	0
Lycopersicon esculentum(field-a)	90	7	3	0
Momordica charantea	95	5	0	0
Capsicum frutescens	90	8	2	0
Rhaphnus sativus	100	0	0	0

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

i. Comparison

➢ Comparison of total percent infection of the crops among the eight villages

Comparison of some crops among the eight villages is presented in Figure 9. The average percent root infection of different crops was varied from village to village. The highest value of percent root infection was found from Raisdia village 18.25% followed by Borokandi (17.38%) and Darpat (7.14%).

➢ Comparison of total percent infection of *Zea mays* among the six villages

Comparison of total infection percentage of *Zea mays* among the six villages is presented in Figure 10. The average percent root infection of *Zea mays* was varied from village to village. The highest root infection was found from Fotekandi village and that was 48% followed by Raisdia (42%) and Kattukali (33%). The lowest root infection was found in Boroikandi village 17.38%.

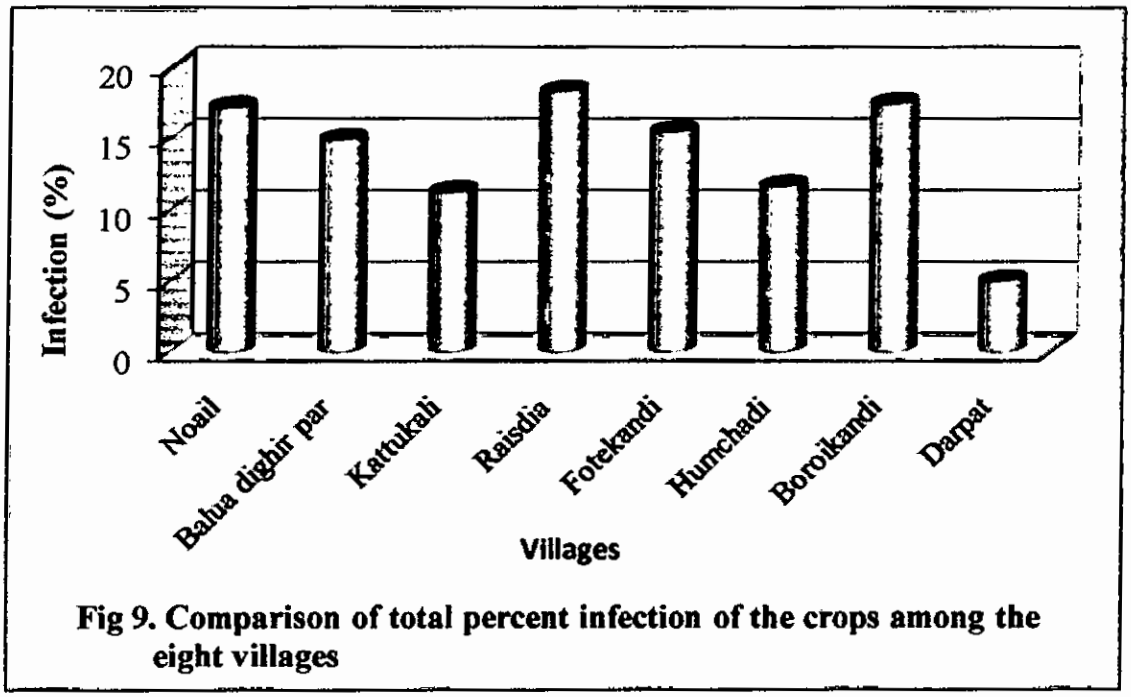


Fig 9. Comparison of total percent infection of the crops among the eight villages

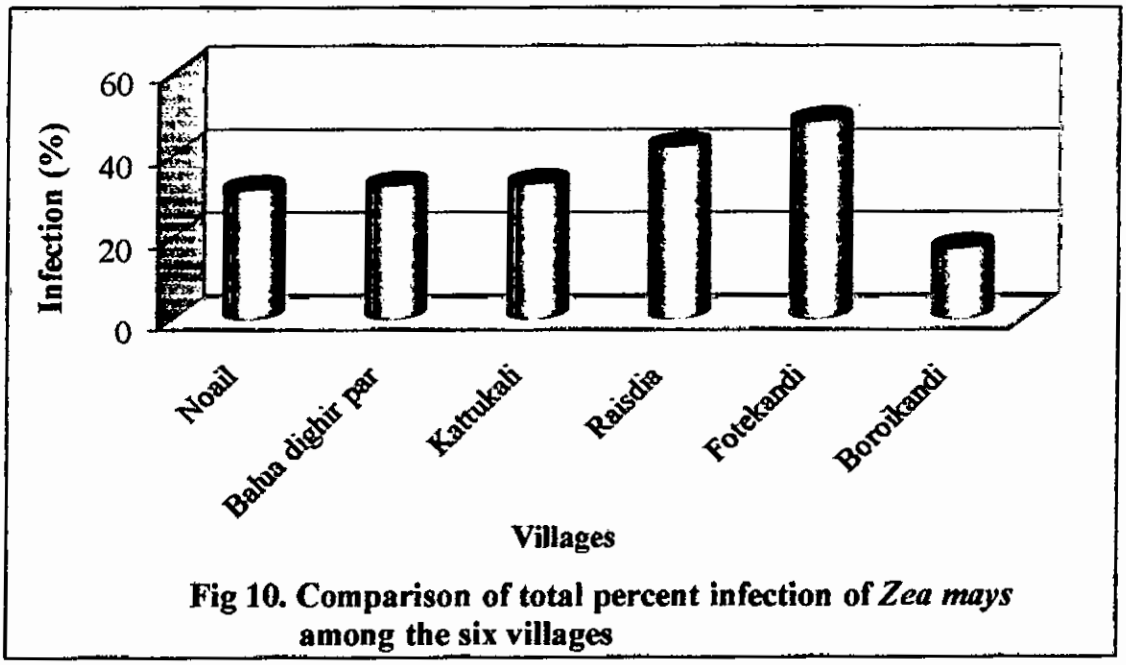


Fig 10. Comparison of total percent infection of *Zea mays* among the six villages

- **Comparison of total % infection of *Ipomea batatas* among the four villages**

Comparison of total infection percentage of *Ipomea batatas* among the four villages is presented in Figure 11. The average percent root infection of *Ipomea batatas* was varied from village to village. The highest root infection was found in Humchadi village 31% followed by Boroikandi (29%). The lowest percent root infection was found in Noail village 19%.

- **Comparison of total percent infection of *Amaranthus gangeticus* among the four villages**

Comparison of total infection percentage of *Amaranthus gangeticus* among the four villages is presented in Figure 12. Varied percent root infection of *Amaranthus gangeticus* was found from village to village. The highest percent root infection was found in Kattukali village (21%) followed by Noial (19%). The lowest percent root infection (9%) was found in Balua digir par and Raisdia village.

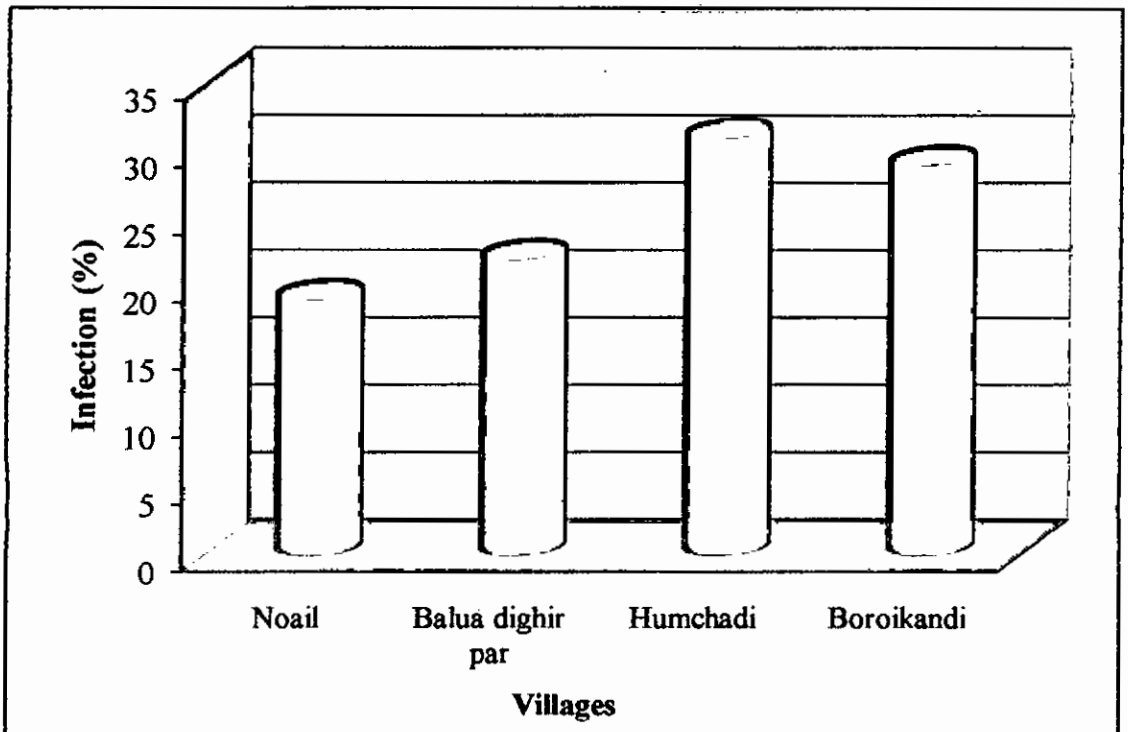


Fig 11. Comparison of total % infection of *Ipomea batatas* among the four villages

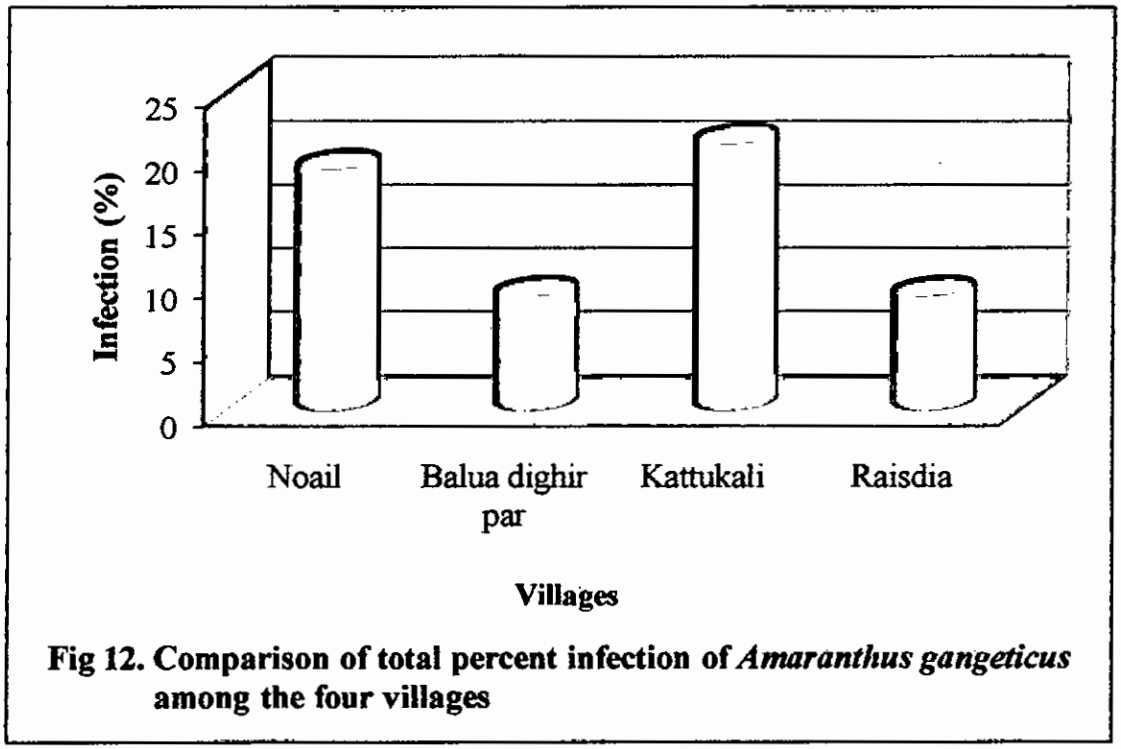


Fig 12. Comparison of total percent infection of *Amaranthus gangeticus* among the four villages

➢ Comparison of total percent infection of *Capsicum frytescens* among the four villages

Comparison of total infection percentage of *Capsicum frutescens* among the four villages is presented in Figure 13. The average percent root infection of *Capsicum frutescens* was varied from village to village. The highest value of percent root infection was found in Humchadi village 21% followed by Boroikandi (19%) and Fotekandi (17%). The lowest percent root infection was found in Balua digir par village and that was 8%. There was no infection percentage found in Noail village.

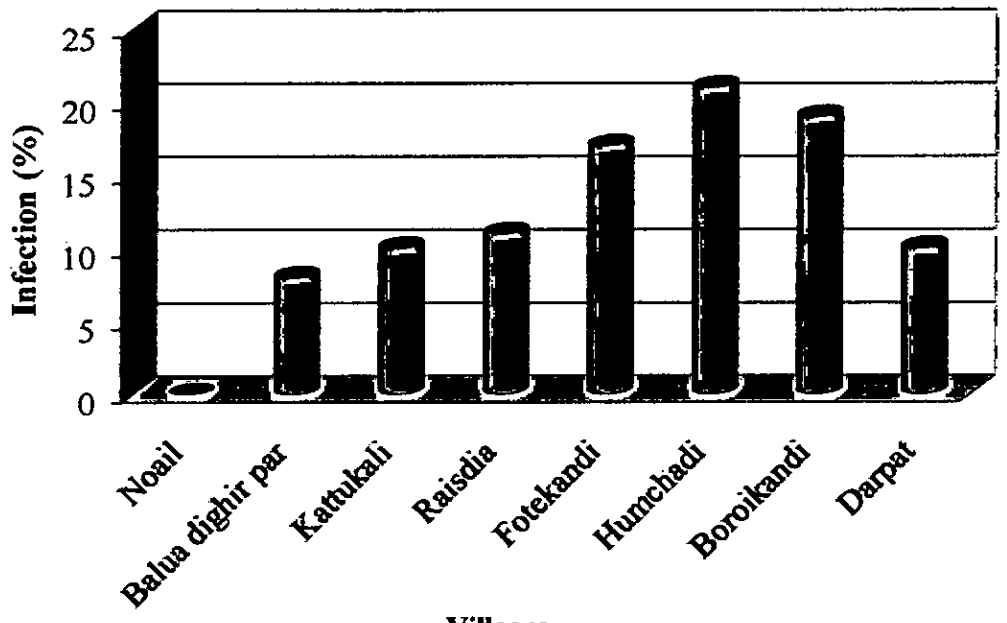


Fig 13. Comparison of total percent infection of *Capsicum frytescens* among the four villages

4.2. Effect of arsenic on seed germination:

Role of arsenic on seed germination of different crops were studied in this section.

4.2.1. Blotter method:

A. Red amaranthus:

The role of different concentrations of arsenic solution on seed germination of red amaranthus at different growth periods is shown in Figure 14. The seed germination was recorded after 3, 5 and 7 days of sowing. It was exposed from the Figure 14 that the performances of the most of the treatments differed significantly from each other. Seed germination of red amaranthus was decreased with the increase of arsenic solution. With the increase of incubation periods the seed germination percentage was increased. Result revealed that treatment T_1 (control) gave the highest seed germination which was better in comparison to each of the other treatments. Among the four different treatments T_1 gave the highest result followed by treatment T_2 and T_3 whereas treatment T_3 (100 ppm arsenic solution) gave the lowest result. No germination was found in case of treatment T_4 (500 ppm arsenic solution).

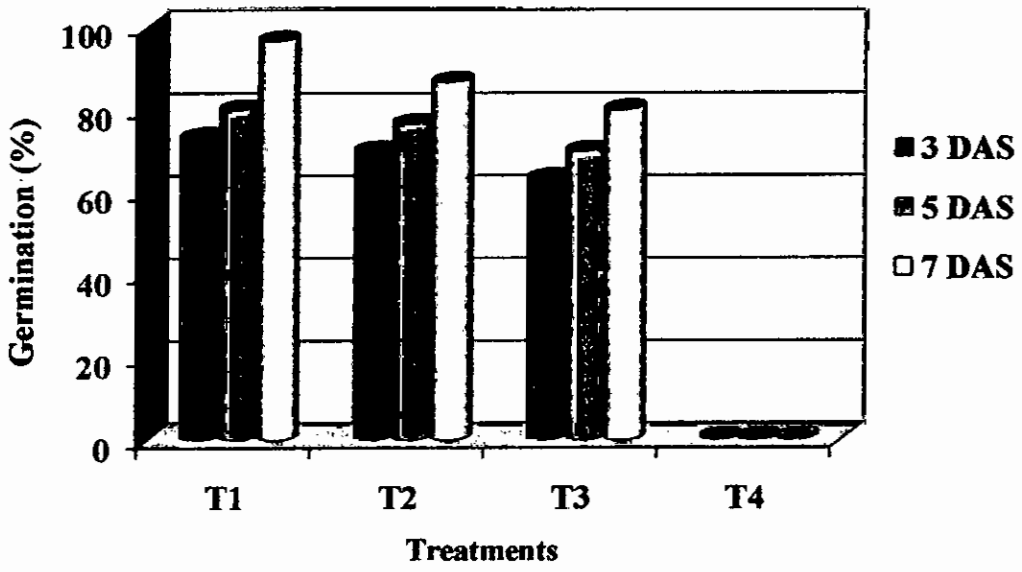


Fig-14. Role of different concentrations of arsenic solution on seed germination of red amaranthus at different growth periods

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 100 ppm arsenic solution

T_4 = 500 ppm arsenic solution

B. Wheat:

The role of different concentrations of arsenic solution on seed germination of wheat at different growth periods is shown in Figure 15. The performances of the most of the treatments differed significantly from each other. Seed germination of wheat was decreased with the increase of arsenic concentration. Result revealed that treatment T_1 (control) gave the highest seed germination among the treatments. It followed by T_2, T_3 and T_4. Treatment T_4 (500ppm arsenic solution) gave the lowest germination where the highest level of arsenic concentration was used.

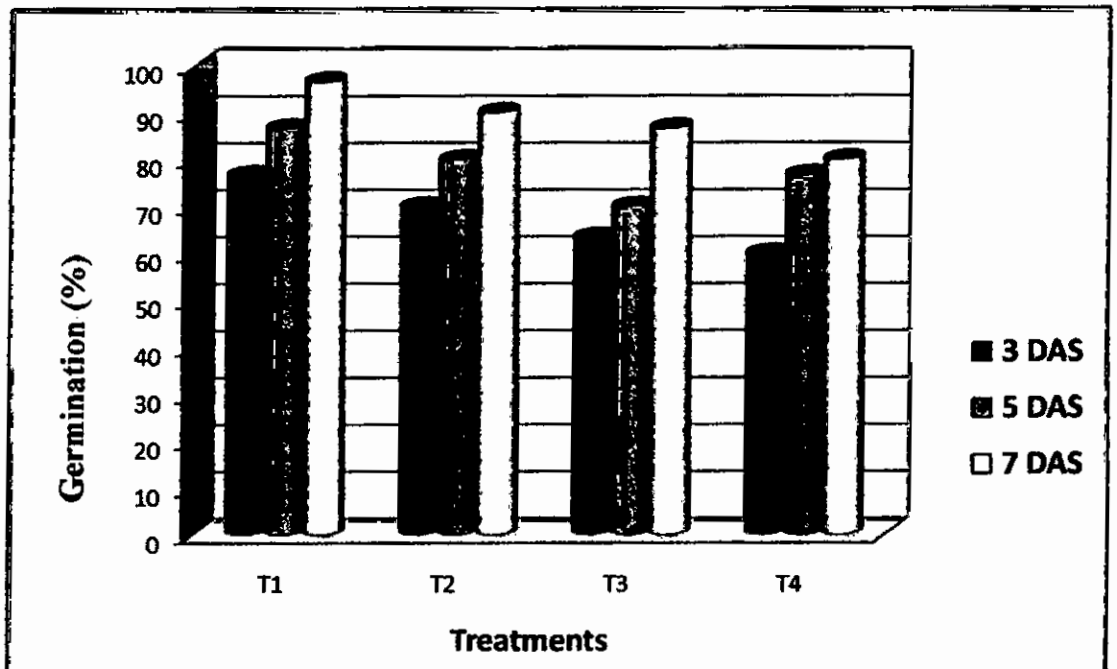


Fig-15. Role of different concentrations of arsenic solution on seed germination of wheat at different growth periods

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 =100 ppm arsenic solution

T_4 =500 ppm arsenic solution

C. Spinach:

The role of different concentrations of arsenic solution on seed germination of spinach at different growth periods by using blotter method was presented in Figure 16. The seed germination was recorded after 3, 5 and 7 days of sowing. It was exposed from the Figure 16 that the performances of the most of the treatments differed significantly from each other and the seed germination of spinach was decreased with the increase of arsenic solution. Result revealed that treatment T_1 (control) gave the highest seed germination which was better in comparison to each of the other treatments. Among the four different treatments T_1 gave the highest result followed by treatment T_2, T_3 and T_4. Among the four different treatments, treatment T_4 (500 ppm arsenic solution) gave the lowest result.

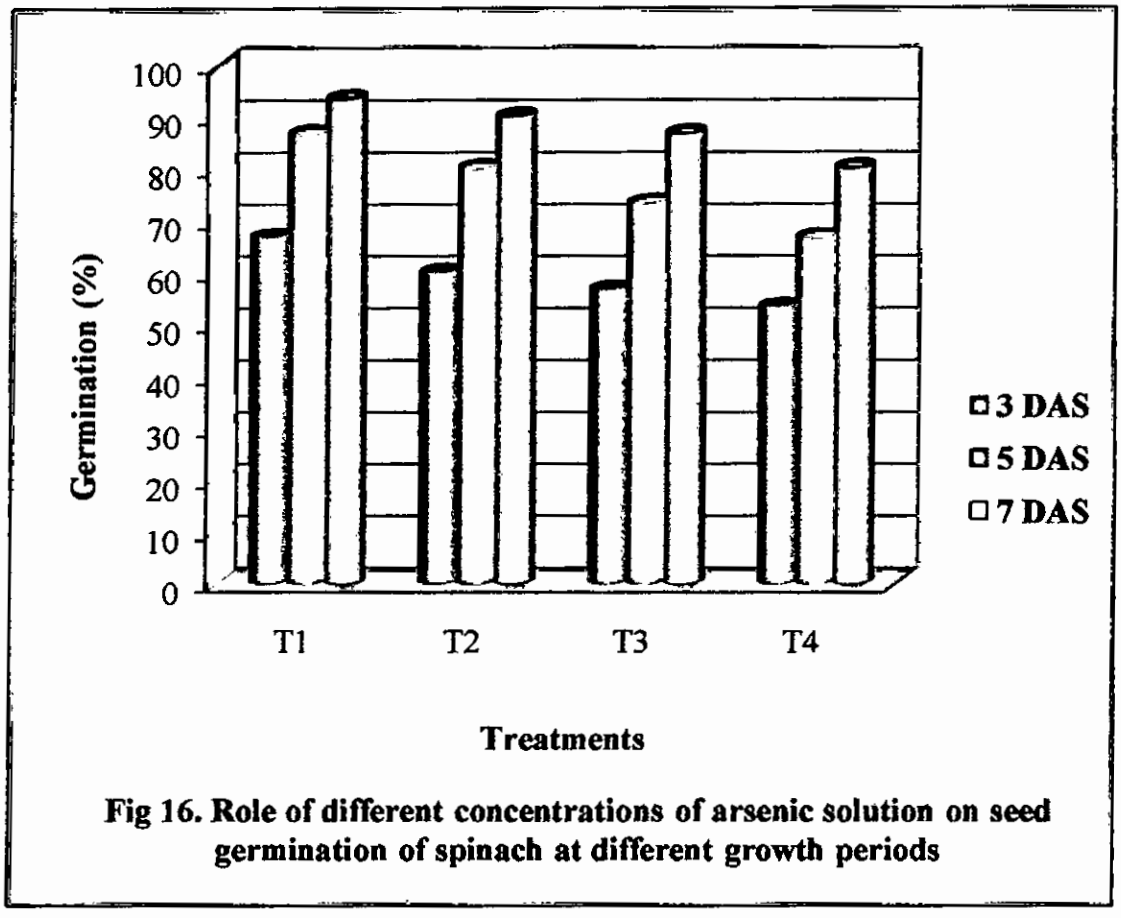


Fig 16. Role of different concentrations of arsenic solution on seed germination of spinach at different growth periods

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 =100 ppm arsenic solution

T_4 =500 ppm arsenic solution

4.3. Role of mycorrhiza on plant growth in arsenic amended soil:

4.3.1. Plastic tray:

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. Red amaranthus

- **Seed germination:**

The influence of AMF inoculation on seedling emergence of red amaranthus seeds sown in soil amended with different concentrations of arsenic solution is represented in Figure 17. The seedling emergence was varied at different concentrations but the emergence was increased with the increase of incubation period. The highest seedling emergence was recorded in treatment T_3 (10 ppm arsenic solution + mycorrhiza) and those were 83.33%, 93.33% and 96.66% at 3DAS, 5DAS and 7DAS, respectively and the lowest was recorded in treatment T_4 (100 ppm arsenic solution). On the other hand, among the only arsenic treatments T_2, T_4 and T_6, seedling emergence was the highest in treatment T_2 (10 ppm arsenic solution) and in 500 ppm no germination was found. In all the three recorded periods it was observed that seedling emergence of red amaranthus decreased with the increase of arsenic concentration.

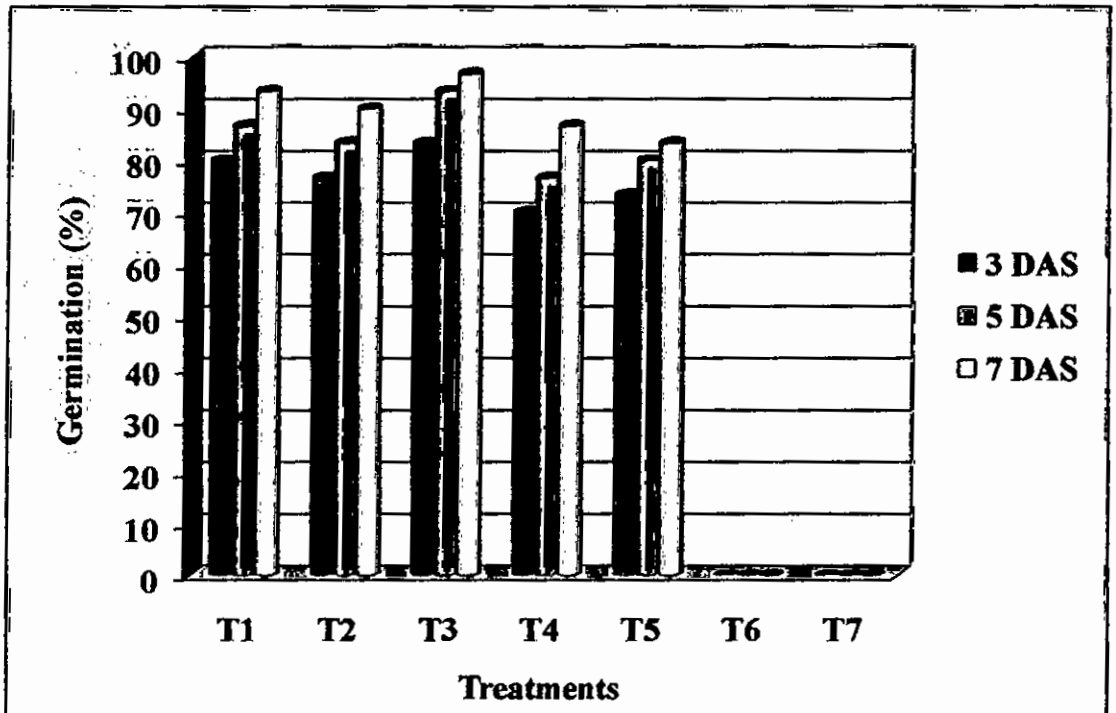


Fig 17. Influence of AMF on seedling emergence of red amaranthus at different growth periods in soil amended with different concentrations of arsenic solution

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 =10 ppm arsenic solution + mycorrhiza

T_4 =100 ppm arsenic solution

T_5 = 100 ppm arsenic solution + mycorrhiza

T_6 =500 ppm arsenic solution

T_7 =500 ppm arsenic solution+ mycorrhiza

➢ Shoot height:

The influence of AMF inoculation on shoot height of red amaranthus at different growth periods, seeds sown in soil amended with different concentrations of arsenic solution are shown in Table 9. Shoot height of red amaranthus differed significantly due to the application of different concentrations of arsenic solution and inoculation of mycorrhiza at 20 and 30 days after sowing. Result revealed that treatment T_3 (10 ppm arsenic solution + mycorrhiza) gave the highest shoot height and those were 14.56cm and 17.89cm at 20 DAS and 30 DAS, respectively which was significantly better in comparison to other treatments. Treatment T_1 (Control) gave the second highest shoot height. The lowest shoot height of red amaranthus was recorded in the treatment T_4 and those were 8.79cm and 10.57cm at 20 DAS and 30 DAS respectively and shoot height decreased when the rate of arsenic concentration increased.

Table 9. Influence of AMF inoculation on shoot height of red amaranthus at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Shoot height (cm)	
	20 DAS	30 DAS
T_1	12.32 e (3.58)	15.34 e (3.97)
T_2	10.33 d (3.29)	13.36 d (3.72)
T_3	**14.56 f (3.94)**	**17.89 f (4.28)**
T_4	8.79 b (3.04)	10.57 b (3.32)
T_5	9.67 c (3.18)	11.99 c (3.53)
T_6	0 a (0.71)	0 a (0.71)
T_7	0 a (0.71)	0 a (0.71)

* The values in the parenthesis are the square root transformed value

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 =10 ppm arsenic solution + mycorrhiza

T_4 =100 ppm arsenic solution

T_5 = 100 ppm arsenic solution + mycorrhiza

T_6 =500 ppm arsenic solution

T_7 =500 ppm arsenic solution+ mycorrhiza

➢ Root length:

Influence of AMF inoculation on root length of red amaranthus, In soil, amended with different concentrations of arsenic solution is shown in Table 10. The performances of the most of the treatments differed significantly from each other. Mycorrhiza inoculated treatments significantly enhanced root length in comparison to non-inoculated. Treatment T_3 (10 ppm arsenic solution + mycorrhiza) gave the highest root length of red amaranthus and those were 6.78cm and 8.02cm at 20 DAS and 30 DAS, respectively. T_2 gave the highest result among the treatment T_2, T_4 and T_6 treatment where low concentration of arsenic was used. The root length of red amaranthus decreased with the increase of arsenic concentration used.

Table 10. Influence of AMF inoculation on root length of red amaranthus at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Root length (cm)	
	20 DAS	30 DAS
T_1	5.86 e (2.52)	6.78 e (2.69)
T_2	4.99 d (2.34)	5.89 d (2.52)
T_3	**6.78 f (2.69)**	**8.02 f (2.91)**
T_4	3.01 b (1.87)	4.29 b (2.18)
T_5	4.01 c (2.12)	5.02 c (2.34)
T_6	0 a (0.71)	0 a (0.71)
T_7	0 a (0.71)	0 a (0.71)

* The values in the parenthesis are the square root transformed value

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 =10 ppm arsenic solution + mycorrhiza

T_4 =100 ppm arsenic solution

T_5 = 100 ppm arsenic solution + mycorrhiza

T_6 = 500 ppm arsenic solution

T_7 = 500 ppm arsenic solution+ mycorrhiza

Table 11. Influence of AMF inoculation on fresh weight of shoot of red amaranthus at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Fresh weight of shoot (gm)	
	20 DAS	30 DAS
T_1	6.28 e (2.60)	7.31 d (2.79)
T_2	5.15 d (2.38)	6.29 c (2.61)
T_3	**7.29 f** (2.79)	**8.48 e** (3.00)
T_4	3.09 b (1.89)	4.17 b (2.16)
T_5	4.10 c (2.14)	5.23 b (2.39)
T_6	0 a (0.71)	0 a (0.71)
T_7	0 a (0.71)	0 a (0.71)

* The values in the parenthesis are the square root transformed value

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution + mycorrhiza

T_4 = 100 ppm arsenic solution

T_5 = 100 ppm arsenic solution + mycorrhiza

T_6 = 500 ppm arsenic solution

T_7 = 500 ppm arsenic solution + mycorrhiza

➢ Fresh weight of root:

The role of AMF inoculation on fresh weight of root of red amaranthus seeds sown in soil amended with different concentrations of arsenic solution was investigated. The performances of the most of the treatments differed significantly from each other. Result revealed that treatment T_3 (10 ppm arsenic solution + mycorrhiza) gave the highest fresh weight of root of red amaranthus and those were 1.85gm and 2.21gm at 20 DAS and 30 DAS, respectively. The lowest fresh weight of root of red amaranthus was recorded from only the arsenic treated treatments (T_2, T_4 and T_6) and fresh weight of root of red amaranthus decreased when the rate of arsenic concentration increased. The fresh weight of root of red amaranthus increased when mycorrhiza inoculated on those treatments.

Table 12. Influence of AMF inoculation on fresh weight of root of red amaranthus at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Fresh weight of root (gm)	
	20 DAS	30 DAS
T_1	1.45 e (1.40)	1.89 e (1.55)
T_2	1.26 d (1.33)	1.68 d (1.48)
T_3	**1.85 f (1.53)**	**2.21 f (1.65)**
T_4	0.86 b (1.17)	1.19 b (1.30)
T_5	1.01 c (1.23)	1.38 c (1.37)
T_6	0 a (0.71)	0 a (0.71)
T_7	0 a (0.71)	0 a (0.71)

* The values in the parenthesis are the square root transformed value

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution + mycorrhiza

T_4 = 100 ppm arsenic solution

T_5 = 100 ppm arsenic solution + mycorrhiza

T_6 = 500 ppm arsenic solution

T_7 = 500 ppm arsenic solution + mycorrhiza

B. Wheat

➢ Seed germination:

Seedling emergence of wheat influenced by AMF inoculation in soil amended with different concentrations of arsenic solution is presented in Figure 18. Seedling emergence of wheat differed significantly due to the different concentrations of arsenic solution and inoculation of mycorrhizal fungi at 3, 5 and 7 days after sowing. Among the seven different treatments, treatment T_3 (10 ppm arsenic + mycorrhiza) gave the highest result and treatment T_6 (500 ppm arsenic solution) gave the lowest result at 30, 45 and 60 days after sowing. In all the three recorded periods it was observed that the seedling emergence of wheat decreased with the increase of arsenic concentration and seedling emergence of wheat was higher in mycorrhiza inoculated poly bags than non-inoculated.

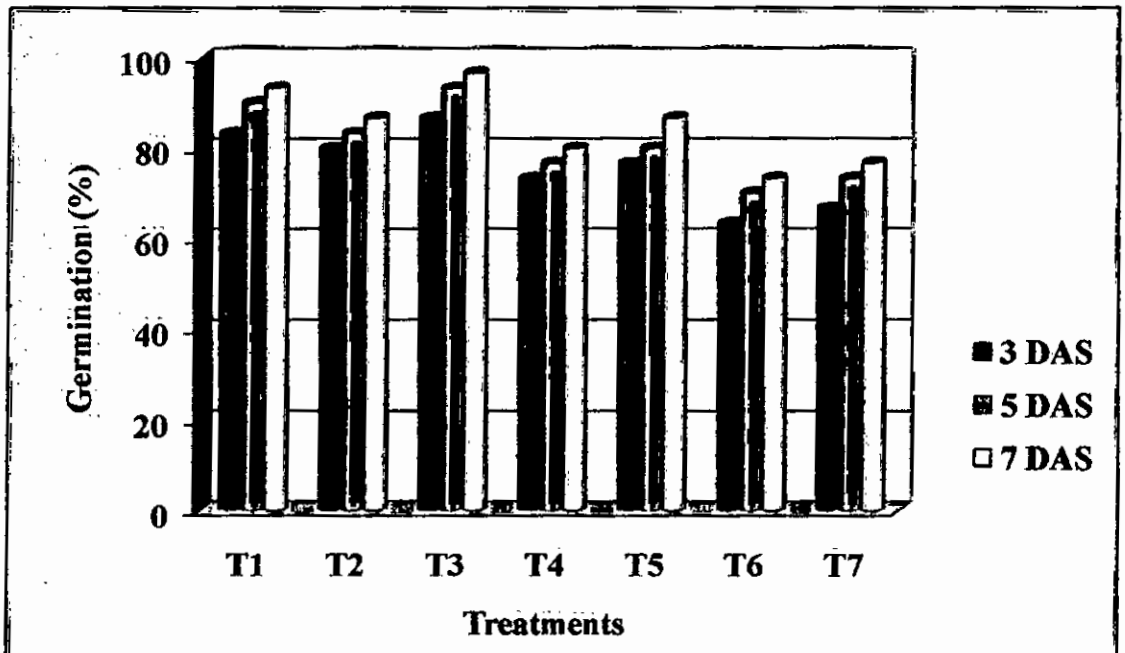


Fig 18. Influence of AMF on seedling emergence of wheat at different growth periods in soil amended with different concentrations of arsenic solution

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 =10 ppm arsenic solution + mycorrhiza

T_4 =100 ppm arsenic solution

T_5 = 100 ppm arsenic solution + mycorrhiza

T_6 =500 ppm arsenic solution

T_7 =500 ppm arsenic solution+ mycorrhiza

➢ Shoot height:

The influence of AMF inoculation on shoot height of wheat, Seeds sown in soil amended with different concentrations of arsenic are shown in Table 13.The shoot height of wheat was ranged from 15.12-23.35cm and 17.80-28.09cm at 20 DAS and 30 DAS, respectively. The highest shoot height of wheat was recorded in case of treatment T_3 (10 ppm arsenic + mycorrhiza) and those were 23.35cm and 28.09cm at 20 DAS and 30 DAS, respectively. The lowest result was recorded in case of treatment T_6 (500 ppm arsenic solution) and those were 15.12cm and 17.80cm at 20 DAS and 30 DAS, respectively. Among the rest of the treatments the shoot height of wheat decreased with the increase of arsenic concentration The shoot height of wheat was 19.69cm in treatment T_2 (10 ppm arsenic solution) but it was increased to 23.35cm in treatment T_3 (10 ppm + mycorrhiza) when mycorrhiza inoculated with 10 ppm arsenic solution.

Table 13. Influence of AMF inoculation on shoot height of wheat at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Shoot height (cm)	
	20 DAS	30 DAS
T_1	21.34 e	25.32 e
T_2	19.69 d	22.03 d
T_3	**23.35 f**	**28.09 f**
T_4	17.73 c	20.00 b
T_5	19.67 d	21.23 c
T_6	15.02 a	17.80 a
T_7	16.34 b	19.34 b

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution + mycorrhiza

T_4 = 100 ppm arsenic solution

T_5 = 100 ppm arsenic solution + mycorrhiza

T_6 = 500 ppm arsenic solution

T_7 = 500 ppm arsenic solution+ mycorrhiza

Table 14. Influence of AMF inoculation on root length of wheat at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Root length (cm)	
	20 DAS	30 DAS
T_1	5.13 f (2.35)	6.12 f (2.57)
T_2	4.22 e (2.17)	5.10 e (2.37)
T_3	**6.12 g (2.57)**	**7.33 g (2.80)**
T_4	2.45 c (1.71)	3.41 c (1.98)
T_5	3.19 d (1.92)	4.23d (2.17)
T_6	1.01 a (1.22)	1.46 a (1.40)
T_7	1.69 b (1.47)	2.46 b (1.70)

* The values in the parenthesis are the square root transformed value

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution + mycorrhiza

T_4 = 100 ppm arsenic solution

T_5 = 100 ppm arsenic solution + mycorrhiza

T_6 = 500 ppm arsenic solution

T_7 = 500 ppm arsenic solution+ mycorrhiza

➢ Fresh weight of shoot:

Seven different treatments were taken to evaluate the role of AMF inoculation on fresh weight of shoot of wheat, which was presented in Table 15. The performances of the most of the treatment differed significantly from each other. Results revealed that treatment T_3 (10 ppm arsenic solution + mycorrhiza) gave the highest fresh weight of shoot of wheat (6.23gm and 7.43gm) at 20 DAS and 30 DAS, respectively which is significantly better in comparison to other treatments. Control T_1 showed the second highest fresh weight of shoot of wheat. The lowest fresh weight of shoot of wheat was recorded from the treatment T_6. The fresh weight of shoot of wheat decreased when the rate of arsenic concentration increased and the fresh weight of shoot of wheat increased when we mycorrhiza inoculated on arsenic treated soil.

Table 15. Influence of AMF inoculation on fresh weight of shoot of wheat at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Fresh weight of shoot (gm)	
	20 DAS	30 DAS
T_1	5.12 e (2.37)	5.89 d (2.53)
T_2	4.89 d (2.32)	5.85 c (2.52)
T_3	**6.23 f** (2.59)	**7.43 e** (2.82)
T_4	3.01 c (1.87)	3.79 b (2.07)
T_5	3.89 d (2.10)	4.89 d (2.32)
T_6	1.29 b (1.34)	2.01 b (1.58)
T_7	2.34 a (1.71)	2.80a (1.82)

* The values in the parenthesis are the square root transformed value

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution + mycorrhiza

T_4 = 100 ppm arsenic solution

T_5 = 100 ppm arsenic solution + mycorrhiza

T_6 = 500 ppm arsenic solution

T_7 = 500 ppm arsenic solution + mycorrhiza

➢ **Fresh weight of root:**

Fresh weight of root of wheat influenced by AMF inoculation in soil amended with different concentrations of arsenic solution presented in Table 16. The fresh weight of root of wheat was varied significantly at different concentrations but with the increase of days the fresh weight of root was increased. The highest fresh weight of root of wheat was found in treatment T_3 (10 ppm arsenic solution + mycorrhiza). Among the treatments T_2, T_4 and T_6, the highest fresh weight of root of wheat was recorded in treatment T_2 (10 ppm arsenic solution) and the lowest fresh weight of root of wheat was found from T_6 (500 ppm arsenic solution). It was found that the fresh weight of root of wheat decreased with the increase of arsenic concentration. The fresh weight of root of wheat increased when inoculated AMF on those treatments,.

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

Treatments	Fresh weight of root (gm)	
	20 DAS	30 DAS
T_1	1.21 f (1.31)	1.54 f (1.43)
T_2	1.08 e (1.26)	1.29 e (1.34)
T_3	**1.42 g (1.39)**	**1.74 g (1.50)**
T_4	0.85 c (1.16)	1.02 c (1.23)
T_5	0.92 d (1.19)	1.11 d (1.27)
T_6	0.51 a (1.00)	0.85 a (1.16)
T_7	0.67 b (1.08)	0.92 b (1.19)

* The values in the parenthesis are the square root transformed value

T_1 = Control

T_2 =10 ppm arsenic solution

T_3 =10 ppm arsenic solution + mycorrhiza

T_4 =100 ppm arsenic solution

T_5 =100 ppm arsenic solution + mycorrhiza

T_6 = 500 ppm arsenic solution

T_7 = 500 ppm arsenic solution + mycorrhiza

C. Spinach

➢ Seed germination:

The influence of AMF inoculation on seedling emergence of spinach, sown in soil, amended with different concentrations of arsenic is shown in Figure 19. Germination percentage of spinach differed significantly due to the effect of different concentrations of arsenic solution and mycorrizal inoculation. Result revealed that treatment T_3 (10 ppm arsenic solution + mycorrhiza) gave the highest percentage of seedling emergence of spinach and those were 86.66%, 90.00% and 96.66% at 3 DAS, 5 DAS and 7 DAS, respectively which was significantly better in comparison to other treatments. The lowest percentage of seedling emergence of spinach was recorded from 500 ppm arsenic treated treatments and percent seedling emergence of spinach decreased when the rate of arsenic concentration increased. The percent seedling emergence of spinach increased when inoculated mycorrhiza with those treatments.

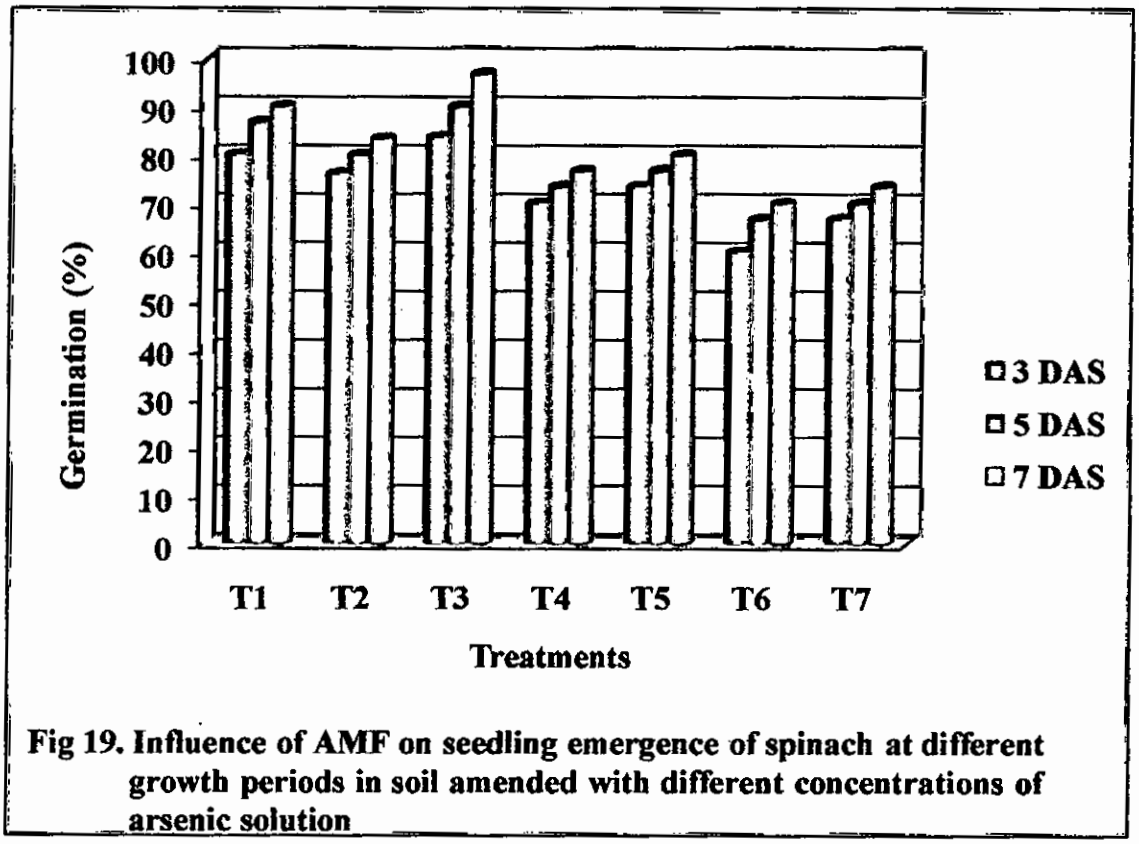


Fig 19. Influence of AMF on seedling emergence of spinach at different growth periods in soil amended with different concentrations of arsenic solution

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution + mycorrhiza

T_4 = 100 ppm arsenic solution

T_5 = 100 ppm arsenic solution + mycorrhiza

T_6 = 500 ppm arsenic solution

T_7 = 500 ppm arsenic solution+ mycorrhiza

➢ Shoot height:

Shoot height of spinach influenced by AMF inoculation in soil amended with different concentrations of arsenic solution is presented in Table 17. There was a remarkable variation of shoot height of spinach among the seven treatments. Shoot height of spinach ranged from 7.54cm-15.10cm and 8.48cm -17.45cm at 20DAS and 30DAS, respectively. In comparison to all the treatments mycorrhizal treatment T_3 (10 ppm arsenic solution + mycorrhiza) gave the highest shoot height and those were 15.10cm and 17.45cm at 20DAS and 30DAS, respectively and the lowest shoot height of spinach was recorded in treatment T_6 (500 ppm arsenic solution) and those were 7.12cm and 8.82cm at 20DAS and 30DAS, respectively. The shoot height of spinach was the lowest in only arsenic treated poly bags but when mycorrhiza added with those treatments, the shoot height of spinach increased. Among the treatment T_2, T_4 and T_6, treatment T_6 gave the lower shoot height due to arsenic concentration.

Table 17. Influence of AMF inoculation on shoot height of spinach at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Shoot height (cm)	
	20 DAS	30 DAS
T_1	13.34 f (3.72)	15.30 f (3.97)
T_2	11.97 e (3.53)	12.99 e (3.67)
T_3	**15.10 g (3.94)**	**17.45 g (4.23)**
T_4	9.87 c (3.32)	10.89 c (3.37)
T_5	10.88 d (3.37)	11.34 d (3.44)
T_6	7.54 a (2.83)	8.48 a (2.99)
T_7	8.23 b (2.95)	9.99 b (3.23)

* The values in the parenthesis are the square root transformed value

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution + mycorrhiza

T_4 = 100 ppm arsenic solution

T_5 = 100 ppm arsenic solution + mycorrhiza

T_6 = 500 ppm arsenic solution

T_7 = 500 ppm arsenic solution+ mycorrhiza

➢ Root length:

The influence of AMF inoculation on root length of spinach, sown in soil, amended with different concentrations of arsenic is shown in Table 18.The root length of spinach was recorded after 20 and 30 days of sowing. The root length of spinach was varied at different concentrations. The highest root length of spinach was recorded in treatment T_3 (10 ppm arsenic solution + mycorrhiza) and the lowest root length of spinach was recorded in treatment T_6 (500 ppm arsenic solution) in all the three recorded time. In comparison to all the treatments better result was obtained where mycorrhiza was inoculated than non-inoculated. Among the mycorrhizal treatments T_3 (10 ppm arsenic solution + mycorrhiza), T_5 (100 ppm arsenic solution+ mycorrhiza) and T_7 (500 ppm arsenic solution+ mycorrhiza); T_3 gave the highest root length of spinach and those were 4.89cm and 6.76cm at 20 DAS and 30 DAS respectively. So the increase of arsenic concentration the root length of spinach was decreasing.

Table 18. Influence of AMF inoculation on root length of spinach at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Root length (cm)	
	20 DAS	30 DAS
T_1	3.97f (2.11)	5.39 f (2.42)
T_2	3.00 e (1.87)	4.45 e (2.22)
T_3	**4.89 g (2.32)**	**6.76 g (2.69)**
T_4	2.03 d (1.59)	3.05 d (1.88)
T_5	2.88 c 1.83)	3.97 c (2.11)
T_6	1.65 b (1.46)	2.45 b (1.71)
T_7	0.96 a (1.20)	1.52 a (1.42)

* The values in the parenthesis are the square root transformed value

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution + mycorrhiza

T_4 = 100 ppm arsenic solution

T_5 = 100 ppm arsenic solution + mycorrhiza

T_6 = 500 ppm arsenic solution

T_7 = 500 ppm arsenic solution+ mycorrhiza

➢ **Fresh weight of shoot:**

Effect of seven different treatments on fresh weight of shoot of spinach sown in soil amended with different concentrations of arsenic solution is presented in Table 19. The fresh weight of shoot of spinach was varied significantly at different concentrations that ranged from 1.06gm to 6.87gm, 2.00gm to 7.76gm at 20 DAS and 30 DAS respectively where treatment T_3 (10 ppm arsenic solution + mycorrhiza) gave the highest weight and those were 6.87gm and 7.76gm at 20 DAS and 30 DAS, respectively. In comparison to all the treatments, mycorrhizal treatments showed the best result. The lowest fresh weight of shoot of spinach was found from treatment T_6 (500 ppm arsenic solution) and those were 1.06gm and 2.00gm at 20 DAS and 30 DAS, respectively. In treatment, T_2, T_4 and T_6 it was found that the fresh weight of shoot of spinach decreased with the increase of arsenic concentration.

Table 19. Influence of AMF inoculation on fresh weight of shoot of spinach at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Fresh weight of shoot (gm)	
	20 DAS	30 DAS
T_1	5.79 f (2.51)	6.66 f (2.68)
T_2	4.89 d (2.32)	5.61 e (2.47)
T_3	**6.87 g** (2.71)	**7.76 g** (2.87)
T_4	3.00 c (1.87)	3.70 c (2.05)
T_5	3.99 e (2.12)	4.60 d (2.26)
T_6	1.06 a (1.25)	2.00 a (1.58)
T_7	2.11 b (1.62)	3.01 b (1.87)

* The values in the parenthesis are the square root transformed value

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution + mycorrhiza

T_4 = 100 ppm arsenic solution

T_5 = 100 ppm arsenic solution + mycorrhiza

T_6 = 500 ppm arsenic solution

T_7 = 500 ppm arsenic solution + mycorrhiza

➢ Fresh weight of root:

Fresh weight of root of spinach influenced by AMF inoculation in soil amended with different concentrations of arsenic solution presented in Table 20. The fresh weight of root of spinach was varied significantly at different concentrations but with the increase of days, the fresh weight of spinach was increased. The fresh weight of root of spinach was recorded after 20 and 30 days of sowing. Higher fresh weight of root of spinach was recorded in mycorrhizal treatment in two recorded time. Among the arsenic treatment T_2, T_4 and T_6, the highest fresh weight of root of spinach was recorded in treatment T_2 (10 ppm arsenic solution) while the lowest was found in T_6 (500 ppm arsenic solution). It was revealed that the fresh weight of root of spinach decreased with the increase of arsenic concentration. In another cases, the fresh weight of root of spinach increased when inoculated AMF on those treatments.

Table 20. Influence of AMF inoculation on fresh weight of root of spinach at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Fresh weight of root (gm)	
	20 DAS	30 DAS
T_1	1.42 e (2.39)	1.88 d (1.54)
T_2	1.01 d (1.23)	1.67 cd (1.47)
T_3	**1.95 f (1.57)**	**2.35 e (1.69)**
T_4	0.77 c (1.13)	0.99 ab (1.22)
T_5	0.99 d (1.22)	1.38 bc (1.37)
T_6	0.38 a (0.94)	0.77 a (1.13)
T_7	0.67 b (1.08)	0.99 ab (1.22)

* The values in the parenthesis are the square root transformed value

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution + mycorrhiza

T_4 = 100 ppm arsenic solution

T_5 = 100 ppm arsenic solution + mycorrhiza

T_6 = 500 ppm arsenic solution

T_7 = 500 ppm arsenic solution + mycorrhiza

4.3.2. Poly bag experiment

To know the role of AMF inoculation on plant growth in soil amended with different concentrations of arsenic solution two experiments were performed. First experiment was performed in plastics tray. The soil holding capacity of tray was too little that the experiment was not continuing for many days. For that a little amount of biomass was found. Therefore, it is necessity to pull out the experiment in the poly bags.

A. Red amaranthus:

- **Seedling emergence:**

The influence of AMF inoculation on seedling emergence of red amaranthus seeds sown in soil amended with different concentrations of arsenic is shown in Figure 20. The seed germination was recorded after 3, 5 and 7 of sowing. The seedling emergence varied at different concentrations and with increase of incubation period the emergence was increased. Higher seed germination was recorded in control treatment in all the three recorded time. In treatment T_8, T_9 and T_{10} there was no germination of red amaranthus seeds in all the three recorded periods. Among the treatment T_2, T_3 and T_4, higher germination was recorded in treatment T_4. Among the treatment T_5, T_6 and T_7, higher germination was recorded in treatment T_7, where mycorrhiza was added with 100 ppm arsenic solution. No germination was noticed in case of 500 ppm arsenic solution but in 10 ppm and 100 ppm above 65% germination was recorded. In comparison to 10 ppm arsenic + mycorrhiza gave higher germination than 100 ppm arsenic + mycorrhiza treatment. The mycorrhiza added with 10 ppm and 100 ppm arsenic solution did not differ so much.

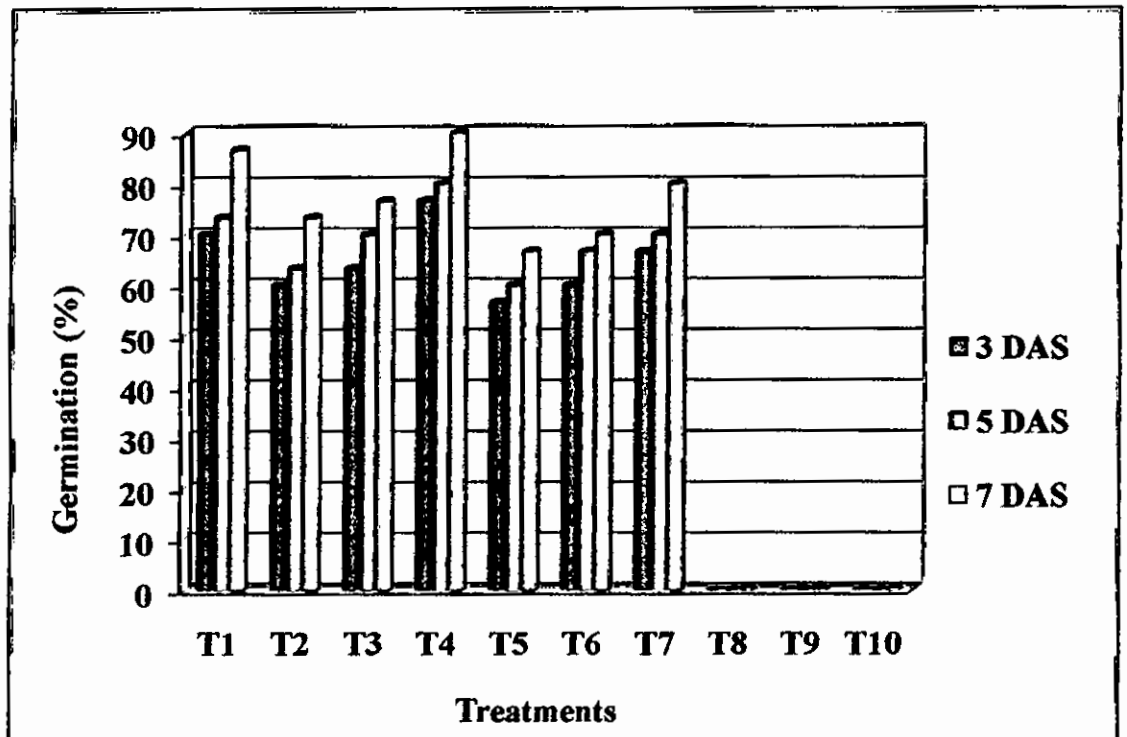


Fig 20. Influence of AMF inoculation on seedling emergence of red amaranthus at different growth periods in soil amended with different concentrations of arsenic solution

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution + sterilized mycorrhiza

T_4 = 10 ppm arsenic solution + mycorrhiza

T_5 = 100 ppm arsenic solution

T_6 = 100 ppm arsenic solution+ sterilized mycorrhiza

T_7 = 100 ppm arsenic solution+ mycorrhiza

T_8 = 500 ppm arsenic solution

T_9 = 500 ppm arsenic solution+ sterilized mycorrhiza

T_{10} = 500 ppm arsenic solution+ mycorrhiza

➢ **Number of leaves:**

The influence of AMF inoculation on number of leaves of red amaranthus at different growth periods in soil amended with different concentrations of arsenic solution is presented in Table 21. The number of leaves of red amaranthus was recorded after 15, 30 and 45 days of sowing. It was revealed that the performances of most of the treatments differed significantly from each other. The highest number of leaves of red amaranthus was recorded in treatment T_4 and the lowest number of leaves of red amaranthus was recorded in treatment T_5 at 15 DAS. Similar results were also obtained at 30 DAS and 45 DAS. It was observed that the number of leaves per plant of mycorrhizal treatment was more than the other treatment. Among the treatment T_2 and T_5 it was revealed that with the increase of arsenic concentration the number of leaves of red amaranthus was decreasing.

Table 21. Influence of AMF inoculation on number of leaves of red amaranthus at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Number of leaves		
	15 DAS	30 DAS	45 DAS
T_1	5.01 d (2.24)	6.04 d (2.55)	6.43 d (2.63)
T_2	4.09 c (2.03)	5.03 c (2.35)	4.88 b (2.31)
T_3	4.32 c (2.19)	5.24 c (2.39)	5.23 c (2.39)
T_4	**6.12 e (2.57)**	**7.00 e (2.73)**	**7.21 e (2.77)**
T_5	3.09 b (1.89)	4.02 b (2.12)	4.45 b (2.22)
T_6	3.85 b (2.08)	4.31 b (2.19)	4.82 b (2.30)
T_7	4.76 c (2.29)	5.05 c (2.35)	5.80 c (2.50)
T_8	0 a (0.71)	0 a (0.71)	0 a (0.71)
T_9	0 a (0.71)	0 a (0.71)	0 a (0.71)
T_{10}	0 a (0.71)	0 a (0.71)	0 a (0.71)

* The values in the parenthesis are the square root transformed value

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution + sterilized mycorrhiza

T_4 = 10 ppm arsenic solution + mycorrhiza

T_5 = 100 ppm arsenic solution

T_6 = 100 ppm arsenic solution+ sterilized mycorrhiza

T_7 = 100 ppm arsenic solution+ mycorrhiza

T_8 = 500 ppm arsenic solution

T_9 = 500 ppm arsenic solution+ sterilized mycorrhiza

T_{10} = 500 ppm arsenic solution+ mycorrhiza

➢ Shoot height:

The influence of AMF inoculation on shoot height of red amaranthus is presented in Table 22. The shoot height was varied significantly at different concentrations. The shoot height was recorded at 30, 45 and 60 days after sowing. The highest shoot height was recorded in T_4 treatment in all the three recorded time. Among the treatment T_4, T_7 and T_{10}, higher seed germination was recorded in treatment T_4. Among the treatment T_2, T_5 and T_8, shoot height was the highest in treatment T_2 (10 ppm arsenic solution). In all the three recorded periods it was observed that the shoot height of red amaranthus decreased with the increase of arsenic concentration and shoot height was higher in mycorrhiza inoculated poly bags than non-inoculated. It was observed from the Plate 1 (T_2) and Plate 2 (T_4); Plate 3 (T_5) and Plate 4 (T_7) that mycorrhizal inoculation helped to increase plant growth in arsenic amended soil.

Table 22. Influence of AMF inoculation on shoot height of red amaranthus at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Shoot height (cm)		
	30 DAS	45 DAS	60 DAS
T_1	21.98 g (4.74)	23.79 g (4.93)	25.67 f (5.12)
T_2	19.86 d (4.51)	21.03 d (4.64)	22.79 c (4.83)
T_3	20.01 e (4.53)	21.85 e (4.73)	22.99 d (4.85)
T_4	**22.86 h** (4.83)	**24.79 h** (5.03)	**26.86 g** (5.23)
T_5	18.69 b (4.38)	19.05 b (4.42)	20.57 b (4.59)
T_6	18.85 c (4.40)	19.67 c (4.49)	20.64 b (4.60)
T_7	20.67 f (4.60)	22.13 f (4.76)	23.95 e (4.94)
T_8	0 a (0.71)	0 a (0.71)	0 a (0.71)
T_9	0 a (0.71)	0 a (0.71)	0 a (0.71)
T_{10}	0 a (0.71)	0(a 0.71)	0 a (0.71)

* The values in the parenthesis are the square root transformed value

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution + sterilized mycorrhiza

T_4 = 10 ppm arsenic solution + mycorrhiza

T_5 = 100 ppm arsenic solution

T_6 = 100 ppm arsenic solution+ sterilized mycorrhiza

T_7 = 100 ppm arsenic solution+ mycorrhiza

T_8 = 500 ppm arsenic solution

T_9 = 500 ppm arsenic solution+ sterilized mycorrhiza

T_{10} = 500 ppm arsenic solution+ mycorrhiza

Plate 1. Effect of 10 ppm arsenic solution on growth of red amaranthus at 60 DAS

Plate 2. Influence of AMF on growth of red amaranthus in 10 ppm arsenic amended soil at 60 DAS

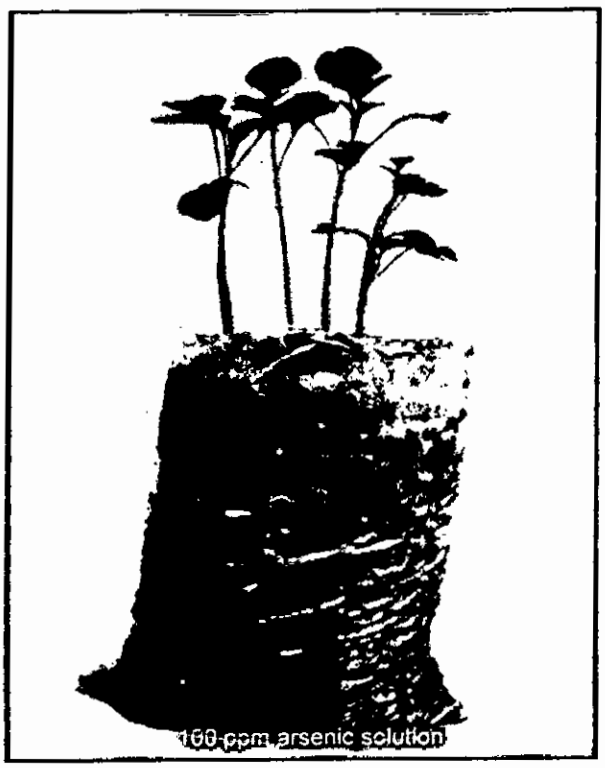


Plate 3. Effect of 100 ppm arsenic solution on growth of red amaranthus at 60 DAS

Plate 4. Influence of AMF on growth of red amaranthus in 100 ppm arsenic amended soil at 60 DAS

➢ Root length:

Root length of red amaranthus ranged from 18.99cm-22.45cm, 19.97cm-25.07cm and 20.34cm-26.24cm at 30 DAS, 45DAS and 60DAS, respectively. In comparison to all the treatments mycorrhizal treatment T_4 (10 ppm arsenic solution + mycorrhiza) gave the highest result and those were 22.45cm, 25.07cm and 26.24cm at 30 DAS, 45DAS and 60DAS, respectively and the lowest root length of red amaranthus was recorded in case of treatment T_8 (500 ppm arsenic solution) and those were 18.99cm, 19.97cmand 20.34cm at 30 DAS, 45DAS and 60DAS, respectively. It was revealed that the root length of red amaranthus was the lowest in only arsenic treated poly bags but when added mycorrhiza with those treatments, the root length of red amaranthus increased. Among the treatment T_2, T_5 and T_8 treatment T_8 gave the lowest result. It was clearly exposed that with the increase of arsenic concentration the root length of red amaranthus was decreasing.

Table 23. Influence of AMF inoculation on root length of red amaranthus at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Root length (cm)		
	30 DAS	45 DAS	60 DAS
T_1	21.80 f (4.72)	23.98 e (4.95)	24.89 f (5.04)
T_2	20.30 d (4.56)	21.87 c (4.73)	22.96 d (4.84)
T_3	20.70 e (4.60)	21.99 c (4.74)	23.00 d (4.85)
T_4	**22.45 g (4.79)**	**25.03 f (5.05)**	**26.24 g (5.17)**
T_5	18.99 b (4.41)	19.97 b (4.52)	20.34 b (4.57)
T_6	19.29 c (4.45)	20.03 b (4.53)	20.87 c (4.62)
T_7	20.98 e (4.63)	22.17 d (4.76)	23.95 e (4.94)
T_8	0 a (0.71)	0 a (0.71)	0 a (0.71)
T_9	0 a (0.71)	0 a (0.71)	0 a (0.71)
T_{10}	0 a (0.71)	0 a (0.71)	0 a (0.71)

* The values in the parenthesis are the square root transformed value

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution + sterilized mycorrhiza

T_4 = 10 ppm arsenic solution + mycorrhiza

T_5 = 100 ppm arsenic solution

T_6 = 100 ppm arsenic solution+ sterilized mycorrhiza

T_7 = 100 ppm arsenic solution+ mycorrhiza

T_8 = 500 ppm arsenic solution

T_9 = 500 ppm arsenic solution+ sterilized mycorrhiza

T_{10} = 500 ppm arsenic solution+ mycorrhiza

➢ **Fresh weight of shoot:**

The influence of AMF inoculation on fresh weight of shoot of red amaranthus at different growth periods in soil amended with different concentrations of arsenic solution is presented in Table 24. Fresh weight of shoot of red amaranthus ranged from 23.90-30.50, 27.00-34.60 and 29.36-36.00 at 30 DAS, 45 DAS and 60 DAS, respectively. The highest fresh weight of shoot of red amaranthus was recorded in treatment T_4 followed by treatment T_1, T_3 and the lowest fresh weight of shoot of red amaranthus was recorded in treatment T_5 at 30 DAS. Similar results were also obtained at 45 DAS and 60 DAS. Incase of treatment T_2 and T_5 it was clearly showed that with the increase of arsenic concentration the fresh weight of shoot of red amaranthus decreased. Incase of only arsenic treatment T_2 (10 ppm arsenic solution) the fresh weight of shoot of red amaranthus was 26.00 gm but it increased to 30.50 gm when we inoculated mycorrhiza with that 10 ppm arsenic solution.

Table 24. Influence of AMF inoculation on fresh weight of shoot of red amaranthus at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Fresh weight shoot (gm)		
	30 DAS	45 DAS	60 DAS
T_1	29.80 f (5.50)	33.80 e (5.85)	35.90 e (6.03)
T_2	26.00 c (5.14)	31.90 c (5.69)	33.20 c (5.80)
T_3	28.90 e (5.42)	32.80 d (5.77)	34.20 d (5.89)
T_4	**30.50 g (5.52)**	**34.60 f (5.92)**	**36.97 f (6.12)**
T_5	23.90 b (5.51)	27.00 a (5.24)	29.36 a (5.46)
T_6	26.10 c (5.15)	29.00 b (5.43)	30.10 b (5.53)
T_7	28.20 d (5.35)	31.90 c (5.69)	32.90 c (5.77)
T_8	0 a (0.71)	0 a (0.71)	0 a (0.71)
T_9	0 a (0.71)	0 a (0.71)	0 a (0.71)
T_{10}	0 a (0.71)	0 a (0.71)	0 a (0.71)

* The values in the parenthesis are the square root transformed value

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution + sterilized mycorrhiza

T_4 = 10 ppm arsenic solution + mycorrhiza

T_5 = 100 ppm arsenic solution

T_6 = 100 ppm arsenic solution+ sterilized mycorrhiza

T_7 = 100 ppm arsenic solution+ mycorrhiza

T_8 = 500 ppm arsenic solution

T_9 = 500 ppm arsenic solution+ sterilized mycorrhiza

T_{10} = 500 ppm arsenic solution+ mycorrhiza

➢ Fresh weight of root:

The role of AMF inoculation on fresh weight of root of red amaranthus, which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 25. There was a remarkable variation of fresh weight of root of red amaranthus among the 10 different treatments. The variation of fresh weight of root of red amaranthus was recorded due to the effect of mycorrhiza inoculation against arsenic solution. Result revealed that treatment T_4 (10 ppm arsenic solution + mycorrhiza) gave the highest results and those were 6.10gm, 7.21gm and 8.51gm at 30 DAS, 45 DAS and 60 DAS, respectively which was significantly better in comparison to each of the other treatments. Treatment T_1 (Control) showed the second highest fresh weight of root of red amaranthus. The lowest fresh weight of root of red amaranthus was recorded from the only arsenic treated treatments (T_2, T_5 and T_8) and Fresh weight of root of red amaranthus decreased when the rate of arsenic concentration increased.

Table 25. Influence of AMF inoculation on fresh weight of root of red amaranthus at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Fresh weight of root (gm)		
	30 DAS	45 DAS	60 DAS
T_1	5.43 f (2.43)	6.30 f (2.60)	7.45 e(2.81)
T_2	3.31 c (2.00)	4.63 c (2.26)	5.00 c(2.34)
T_3	3.87 d (2.09)	5.03 d (2.34)	5.29 c(2.40)
T_4	**6.10 g (2.56)**	**7.21 g (2.77)**	**8.51 f(3.00)**
T_5	2.90 b (1.84)	3.90 b (2.09)	4.25 b(2.17)
T_6	3.19 bc (1.89)	4.23 c (2.17)	4.46 b (2.21)
T_7	4.80 e (2.30)	5.27 e (2.40)	6.12 d (2.57)
T_8	0 a (0.71)	0 a (0.71)	0 a (0.71)
T_9	0 a (0.71)	0 a (0.71)	0 a (0.71)
T_{10}	0 a (0.71)	0 a (0.71)	0 a (0.71)

* The values in the parenthesis are the square root transformed value

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution + sterilized mycorrhiza

T_4 = 10 ppm arsenic solution + mycorrhiza

T_5 = 100 ppm arsenic solution

T_6 = 100 ppm arsenic solution+ sterilized mycorrhiza

T_7 = 100 ppm arsenic solution+ mycorrhiza

T_8 = 500 ppm arsenic solution

T_9 = 500 ppm arsenic solution+ sterilized mycorrhiza

T_{10} = 500 ppm arsenic solution+ mycorrhiza

➢ Vigor percent:

The role of AMF inoculation on vigor percentage of red amaranthus, is presented in Table 26. There was a remarkable variation of vigor percentage of red amaranthus among the 10 different treatments. Result revealed that treatment T_4 (10 ppm Arsenic solution + mycorrhiza) gave the highest percent vigor of red amaranthus and those were 31.71%, 35.87% and 45.13% at 30 DAS, 45 DAS and 60 DAS, respectively which was significantly better in comparison to each of the other treatments. Treatment T_1 (Control) showed the second highest percent vigor. The lowest vigor percentage of red amaranthus was recorded from the 500 ppm arsenic treated treatments (T_8, T_9 and T_{10}) and percent vigor of red amaranthus decreased when the rate of arsenic concentration increased.

Table 26. Influence of AMF inoculation on percent vigor of red amaranthus at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Vigor (%)		
	30 DAS	45 DAS	60 DAS
T_1	30.85 g(5.59)	34.21 g(5.89)	41.50 g(2.23)
T_2	24.89 d(5.03)	27.80 d(5.31)	32.72 e(5.76)
T_3	25.64 e(5.11)	28.37 e(5.37)	33.11 f(5.80)
T_4	**32.71 h(5.76)**	**38.87 h(6.27)**	**45.13 h(6.5)**
T_5	22.60 b(4.80)	24.19 b(4.96)	26.59 b(5.20)
T_6	23.64 c(4.91)	25.80 c(5.12)	27.81 c(5.32)
T_7	27.07 f(5.25)	31.01 f(5.61)	31.32d(5.61)
T_8	0(0.71) a	0(0.71) a	0(0.71) a
T_9	0(0.71) a	0(0.71) a	0(0.71) a
T_{10}	0(0.71) a	0(0.71) a	0(0.71) a

* The values in the parenthesis are the square root transformed value

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution + sterilized mycorrhiza

T_4 = 10 ppm arsenic solution + mycorrhiza

T_5 = 100 ppm arsenic solution

T_6 = 100 ppm arsenic solution+ sterilized mycorrhiza

T_7 = 100 ppm arsenic solution+ mycorrhiza

T_8 = 500 ppm arsenic solution

T_9 = 500 ppm arsenic solution+ sterilized mycorrhiza

T_{10} = 500 ppm arsenic solution+ mycorrhiza

➢ **Dry weight of shoot:**

The role of AMF inoculation on dry weight of shoot of red amaranthus, which was sown in soil, amended with different concentrations of arsenic solution is presented in Table 27. There was a remarkable variation of dry weight of shoot among the 10 different treatments. The variation of dry weight of shoot of red amaranthus was recorded due to the effect of mycorrhiza inoculation against arsenic solution. Result revealed that treatment T_4 (10 PPM arsenic solution + mycorrhiza) gave the highest 2.01gm 2.89gm 3.00gm dry weight of shoot at 30 DAS, 45 DAS and 60 DAS, respectively which was significantly better in comparison to each of the other treatments. Treatment T_1 (Control) showed the second highest dry weight of shoot of red amaranthus. The dry weight of shoot of red amaranthus decreased when the rate of arsenic concentration increased. The variation of dry weight of shoot of red amaranthus was recorded due to the effect of mycorrhiza inoculation against arsenic solution.

Table 27. Influence of AMF inoculation on dry weight of shoot of red amaranthus at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Dry weight shoot(gm)		
	30 DAS	45 DAS	60 DAS
T_1	2.05 g (1.60)	2.75 e (1.80)	2.95 f (1.86)
T_2	0.89 b (1.18)	1.02 c (1.23)	1.02 bc (1.23)
T_3	1.25 e (1.32)	1.81 d (1.52)	1.95 e (1.57)
T_4	**2.01 f (1.58)**	**2.89 f (1.84)**	**3.00 g (1.87)**
T_5	0.46 b (0.98)	0.92 b (1.19)	0.99 b (1.22)
T_6	0.94 c (1.20)	0.99 c (1.22)	1.03 c (1.24)
T_7	1.03 d (1.24)	1.01 c (1.23)	1.41 d (1.38)
T_8	0 a (0.71)	0 a (0.71)	0 a (0.71)
T_9	0 a (0.71)	0 a (0.71)	0 a (0.71)
T_{10}	0 a (0.71)	0 a (0.71)	0 a (0.71)

* The values in the parenthesis are the square root transformed value

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution + sterilized mycorrhiza

T_4 = 10 ppm arsenic solution + mycorrhiza

T_5 = 100 ppm arsenic solution

T_6 = 100 ppm arsenic solution+ sterilized mycorrhiza

T_7 = 100 ppm arsenic solution+ mycorrhiza

T_8 = 500 ppm arsenic solution

T_9 = 500 ppm arsenic solution+ sterilized mycorrhiza

T_{10} = 500 ppm arsenic solution+ mycorrhiza

➢ Dry weight of root:

The role of AMF inoculation on dry weight of root of red amaranthus, which was sown in soil, amended with different concentrations of arsenic solution is shown in Table 28. There was a remarkable variation of dry weight of root among the 10 different treatments. Result revealed that treatment T_4 (10 ppm arsenic solution + mycorrhiza) gave the highest dry weight of root of red amaranthus and those were 0.94gm 1.26gm 1.57gm at 30 DAS, 45 DAS and 60 DAS, respectively which was significantly better in comparison to each of the other treatments. Treatment T_1 (Control) showed the second highest dry weight of root of red amaranthus. Dry weight of root of red amaranthus decreased when the rate of arsenic concentration increased. The variation of dry weight of root of red amaranthus was recorded due to the effect of mycorrhiza inoculation against arsenic solution.

Table 28. Influence of AMF inoculation on dry weight of root of red amaranthus at different growth periods in soil amended with different concentrations of arsenic solution

Treatments	Dry weight root(gm)		
	30 DAS	45 DAS	60 DAS
T_1	0.84 g (1.16)	1.02 g (1.23)	1.32 g (1.35)
T_2	0.68 e (1.04)	0.72 d (1.10)	0.97 d (1.21)
T_3	0.72 f (1.10)	0.98(f 1.22)	1.02 f (1.23)
T_4	**0.94 h (1.20)**	**1.26 h (1.33)**	**1.57 h (1.44)**
T_5	0.10 b (0.77)	0.52 b (1.01)	0.67 b (1.08)
T_6	0.29 c (0.89)	0.67 c (1.08)	0.75 c (1.12)
T_7	0.59(d 1.04)	0.89 e (1.18)	0.99 e (1.22)
T_8	0 a (0.71)	0 a (0.71)	0 a (0.71)
T_9	0 a (0.71)	0 a (0.71)	0 a (0.71)
T_{10}	0 a (0.71)	0 a (0.71)	0 a (0.71)

* The values in the parenthesis are the square root transformed value

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution + sterilized mycorrhiza

T_4 = 10 ppm arsenic solution + mycorrhiza

T_5 = 100 ppm arsenic solution

T_6 = 100 ppm arsenic solution+ sterilized mycorrhiza

T_7 = 100 ppm arsenic solution+ mycorrhiza

T_8 = 500 ppm arsenic solution

T_9 = 500 ppm arsenic solution+ sterilized mycorrhiza

T_{10} = 500 ppm arsenic solution+ mycorrhiza

➢ Nutrient uptake:

The inoculation of arbuscular mycorrhizal fungi in response to nutrient uptake (N, P, K and S) by red amaranthus shoots at 60 DAS is represented in Table 29. It is revealed from the study that mycorrhizal fungi had a positive role in response to nutrient uptake and mycorrhizal fungi inoculated treatments significantly enhanced nutrient uptake by red amaranthus shoot in comparison to other treatment. The highest nutrient uptake was recorded in case of treatment T_4 (10 ppm arsenic solution + mycorrhiza) and those were 2.48 % total N, 0.78 % P, 2.89 % K and 0.49 % S and the lowest result was found in case of treatment T_2 (10 ppm arsenic solution) and those were 1.89 % total N, 0.61 % P, 2.37 % K and 0.42 % S and statistically similar result was found from the treatment T_3 (10 ppm arsenic solution + sterilized mycorrhiza). It was clearly showed from the table that due to toxicity of arsenic the percent nutrient uptake by shoots of red amaranthus was the lowest but when we inoculated mycorrhiza we got the highest percentage of nutrient uptake.

Table 29. Influence of AMF inoculation on nutrient uptake by shoots of red amaranthus at 60 DAS in soil amended with different concentrations of arsenic solution

Treatments	Nutrient uptake			
	Total N %	P %	K %	S %
T_1	2.37 e (1.69)	0.72 d (1.10)	2.82 g (1.82)	0.44 e (.97)
T_2	1.89 b (1.56)	0.61 c (1.05)	2.37 d (1.69)	0.42 d (.96)
T_3	1.89 b (1.55)	0.61 c (1.05)	2.58 e (1.75)	0.43 de (.95)
T_4	2.48 f (1.73)	0.78 e (1.13)	2.89 f (1.84)	0.49 f (.99)
T_5	1.96 b (1.57)	0.45 b (.97)	2.16 b (1.63)	0.30 b (.89)
T_6	2.10 c (1.61)	0.53 b (1.01)	2.20 b (1.64)	0.32 b (.90)
T_7	2.21 d (1.65)	0.61 c (1.05)	2.28 c (1.67)	0.35 c (.92)
T_8	0 a (0.71)	0 a (0.71)	0 a (0.71)	0 a (0.71)
T_9	0 a (0.71)	0 a (0.71)	0 a (0.71)	0 a (0.71)
T_{10}	0 a (0.71)	0 a (0.71)	0 a (0.71)	0 a (0.71)

* The values in the parenthesis are the square root transformed value

T_1 = Control

T_2 = 10 ppm arsenic solution

T_3 = 10 ppm arsenic solution + sterilized mycorrhiza

T_4 = 10 ppm arsenic solution + mycorrhiza

T_5 = 100 ppm arsenic solution

T_6 = 100 ppm arsenic solution+ sterilized mycorrhiza

T_7 = 100 ppm arsenic solution+ mycorrhiza

T_8 = 500 ppm arsenic solution

T_9 = 500 ppm arsenic solution+ sterilized mycorrhiza

T_{10} = 500 ppm arsenic solution+ mycorrhiza

➢ Arsenic uptake:

Influence of AMF inoculation in response to arsenic uptake by shoot of red amaramthus, sown in soil, amended with different concentrations of arsenic solution is presented in Table 30. The amount of arsenic uptake by shoots of red amaramthus 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 of arsenic concentrations but in all cases of AMF, inoculation the rate of arsenic uptake decreased. The lowest amount of arsenic was found in treatment T_3 (10 ppm arsenic solution + mycorrhiza) and that was 0.215 ppm . The highest amount of arsenic was found in treatment T_4 (100 ppm arsenic solution) and that was 0.763 ppm. Among the treatment T_1, T_2 and T_3, the amount of arsenic was higher in treatment T_1 and that was 0.430 ppm but inoculation of mycorrhiza on that treatment, the amount of arsenic decreased significantly to 0.215 ppm. Similar result was found from other treatments. Inoculation of mycorrhizal fungi helped to reduce arsenic uptake up to 50% than non-inoculated treatments when lower amount of arsenic concentration used, but at higher amount of arsenic concentration, it helps to reduce only 34.33%.

Table 30. Influence of AMF on arsenic uptake by shoots of red amaramthus at 60 DAS in soil amended with different concentrations of arsenic solution

Treatments	Arsenic uptake by red amaramthus shoots at 60 DAS (ppm)
T_1	0.430 c
T_2	0.302 b
T_3	**0.215 a**
T_4	0.763 f
T_5	0.695 e
T_6	0.501 d

T_1 = 10 ppm arsenic solution

T_2 = 10 ppm arsenic solution + sterilized mycorrhiza

T_3 = 10 ppm arsenic solution + mycorrhiza

T_4 = 100 ppm arsenic solution

T_5 = 100 ppm arsenic solution+ sterilized mycorrhiza

T_6 = 100 ppm arsenic solution+ mycorrhiza

➢ Root colonization:

Colonization was demonstrated by the presence of vesicles, arbuscules and/or hyphae in root tissue. The highest percent root colonization was 23.00% of 10 ppm + mycorrhiza inoculated plants were recorded at 60 DAS and the lowest was 19.36% of 100 ppm + mycorrhiza inoculated plants were recorded at 30DAS. On the other hand no root colonization was found in non-inoculated poly bags.

CHAPTER 5
DISCUSSIONS

CHAPTER 5
DISCUSSION

The occurrence of AM fungi association in the roots of different selected crops collected from different arsenic affected villages of Sonargaon area are reported here. Preliminary studies were done to know the occurrence of AM fungi association in the selected 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 and the extent of infection varied from species to species and within the same species from location to location. Among the eight villages, the highest percentage of infection was found in Raisdia village and that was 18.25% and the lowest was in Darpat village and that was 7.14%. Among the selected plant species, *Zea mays* roots collected from different locations showed the highest percentage of infection and intensity of infection of AM fungi. The highest percentage of infection was found in Fotekandi village and that was 51% in "field-a" and the lowest was 33% in kattukali village. *Zea mays* root collected from other locations like Noail, Balua dighir par and Boroikandi village, the percent root infection were 36% in "field-a" and 27% in "field-b", 36% in "field-a" and 29% in "field-b" and 33% in "field-a" and 41% in "field-b" respectively. The second highest percent root infection was found in *Ipomoea batatus*, collected from Humchadi village and that was 31%. Some of the selected plant species like

Ipomoea batatas, *Corcorus capsularis*, *Amaranthus gangeticus capsicum frutescens* etc collected from different field of same location showed a wide range of variation. The percent root infection in different plant species recorded in the present study is in agreement with others. 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 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 selected 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 selected crops root collected from different arsenic affected locations of Sonargaon 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 was depended on the indigenous VAM fungi , presence of host plant and different edaphic factors (e.g. soil texture, pH, conductivity, organic matter, phosphorus etc.).

Germination percentage was tested in three different experiments under different conditions like Petri plate, plastic tray and poly bag. In Petri plate experiment the seedling emergence was varied significantly at different concentrations. It was revealed from the study that the seedling emergence of the three selected crops decreased with the increase of arsenic concentrations.

In plastic tray experiment, the seedling emergence of red amaranthus, wheat and spinach differed significantly due to the application of different concentrations of arsenic solution and inoculation of mycorrhiza. In this study, it was observed that a significant reduction of seedling emergence due to arsenic toxicity.

Germination test were done in poly bag experiment and data were recorded at 3, 5 and 7 days after sowing. It was observed that seedling emergence of red amaranthus decreased with the increase of arsenic concentrations and it was the highest in AMF inoculated poly bags than non-inoculated ones. The seedling emergence was varied significantly at different concentrations in all the three recorded periods (3, 5 and 7 days after sowing). The percent seedling emergence of red amaranthus increased by AMF inoculation when the crops were grown in arsenic amended soil. Regarding germination percentage, few works yet have been done so far. Our results are more or less similar with Vishwakarma and Singh, 1996 and

Matsubara *et al.* (1994) who investigated the effects of inoculation with Vesicular-arbuscular mycorrhizal fungi (*Glomus etunicatum* or *Glomus intraradices*) on seedling growth of 17 vegetable crop species and reported that the growth was noticeably enhanced by AMF inoculation to some of the selected crops studied in the present investigation. AM fungi promote phosphate uptake in low phosphate soils during the early stages of plant growth. Under nursery conditions, mycorrhizal inoculation improved growth of seedlings (Giri *et al.*, 2005)

The highest germination percent of red amaranthus was (90.00%) obtained in case of 10 ppm arsenic solution with mycorrhizal treated poly bags. The germination per cent significantly reduced when arsenic added above 10 ppm. No germination was found in red amaranthus when poly bags were amended with 500 ppm arsenic solution.

Number of leaves was determined in poly bag experiment. It was observed that, in mycorrhizal inoculated treatments the number of leaves of red amaranthus was higher than non-inoculated. In this study treatment T_4 (10 ppm arsenic solution + mycorrhiza) gave the highest result and the lowest number of leaves of red amaranthus was recorded in case of treatment T_8 (500 ppm arsenic solution) in all the three recorded time in comparison to other treatments. It was exposed from the study that the number of leaves of red amaranthus was the lowest when poly bags were treated with only arsenic but when added mycorrhiza with those treatments, the number of leaves of red amaranthus increased. Number of leaves of red amaranthus was decreased gradually with the increase of arsenic concentration

(10ppm to 500 ppm). The result of present studies are in agreement with the literature of Ahmed *et al.* (2005) who reported that the number of leaves of lentil decreased significantly with the increase of arsenic concentrations. He also reported that mycorrhizal inoculation reduced As concentration in roots and shoots and growing lentil with compatible AM inoculums can minimize As toxicity and increased number of leaves.

In plastic tray experiment both shoot height and root length were observed. In all the two recorded periods it was observed that shoot height and root length of red amaranthus, wheat and spinach increased in mycorrhiza inoculated poly bags than non-inoculated and shoot height and root length of red amaranthus, wheat and spinach decreased with the increase of arsenic concentrations.

In poly bag experiment the results indicate that, shoot height and root length of red amaranthus were higher in all the mycorrhiza inoculated treatments than non-inoculated treatments. Shoot height and root length of red amaranthus differed significantly due to the relevance of different concentrations of arsenic solution and inoculation of mycorrhiza. Among the 10 treatments, treatment T_4 (10 ppm arsenic +mycorrhiza) gave the best result whereas, treatment T_8 (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 red amaranthus in comparison to non-inoculated. This was probably due to uptake of nutrient, which increased vegetative growth. Shoot height and root length of red amaranthus was decreased with the increase of arsenic

concentrations. Present results are more or less similar with Gaur and Adholeya (2000). They reported the higher growth of some crops such as onion, potato and garlic when inoculated with AM fungi. Ultra *et al.* (2007) reported the AM inoculation as well as P application reduced As toxicity symptoms.They also reported that Plant growth was highest in the +AM + P treatment. The present study also keep in with (Xia *et al.*, 2007). They conducted an experiment under glasshouse condition in an As-contaminated soil and they reported arbuscular mycorrhizal (AM) fungus (*Glomus mosseae*) increased both root length markedly under the zero-P treatments. Ahmed *et al.* (2005) 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.

Fresh weight of shoots and roots were observed in plastic tray experiment. In this study mycorrhiza inoculation significantly increased fresh weight of shoots and roots. In case of red amaranthus, wheat and spinach, fresh weight of shoot and root decreased gradually with the increase of arsenic concentrations.

Both fresh and dry weight of shoots and roots were observed in poly bag experiment. In this study, It was observed that a significant reduction of fresh and dry weight of shoot and root of red amaranthus due to arsenic concentrations. In comparison to all the treatments, better result was obtained where mycorrhiza was inoculated than non-inoculated. Our results indicate 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 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 A M fungi inoculated plants. The results are 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).

Our results is similar with Xia *et al.* (2007) who reported that dry weight and root biomass both increased markedly when maize plants inoculated with arbuscular mycorrhizal (AM) fungus (*Glomus mosseae*) under glasshouse condition in an arsenic amended soil. Plant biomass and shoot and root P concentration/off take increased significantly due to mycorrhizal infection. 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

markedly decreased root to shoot As translocation and shoot As concentrations. Khan *et al.* (1995) identified 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.

A positive response of AMF was found in terms of arsenic uptake by shoots of red amaranthus in poly bag experiment. AMF inoculation significantly reduced arsenic uptake. The result is similar with Ahmed *et al.* (2006) who found that shoot arsenic concentration and offtake in mycorrhizal plants were significantly lower than in non-mycorrhizal plants. They also observed that arsenic significantly reduced shoot P concentration and offtake because of the reduction in root growth. Xia *et al.* (2007) examined the effects of arbuscular mycorrhizal fungus and phosphorus addition on arsenic uptake by maize plants from an As-contaminated soil. Their results indicated that AM fungal inoculation decreased shoot As concentrations when no P was added. Chen *et al.* (2007) reported that mycorrhizal

fungi may play an important role in protecting plants against arsenic contamination. They also reported that 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. 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 experimentally contaminated with five As levels. They informed that shoot As concentration increased with increasing As addition up to 50 mg kg^{-1} but decreased with mycorrhizal colonization. Mycorrhizal colonization may have increased plant resistance to potential As toxicity at the highest level of As contamination.

CHAPTER 6
SUMMARY AND CONCLUSION

CHAPTER 6

SUMMARY AND CONCLUSION

Arsenic pollution has created a serious health problem especially in Bangladesh, Vietnam, Taiwan and the west Bengal district in India (Abedin *et al.*, 2002; Meharg 2004). Arsenic enters in to human body not only the use of drinking water containing high concentrations of arsenic but also the consumption of agricultural products grown in arsenic contaminated sites. Recently it is found that crops and vegetables were a sustainable source of arsenic to the Bangladeshi people and it is hazardous to humans. With respect of those problems, a series of experiments were performed to know the role of arsenic on growth of some agricultural crops, particularly vegetables. The variation of arbuscular mycorrhizal root infection and intensity of infection of different agricultural crops root collected from different arsenic affected village of Sonargaon area were investigated. The percentage root infection and intensity of infection were varied from location to location and crop to crop. To determine the infection percentage, agricultural crops roots collected from eight different villages of Sonargaon area. Among the eight villages, the highest infection percentage was recorded from Raisdia village and that was 18.25%. The lowest infection percentage was recorded from Darpat village and that was 7.14%. The comparison of some specific crops (Zea *mays*, *Ipomea batatas*, *Amaranthus gangeticus*, *Capsicum frutescens*) collected from different location showed varied infection percentage. The intensity of infection was also

varied from location to location. Most of the cases the intensity of infection was found in scale-0, 1 and 2.

In artificial condition, three experiments were performed to know the role of arsenic on seed germination of three selected crops with or without mycorrhiza. Seed germination was varied in different concentrations of arsenic solution. The highest seed germination was recorded when low concentration of arsenic was used and the seed germination was hampered with the increase rate of arsenic concentration. In 500 ppm arsenic treated pots, no seed germination was found in case of red amaranthus but little germination was found in other two crops.

To see the role of AMF on physical and chemical growth of different agricultural crops a number of arsenic concentrations (10 ppm, 100 ppm and 500 ppm) were used. Different physical growth parameters were recorded in both plastic tray and poly bags experiments. In every case, 10 ppm arsenic solution with mycorrhiza treated pots gave the highest result. All growth parameters were reduced significantly due to high toxicity of arsenic and increase significantly due to mycorrhizal inoculation.

From poly bag experiment we also determine chemical parameter (Nutrient uptake by shoots). 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 red amaranthus growth was observed when arsenate contaminated irrigation water is applied to the

crop. Importantly, we found reduced translocation of As to aerial plant parts with the inoculation of AM fungi. When red amaranthus grown in arsenic amended soil, then arsenic can be translocated within the shoots of those crops but mycorrhiza inoculation can significantly helps to reduce the translocation of arsenic.

Even though there was a high level of arsenic concentration, mycorrhiza fungi also help to improve different growth parameters. 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 we have not enough technology to meet up those problems. We need to increase our crop production 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 we can not only improve our crop production but also protect our crops against arsenic toxicity. Decreased uptake of As by red amaranthus shoots 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.

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APPENDIX

Appendix I. A poster was presented in the 20th New Phytologist Symposium, University of Aberdeen, Scotland, U.K. 26–27 June 2008.

ROLE OF ARBUSCULAR MYCORRHIZA IN CROP GROWTH IN ARSENIC AMENDED SOIL

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USDA

INTRODUCTION

The agricultural crops are getting contaminated with arsenic because of irrigation with arsenic contaminated ground water and causing significant risk to animal and human health through soil-crop transfer. Since 1993 arsenic contamination in ground water drew attention in Bangladesh. Out of 64 districts, 61 districts are effected with arsenic contamination in ground water (Plate 1) . More than 70-80 million people are threatened with the problem. Arsenic poisoning causes skin pigmentation, development of warts, ulcers, cancer etc. (Plate 2). Mycorrhizal fungi can reduce the uptake of arsenic from the irrigation water to crop growth (Ahmed *et al.*, 2006; Yan-sheng, *et al.*, 2007).

MATERIALS AND METHODS

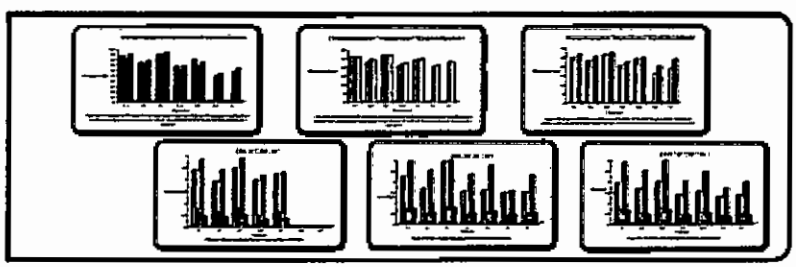
The role of arbuscular mycorrhizal fungi in crop growth in arsenic amended soil was studied with three agricultural crops namely: *Amaranthus gangeticus*, *Raphanus sativus* and *Lycopersicon esculentum*. The plants were grown in arsenic amended soils (10ppm, 100ppm and 500ppm arsenic solution) with or without mycorrhizal inoculation. The treatments were T_1 = Control; T_2 = 10 ppm Arsenic solution; T3 = 10 ppm Arsenic solution + Mycorrhiza; T_4 = 100 ppm Arsenic solution; T_5 = 100 ppm Arsenic solution+ Mycorrhiza; T_6 = 500 ppm Arsenic solution; T_7 = 500 ppm Arsenic solution + Mycorrhiza.

Table 1. Influence of AMF inoculation on fresh and dry weight of shoot and root of *Amaranthus gangeticus*, *Raphanus sativus* and *Lycopersicon esculentum* in soil amended with different concentrations of arsenic solution

	Fresh weight (g) at 60 DAS						Dry weight (g) at 60 DAS					
Treatments	*Amaranthus gangeticus*		*Lycopersicon esculentum*		*Raphanus sativus*		*Amaranthus gangeticus*		*Lycopersicon esculentum*		*Raphanus sativus*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Control	35.90 e (6.03)	7.45 e (2.81)	34.60 f	7.10 f (2.76)	36.00 f	6.80 f (2.61)	2.95 e (1.89)	1.32 e (1.35)	2.77 f (1.97)	1.31 e (1.35)	2.95 f (1.89)	1.25 f (1.32)
10 ppm Arsenic solution	33.20 d (5.81)	6.00 c (2.64)	28.80 c	6.40 d (2.63)	32.80 d	4.20 d (2.17)	1.02 c (1.23)	0.97 c (1.21)	1.21 d (1.31)	0.99 d (1.22)	1.71 c (1.51)	0.85 d (1.17)
10 ppm Arsenic solution + Mycorrhiza	35.00 f (6.04)	8.51 f (3.02)	33.10 f	7.80 g (2.90)	36.80 g	6.90 g (2.72)	3.00 e (1.87)	1.57 f (1.46)	3.10 g (1.85)	1.72 f (1.48)	3.03 g (1.88)	1.39 g (1.36)
100 ppm Arsenic solution	29.38 b (5.48)	4.25 b (2.17)	28.30 b	3.10 b (1.80)	29.40 b	3.10 b (1.83)	0.80 b (1.22)	0.67 b (1.08)	0.83 b (1.10)	0.69 c (1.04)	0.90 b (1.22)	0.62 c (1.09)
100 ppm Arsenic solution + Mycorrhiza	32.36 c (5.78)	6.12 d (2.57)	33.60 e	5.80 e (2.51)	34.10 e	4.80 e (2.32)	1.41 d (1.38)	0.89 d (1.22)	1.41 e (1.38)	1.01 d (1.22)	1.50 e (1.41)	0.98 e (1.27)
500 ppm Arsenic solution	0 a (0.71)	0 a (0.71)	17.47 a	2.80 a (1.84)	27.20 a	2.40 a (1.72)	0 a (0.71)	0 a (0.71)	0.58 a (1.06)	0.29 a (0.88)	0.75 a (1.32)	0.34 a (0.92)
500 ppm Arsenic solution + Mycorrhiza	0 a (0.71)	0 a (0.71)	31.00 d	4.80 c (2.32)	31.60 c	3.80 c (2.19)	0 a (0.71)	0 a (0.71)	1.03 c (1.24)	0.34 b (0.91)	1.25 d (1.32)	0.58 b (1.06)

RESULTS AND DISCUSSION

The overall growth was higher in arsenic contaminated soil inoculated with mycorrhizal fungi (Plate 3) The results indicated that at higher concentrations of arsenic, the seed germination was effected more than the lower concentrations (Fig. 1). A positive germination response to AM was observed in all the selected plants. Root length and shoot height, leaf number, both fresh and dry weight of root and shoot were higher in case of AM inoculated plants in comparison to their respective treatments (Table 1; Fig. 2 & 3). Higher nutrient uptake and less arsenic content were recorded in mycorrhiza inoculated plants (Fig. 4, 5 & 6) Mycorrhizal fungi may play an important role in protecting plants against arsenic contamination (Ahmed *et al.*, 2006; Yan-sheng, *et al.*, 2007).



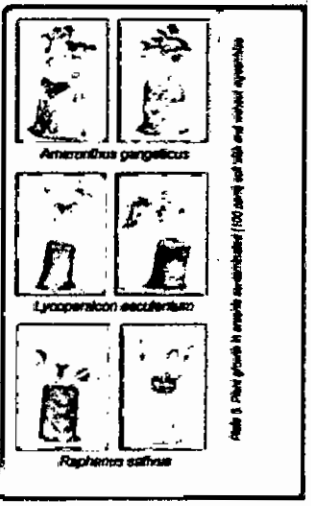


Plate 3. Plant growth in arsenic contaminated (100 ppm) soil with and without mycorrhiza

References

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