ROLE OF ARBUSCULAR MYCORRHIZAL FUNGI ON GROWTH AND NUTRIENT UPTAKE OF SOME VEGETABLE CROPS

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DHAKA-1207

December, 2007

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A Thesis
Submitted to the Faculty of Agriculture,
Sher-e-Bangla Agricultural University, Dhaka,
in partial fulfilment of the requirements
for the degree of

MASTER OF SCIENCE
IN
PLANT PATHOLOGY

SEMESTER: JULY-DECEMBER, 2007

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Dedicated to

My

Beloved Parents

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CERTIFICATE

This is to certify that the thesis entitled **"ROLE OF ARBUSCULAR MYCORRHIZAL FUNGI ON GROWTH AND NUTRIENT UPTAKE OF SOME VEGETABLE CROPS"** 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 **MOST. ARIFUNNAHAR, REG. NO. 06-02165,** 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: 27.12.2007
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ACKNOWLEDGEMENT

All praise are due to Almighty "Allah" the most gracious and merciful Lord of the universe, who enabled the author to conduct and complete the research work and thesis writing leading to Master of Science (MS) in Plant Pathology. The author expresses her grateful respect and wishes, whole hearted gratitude and appreciation to her benevolent teacher and supervisor Dr. Md. Amin Uddin Mridha, Professor, Department of Botany, University of Chittagong for his precious suggestions, constructive criticism, proper guidance and helpful comments through out the study. He took keen interest and intellectually guided the author to develop the conceptual framework of the study.

The author expresses her deepest sense of gratitude, indebtedness and sincere appreciation to her co-supervisor Dr. F. M. Aminuzzaman, Assistant Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for his valuable advices, constant inspiration and helpful suggestions. He took much pain to edit the thesis thoroughly and gave valuable suggestions for its improvement. His scholastic supervision and constant inspiration brought this thesis up to its present standard.

Cordial thanks and honors to Professor Mrs. Nasim Akhtar, Chairman, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for her helpful cooperation providing necessary facilities during the period of the research work.

The author also expresses her cordial thanks and gratefulness to Dr. Md. Rafiqul Islam, Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, for his valuable advices, suggestions and cooperation.

The author also is thankful to all other respected teachers of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, for their valuable advices, suggestions and constructive criticism.

The author is grateful to the office staffs of the Department of Plant Pathology and Farm Division of Sher-e-Bangla Agricultural University, for their cooperation, encouragement and help to complete the research work.

The author extends her heartiest thanks and special gratefulness to her friends, Ummey Sharifun Akhter, Elius, Tanvir Ali Siddiquee, Shib Sankar and many other well wishers for their inspiration, encouragement, help and active co-operation for carrying out the present study.

The author also is grateful to Md. Moniruzzaman, Senior Scientific Officer, Soil Resources Development Institute, Dhaka for his cooperation to analysis of plant nutrient.

The author also convey her special thanks to Md Saiful Alam, Nazmun Naher Tonu, , Nibir Kumar Saha and Sonatan Gupta for their generous help to analyse data and for taking photographs for the research work.

Finally, the author is highly indebted to her beloved parents, brothers and sisters for their immeasurable sacrifices, blessing and constant inspirations to pursue education from beginning to the completion.

December, 2007 ***Author***

CONTENTS

CONTENTS (cont'd)

LIST OF TABLES

LIST OF TABLES (cont'd)

LIST OF FIGURES

ABSTRACT

The present experiment was conducted in the net house of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka during the period from May 2006 to December 2006 with a view to study the role of Arbuscular Mycorrhizal (AM) fungi on growth and nutrient uptake of some vegetable crops. A positive growth response to AM was observed in all the selected vegetables. The seedling emergence, plant height, shoot length and root length of mycorrhiza inoculated vegetables were comparatively higher than that of uninoculated control. The mycorrhizal inoculation suppressed root rot, damping off and leaf spot disease of Spinach (*Spinacia oleracea L.*) and White gourd (*Cucurbita vulgaris*). The mycorrhiza inoculated plants showed more leaves, branches, early flowering and fruiting in comparison to uninoculated control. Mycorrhizal root colonization differed among the crops ranging from 18.65 to 53.48% and mycorrhizal dependency varied from 18.57 to 36.36%. Increased nutrient (N, P, K, Fe and Zn) uptake was recorded with the inoculated plants. Among the inoculated vegetables, comparatively higher N, P and K uptake was observed in Spinach, Water spinach (Kalmishak) and Cucumber where as Zn and Fe uptake was found higher in Spinach and in Cucumber, respectively.

Chapter 1

Introduction

1. INTRODUCTION

"Vegetables" constitute a very important group of crops in Bangladesh. They are important for their low production cost, short production time and high nutritive value. In 2004-2005 about 270.85 ('000') hectare of land were under vegetable cultivation in Bangladesh and produced 1840 ('000') tons of vegetables. Spinach (*Spinacia oleracea L.*), Indian spinach (*Basella alba* L.), Water spinach/ Kalmishak *(Ipomoea aquatica*), White gourd (*Cucurbita vulgaris*) and Cucumber (*Cucumis sativus*) are mainly cultivated as vegetables in our country. Vegetables are an important component of cropping systems in south and west Asia. Total areas under cultivation of Spinach, Indian spinach, Water spinach (Kalmishak), White gourd and Cucumber are 6.47 thousand ha, 5.66 thousand ha, 7.28 thousand ha, 8.50 thousand ha and 7.69 thousand ha with the annual production of 12.95 tons/ha, 12.95 tons/ha, 14.57 tons/ha, 23.48 tons/ha and 15.39 tons/ha, respectively (BBS, 2006). In general, per hectare yield of these crops are low. Even the country relies on farming yet its crop yield is dramatically less than expected. Among the factors responsible for low yield, improper use of fertilizer and pesticides features predominantly. The country requires a sustainable technology where an agricultural out put can be high as well as minimum residual effects of chemical fertilizer.

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). 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 and plant resistance to diseases. They also help in conserving soil productivity for the future. Given that the majority of cultivated plants used for human and animal food purposes are colonized by mycorrhizae. This symbiosis can be considered for the benefit of agriculture by selecting the best plant fungus combinations (Abbott and Robson, 1991). It is then possible to promote healthier cropping systems and to reduce the use of chemical inputs (pesticides, fertilizers), while ensuring crop profitability and environmental quality. This symbiosis association contributes to the success of the plant establishment and survival, increasing uptake of water and osmotic adjustment under drought stress (Masri, 1997) and also improves soil-plant waters relationship (Jastrow *et al.*, 1998).

Diversification of arbuscular mycorrhizal association in various agricultural crops is currently of great interest because of the important role played by various vegetable crops in enriching soil fertility (Mridha, 2002). Out of the different types of mycorrhizae, the AM fungi are by far the most widely occurring mycorrhizae and very important in relation to improvement of agricultural and horticultural crops and forest trees in hilly areas (Mridha *et al.*, 2001).The fungus receives sucrose from the plant in exchange for soil solution derived orthophosphate, which is given up by the fungus at the arbuscules in the root cortex (Smith and Gianinajji-pearson, 1998). The AM association can help in higher production of growth regulating substances (Danneberg *et al.*, 1992) and increase plant resistance against pest and diseases (Bethlerfalvay 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). Reduction of the effects of pathogenic roots is infecting fungi like *Macrophomina phascolina, Rhizoctonia solani* by this fungus, *Glomus mosseae* (Zambolim and Schenok, 1989). This fungus reduced the number of sclerotia produced by *Sclerotium roffsii* while the root pathogen reduced the percentage of root infection and chlamydospore production by *Glomus mosseae* (Krishna and Bagyaraj, 1983). It also appears to decrease the plant susceptibility to disease or increase tolerance against the attach of root pathogens (Jalali and Chand, 1988). Simultaneous inoculation of VAM fungi reduced the *Fusarium* wilt incidence in the wilt susceptible JG62 chickpea variety (Reddy *et al.*, 1988). Introduction of mycorrhizal fungi 15 days before the nematode adversely affected root penetration to a greater extent than simultaneous inoculations (Jain and Sethi, 1989). Plants with VAM were more resistant to *Meloidogyne incognita* than without VAM fungi (Santhi and Sundarababu, 1995).

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). Khan *et al.*, (1995) identified that nitrogen fixation as well as N and P contents in groundnut increased only by dual inoculation with VAM 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). 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). In addition to enhanced P uptake, VAM fungi often also enhance acquisition of relatively immobile micronutrient cations, particularly Zn and Cu (Lambert *et al.*, 1979;

Killham and Firestone, 1983; Gnekow and Marschner, 1989; Swaminathan and Verma, 1983; Gildon and Tinker, 1983; Pacovsky, 1986).

The present study was carried out to evaluate the effect of AM on growth and nutrient uptake ability of vegetables with the following objectives:

1. To evaluate the effect of Arbuscular mycorrhizal (AM) fungus on growth, nutrient uptake and disease suppression of some selected vegetable crops.
2. To determine the effect of Arbuscular mycorrhizal (AM) fungi on yield of some selected vegetable crops.

Chapter 2

Review of Literature

2. REVIEW OF LITERATURE

The literatures available on VA-mycorrhizal association of different vegetable crops are presented in this chapter.

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.

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).

Afek *et al.* (1990) studied the percent root colonization of AM fungi on onion, cotton and capsicum inoculated with *Glomus deserticola, Dlomus intraracices* and *Glomus spp.* They recorded 50%, 37% and 20% colonization after 12 days of inoculation and 60%, 13% and 10% colonization after 21 days of inoculation in onion, cotton and capsicum, respectively. They found that length of time was an important factor for root colonization. Wani and Konde (1996) recorded AM colonization in garlic root ranging from 39 to 62%.

The association of AM fungi increased uptake of immobile nutrients especially phosphorus and micronutrients (Azizah and Martin, 1992; Douds and Miller, 1999). The AM association contributed to the success of the plant establishment, survival and increasing uptake of water and osmotic adjustment under drought stress (Auge *et al.*, 1986; Masri, 1997)

Rosendahl and Rosendahl (1991) examined the interactions between ***Pythium ultimum*** and two strains of vesicular-arbuscular mycorrhizal (VAM) fungi (*Glomus* spp.) with Cucumber (*Cucumis sativus* L.) plants.VAM inoculation before or simultaneous with the inoculation of the pathogen increased survival of the seedlings. Inoculation with *P. ultimum* 14 days after sowing did not kill the plants, but reduced the leaf area. This reduction was almost eliminated by one of the VAM isolates.

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.

Christensen and Jakobsen (1993) conducted an experiment on Cucumber, grown in a partially sterilized sand-soil mixture with the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* or left uninoculated. The presence of VAM decreased the rate of bacterial DNA synthesis, decreased the bacterial biomass, and changed the spatial pattern of bacterial growth compared to non-mycorrhizal cucumbers.

Edathii *et al.* (1994) assessed the VAM status of tomato, brinjal (aubergin) 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 maximum at 45, 50 and 60 days after germination of brinjal, tomato and capsicum seeds, respectively under field conditions and on the 60th day in pot culture.

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 reported 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 seven families with VAM fungus infection.

Joner and Jakobsen (1994) investigated the role of arbuscular mycorrhiza (AM) in utilization of P from organic matter during mineralization in soil. Cucumber (*Cucumis sativus L.*) was inoculated with one or two AM fungi or left uninoculated were grown for 30 days in cross-shaped PVC pots. The experiment confirmed that AM fungi differ in P uptake characteristics, and that mycorrhizal hyphae can intercept some P immobilization by other microorganisms and P-sorbing clay minerals.

Trimble and Knowles (1995) divulged the growth response of cucumber (*Cucumis sativus* L.) to infection by vesicular-arbuscular mycorrhizal (VAM) fungi in greenhouse. 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 non infected plants at all levels of P nutrition. The VAM-enhanced growth was similar to that induced by increases in P nutrition.

Ravnskov and Jakobsen (1995) reported that cucumber (*Cucumis sativus* L.), wheat (*Triticum aestivum* L.) and flax (*Linum usitatissimum* L.) were inoculated with *Glomus caledonium* (Nicol. & Gerd.), Trappe & Gerdemann (isolate RIS42, BEG15) and *Glomus invermaium* Hall (isolate WUM10) or left uninoculated and grown for 28 days in soil divided into three compartments. The symbioses differed markedly with respect to functional compatibility as phosphorus uptake by each fungus depended on the species of host plant. The hyphal transport of P-32 was high in *G. caledonium* in symbioses with all three plant species, whereas *G. invermaium* transported significant amounts of P-32 only when associated with flax. Consequently, to determine the P-transport effectiveness of a mycorrhizal fungus is meaningful only in the context of its associated host plant species.

Sreeramulu *et al.* (1996) observed the growth of *Amaranthus viridis* and *Trigonella foenuni* in an unsterlized sandy loam soil in response to P fertilizers application (0 or 25, 50 or 100% of the recommended rate of 50 kg P_2O_5/ha) in a pot experiment. They recorded significantly greater higher and leaf number of plants when inoculated with *Glomus fasciculatum*. Inoculated plants also had significantly higher shoot and root biomass than corresponding uninoculated plants. Shoot weight with 50% of the recommended P rate + AM inoculation was comparable that with 100% of the recommended P rate without mycorrhizal inoculation. Inoculation of AM in *Amaranthus viridis* and *Trigonella foenuni* reduced the P requirement to obtain maximum leaf yields. Phosphorus uptake in both shoot and root was significantly higher in inoculated plants over control.

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. Loth (1996) recorded spore densities ranging from 31 to 97 per gram of 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.

Tarafdar and Praveen (1996) studied the effect of different vesicular arbuscular mycorrhizal fungi (VAMF) on crops (*Vigna aconitifoli*) under field conditions. Plants growth and nutrient uptake of non-inoculated plants were compared with the growth and nutrient uptake of VAMF-inoculated plants. After 8 weeks of growth, percent root infection 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.

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.

Nedumpara and Mercy (1996) studied the Vesicular Arbuscular Mycorrhizal (VAM) assosiation with many vesicular plant species and the contribution of VAM fungi on uptake N, P, K by crop plant is absent.

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.

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*.

Osonubi *et al.* (`1998) studied on the effect of root exudates from non-mycorrhizal and mycorrhizal cucumber (*Cucumis sativus* L.) plants colonized by one of three arbuscular mycorrhizal fungi *Gigaspora rosea*, *Glomus intraradices* or *Glomus mosseae* on hyphal growth of *G. rosea* and *G. intraradices* in axenic culture and on root colonization by *G. mosseae* in soil. Root exudates from non-mycorrhizal cucumber plants clearly stimulated hyphal growth, whereas root exudates from all mycorrhizal cucumber plants tested showed no stimulation of the hyphal growth of *G. rosea* and only a slight stimulation of the hyphal growth of *G. intraradices*. These results suggest that plants colonized by AM fungi regulate further mycorrhization via their root exudates (Pinior *et al.*, 1999).

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 crops of Bangladesh and the relationship with Vesicular Arbuscular Mycorrhizal (VAM) fungi. They identified *Acaulospora, Entrophosphora, Gigaspora, Glomus* and *Scutellospora*. *Glomus* spp. were the most common followed by *Gigaspora* and *Scutellospora* in vegetables and rice.

Hirrel *et al.* (1978) found the AM colonization in most plant families so far examined, although it may be rare or absent in families such as Cruciferae, Chenopodiaceae, Caryophyllaceae and Cyperaceae. They prevail over a broad ecological range from aquatic to desert environments (Mosse *et al.*, 1981). Limited studies on biodiversity of AM fungi have been done in Bangladesh (Khan *et al.*, 1988; Zahed *et al.*, 1994; Mridha *et al.*, 1999; Mahmud *et al.*, 1999 ; Mridha, 2000).

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.

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.

Bagayoko *et al.* (2000) reported positive effects of Vesicular Arbuscular Mycorrhizae (VAM) on plant growth in temperate soils. A pot experiment was conducted with local genotypes of pearl millet (*Pennisetum glaucum* L.), sorghum (*Sorghum bicolor* L. *Moench*) and cowpea (*Vigna unguiculata*) with and without phosphorus (P) application in a sterilized sandy soil from a farmer's field in Niger shelved large growth-enhancing effects of VAM. Phosphorus application led to 18-fold and 24-fold increases in pearl millet root and shoot dry matter independently of VAM, whereas the shoot and root dry matter of sorghum and cowpea depended largely on the interaction between P application and VAM. With P, VAM increased total uptake of P, K, Ca, Mg and Zn by 2.5- to 6-fold in sorghum and cowpea. On severely P deficient West African soils P application can lead to large increases in early root growth, a prerequisite for early mycorrhizal infection and a subsequent significant contribution of VAM to enhanced plant growth and nutrient uptake.

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.

Estrada-Luna and Davies (2001) studied the effects of a mixed Mexican Arbuscular Mycorrhizal (AM) fungal inoculum (composed of *Glomus albidum, G. diaphanum*, and *G. claroides*) and low phosphate supply on growth and nutrient uptake of micropropagated prickly-pear cactus (*Opuntia albicarpa Scheinvar Reyna*) plantlets. Poorest growth occurred with uninoculated plantlets that lacked supplementary P supply. In contrast, the combination of mycorrhizal colonization and supplementary P significantly increased shoot length, shoot and root DM and surface area of the plantlets. AM fungi enhanced concentration of P and Zn and increased nutrient uptake of P, B and Zn in the cladodes. They conclude that AM fungi can be used as a biotechnological tool that allows more efficient, low P input to enhance ex vitro transplantation of *O. albicarpa*.

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

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 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.

Kubota *et al.* (2005) studied the colonization by arbuscular mycorrhizal (AM) fungi, investigated in cucumber *(Cucumis sativus)*, tomato (*Lycopersicon esculentum*) and *Clethra barbinervis* (Ericales) grown in field-collected soil known from previous studies to generate Paris-type arbuscular mycorrhizae in *C. barbinervis*. The morphology of colonization was strongly influenced by the selection of fungi to colonize the host plant from among those in the soil environment.

Giri *et al.* (2005) evaluated 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. Mycorrhization led to decrease in alkalinity of the rhizosphere soil from pH 8.5 to 7.4. 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.

Chapter 3

Materials and Methods

3. MATERIALS AND METHODS

3.1 Experimental site

The present experiment was conducted in the net house and in the seed health laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh.

3.2 Experimental period

The experiment was carried out during the period from May, 2006 to December, 2006.

3.3 Selection of crops

Different important available vegetable crops were selected for the experiment which grown in different areas of Bangladesh to assess their dependency to AM. The list of the crops included in the experiment is given below.

List of the crops included in the present experiment:

Common Name	**Scientific name**	**Family**
Spinach	*Spinacia oleracea L*	Chenopodiaceae
Indian Spinach	*Basella alba L*	Basellaceae
Water Spinach/Kalmishak	*Ipomoea aquatica*	Convolvulaceae
White Gourd	*Cucurbita vulgaris*	Cucurbitaceae
Cucumber	*Cucumis sativus*	Cucurbitaceae

3.4 Collection of roots for mycorrhizal assessment

For conducting the experiment, inoculum of Mycorrhizal fungi from natural condition was used. For this reason, a survey programme was conducted in the Agronomy farm of Sher-e-Bangla Agricultural University in May 2006. Root samples of 21 plants species (both crops and weeds) growing under natural condition at a depth of 0 to 15 cm in different places of the Agronomy field were collected for the observation of occurrence of vesicular arbuscular mycorrhizal (VAM) association with the root systems. The list of the plants collected for inoculum preparation is given below.

List of plants

Common name	**Scientific Name**	**Family**
White eclipta	*Eclipta prostrate* L.	Composite
Goat weed	*Ageratum conyzoides* L.	Composite
Harkuch	*Enhydra fluctuans Lour.*	Composite
Zirakata ful	*Spilanthes acmella* L.	Composite
Garden spurge	*Euphorbia hirta* L.	Euphorbiaceae
Prostute spurge	*Euphorbia parviflora* F.B.I.	Euphorbiaceae
Croton plant	*Croton sparsiflorus* L.	Euphorbiaceae
Spider wort	*Commelina benghalensis* L.	Commelinaceae
Kanainala	*Cyanotis axillaries* Roem and schutt	Commelinaceae
Sensitive plant	*Mimosa pudica* L.	Leguminosae
Araich	*Cassia tora* L.	Leguminosae
Wild lentil	*Vicia sativa* L.	Leguminosae
Wild mustard	*Brassica kaber (DC)* L. *E. wheeler*	Cruciferae
Block night shade	*Solanum nigrum* L.	Solanaceae
Horse nettle	*Salarum carolinense* L.	Solanaceae
Bondhunia	*Seroparua dulcis* L.	Scrophulariaceae
Spiny pig weed	*Amaranthus spinosus* L.	Amaranthaceae
Pig weed	*Amaranthus Viridis* L.	Amaranthaceae
Alligator weed	*Alternanthera philoxeroides*	Amaranthaceae
Wild clary	*Heliotropium indicum* L.	Boraginaceae
Soybean	*Glycine max*	Leguminosae

Plant roots were dugout, washed thoroughly with water to remove the adhering soil particles and then cut into 1 cm long segment. The root samples were then preserved in screw cap test tubes with 50% ethanol for future use.

3.5 Cleaning of roots

Collected roots were freed from adhering soil, gently washed with water and fine roots were cut into small segments of approximately 1 cm to determine the percentage of VAM root colonization. For these only 100 segments were randomly selected for staining. The root segments were then preserved in screw cup test tubes with 50% ethanol for future use.

3.6 Staining of roots

According to Koske and Gemma (1989), the roots of each plant species were stained with some modifications (Mridha *et al.*, 1999). The root pieces were boiled in 2.5% KOH solution for 30 minutes at 90 ° C temperatures. Later on, the root segments were washed in water 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.

3.7 Mycorrhizal assessment

The stained root segments were mounted in glycerol solution on glass slides and the cover slip was gently pressed to facilitate the observation of different type of structures present in the whole root segment. A root segment was considered to be infected if it showed mycelium, arbuscular, vesicles either alone any other combination of these structural characteristics of AM fungi. When any of these were found in sample, the intensity of infection of VAM fungi was estimated as: Low (*) if only mycelium were present; moderate (**) if mycelium and vesicles or arbuscules were present and High (***) if mycelium, vesicles and arbuscules were present (Mridha and Mohammed, 1989). Out of 21 plant species studied, abundant amount of infections (hundred percent root segments infected) were found with only two plant species *Cassia tora* L. and *Croton sparsiflorus* L. (weeds). For this study the *Cassia tora* weed roots were used as a natural inoculum. The soils of this plant collection sites were sandy loam type. This rhizosphere soils also used as natural inoculum.

3.8 Collection of soils

For the experiment soil was collected from the Agronomy field of Sher-e-Bangla Agricultural University Campus from a depth of 5 to 10 inch. After collecting the soil, clods were broken and weeds, stones, gravels, roots, and other unwanted materials were removed.

3.9 Collection of seeds

For experimental purpose seeds of 5 different vegetable crops namely Spinach, Indian Spinach, Water Spinach/Kalmishak, White Gourd and Cucumber were collected from BARI (Bangladesh Agricultural Research Institute), Gazipur.

3.10 Preparation of inoculum

Cassia tora roots were collected from Agronomy field along with rhizosphere soil for inoculum. The presence of AM fungi within the root sample was confirmed using the staining procedure of Koske and Gemma (1979). Collected root samples were cut into small pieces by the help of chopper. Half of rhizosphere soils and root samples were sterilized in the autoclave at 121° C at 15 PSI for 15 minutes and used as base materials for control pots.

3.11 Preparation of seedling bags

The polythene bags of 8"×12" sized 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. At first $\frac{2}{3}$ rd portion of the seedling bags were filled with substratum. Then a layer of both inoculum i.e. root inoculum 25 g and soil inoculum 100 g were placed in each treated bag. For each crop 10 replications i.e., 10 for inoculated and 10 for non-inoculated were prepared. Both 25 g roots and 100 g soil (rhizosphere) of sterilized inoculum were used in non-inoculated bags to maintain the same nutrient status between the inoculated and non-inoculated bags. The inoculum layer (both sterilized and non-sterilized) of each bag was covered with a thin soil (substratum) layer of 2 cm below the surface in which seeds were sown. Two hundred polythene bags (10 × 2 × 10) were prepared for ten crops in the present study.

3.12 Sowing of seeds

For each crop 10 replications were maintained and in each replication consists same number of seed of same crop plant. For different crops different number of seeds was sown in the bags based on seed size. For Cucumber 7 seeds/bag, Spinach 20 seeds/bag, Indian Spinach 10 seeds/bag, Water Spinach 10 seeds/bag and White Gourd 7 seeds/bag were sown. After 15 days, 5 seedlings in each bag were retained and other seedlings were removed. To avoid the chance of contamination a space of 30 cm was maintained between the inoculated and non-inoculated replications.

3.13 Intercultural operation

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

3.14 Harvesting

The seedlings were harvested when those were 40 DAS and 60 DAS old. In this case 3 seedlings from inoculated bag and 3 seedlings from non-inoculated bag were harvested randomly for each crop. Shoots and roots of five different crops were collected. The roots were washed with tap water to remove the adhering soil. Roots and shoots were separated with the help of sharp scissors and were preserved after necessary processing for determining root mass and shoot mass. Then roots and shoots were dried in an oven for 72 hours at 70° C until the samples gave constant weight.

3.15 Data recording

Data were recorded on seedling emergence (%) (7 DAS, 10 DAS and 15 DAS), plant height (cm) (20 DAS, 40 DAS and 60 DAS), shoot fresh and dry weight (g) (40 DAS, 60 DAS), root fresh and dry weight (g) (40 DAS and 60 DAS), shoot and root length (cm), 1st branching and flowering, number of leaves and branches plant^{-1}, number of flowers plant^{-1} and disease incidence.

3.16 Mycorrhizal dependency (MD)

Mycorrhizal dependency was calculated according to Plenchette *et al.*, (1983).

$$\text{MD}(\%) = \frac{\textit{Dry wt. of Mycorrhizal plant} - \textit{Dry wt. of non mycorrhizal plant}}{\textit{Dry wt. of mycorrhizal plant}} \times 100$$

3.17 Assessment of root colonization

Preserved root samples were assessed. Roots were taken out of the vial and washed 2-3 times with clean water and cut into small segments of approximately one 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). One hundred root segments were examined for each sample. The stained root pieces were mounted in acidic glycerol on slides and the cover slip was placed and slightly pressed. The roots were observed under compound 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:

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

3.18 Nutrient analysis

3.18.1 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.

3.18.2 Sample analysis

3.18.2.1 N, P, K nutrient uptake

The shoot samples were oven dried at 70° C for 72 hours. Dried plant materials were ground and processed for determination of N, P and K.

3.18.2.2 Total Nitrogen

Total nitrogen content in plants samples (shoot) were determined by micro Kjeldahl method (Bremner, 1965).

3.18.2.3 Total phosphorus and potassium

For determination of total phosphorus and potassium contents, dried plant materials were digested with concentrated HNO_3 and $HCLO_4$ mixture as described by Piper (1950).

Total phosphorus content in the extract was determined by Vanado-Molybdate Yellow colour methods as described by Jackson (1973).

Total potassium content was determined by Atomic Absorption Spectrophotometer.

NPK uptake was computed using N, P and K contents and yield data (shoot).

Nutrient uptake was calculated by using the following formula:

$$\text{Nutrient uptake} = \frac{\textit{Nutrient content}\ (\%) \times \textit{yield}}{100}$$

Available other elements like Fe and Zn were determined following ASI method (Hunter, 1984).

3.19 Statistical analysis

All data were analyzed using computer program 'SPSS' Program for T-test.

Chapter 4

Results

4. RESULTS

4.1. SPINACH (*Spinacia oleracea L*)

Seedling emergence

The influence of inoculation of Arbuscular Mycorrhizal (AM) fungi on seedling emergence of Spinach is presented in Table 1. The seedling emergence was calculated in three different times. With the elapse of time the seedling emergence increased in both treatments. But significantly the higher seedling emergence was found in case of inoculated Spinach than non-inoculated. The per cent seedling emergences increased over control in mycorrhizal treated pots was 16.58, 17.10 and 2.27 at 7, 10 and 15 days after sowing, respectively. The highest seedling emergence was 90% in mycorrhiza treated pot at 15 days after sowing and the lowest (56.33%) was counted in control condition at 7days after sowing.

Plant height

Results of table 2 showed the effect of AM fungi on plant height of Spinach. With the increase of growth period, the plant height was increased both mycorrhizal inoculated and non inoculated plants. In both the cases, at 1st 20 days (20 DAS) and 2nd 20 days (20 to 40 DAS) after sowing the growth increment was higher than the 3rd 20 days (40 to 60 DAS) after sowing and the rate of growth was also higher than 1st and 2nd 20 days. The percent plant height increased over control in mycorrhiza inoculated pots was 37.52, 22.65 and 15.78 % at 20, 40 and 60 days after sowing, respectively.

Table 1. Influence of Arbuscular Mycorrhizal Fungi (AMF) inoculation on seedling emergence (%) of Spinach at different periods

	Seedling emergence (%)			% Increased over control		
	7 DAS	10 DAS	15 DAS	7 DAS	10 DAS	15 DAS
Non-inoculated (Control)	56.33b	76.00b	88.00b	----	----	----
Inoculated (Mycorrhiza)	65.67a	89.00a	90.00a	16.58	17.10	2.27

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Table 2. Influence of AMF inoculation on plant height of Spinach at different growth stages

Treatments	Plant height (cm)			% Increased over control		
	20 DAS	40 DAS	60 DAS	20 DAS	40 DAS	60 DAS
Non-inoculated (Control)	5.33b	10.33b	12.67b	----	----	----
Inoculated (Mycorrhiza)	7.33a	12.67a	14.67a	37.52	22.65	15.78

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Root growth

Influence of AM inoculation on root growth is presented in Table 3. The root length of mycorrhizal plants in both harvested period (40 and 60 days) was significantly higher in comparison to non mycorrhizal plants. It is also observed that the rate of root length increment at 40 days after sowing was higher than in 60 days after sowing in both treatments, respectively. With the increase of growth period, the root weight was increased in treated plant and constant condition in control plant, but the percent root weight increased over control in mycorrhizal plant was 101.81, 40.00(Fresh) and 50.00, 83.33(Dry) after 40 and 60 days sowing, respectively.

Shoot growth

Shoot length and shoot weight (Fresh and dry) of Spinach is presented in Table 4. Mycorrhizal inoculation significantly enhanced plant shoot length in comparison to non inoculated plant. Among the mycorrhizal plant, the rate of shoot weight increment in 20 days duration (40 to 60 days) was higher (45.99%) in comparison to non mycorrhizal plant (29.13%) for fresh shoot weight. The percent shoot weight increased over control in mycorrhizal plants was 23.42, 39.53 (Fresh) and 46.87, 50.98 (Dry) after 40 and 60 days sowing, respectively. Some variation in shoot length of Spinach always was found in every growth period and maximum variation (2.33 cm) was found at 40 days after sowing between mycorrhizal and non mycorrhizal plant.

Table 3. Influence of AMF inoculation on root growth of Spinach at different growth stages

Treatments	Root length (cm)		Root weight (g)				% Root weight increased over control			
			Fresh		Dry		Fresh		Dry	
	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS
Non-inoculated (Control)	5.44b	8.66b	0.55b	0.55b	0.12b	0.12b	---	---	---	----
Inoculated (Mycorrhiza)	7.66a	9.77a	1.11a	0.77a	0.18a	0.22a	101.81	40.00	50.00	83.33

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Table 4. Influence of AMF inoculation on shoot growth of Spinach at different growth stages

eatments	Shoot length (cm)		Shoot weight (g)				% Shoot weight increased over control			
			Fresh		Dry		Fresh		Dry	
	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS
n- culated ontrol)	10.33b	12.66b	3.33b	4.30b	0.32b	0.51b	----	----	----	----
culated ycorrhiza)	12.66a	14.66a	4.11a	6.00a	0.47a	0.77a	23.42	39.53	46.87	50.98

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Disease suppression

Inoculated of pot grown Spinach plants with VA mycorrhizal plants resulted by the endophyte by the accompany reduction in the incidence of diseases are presented in Table 5. The damping off disease incidence was 10.66% in non inoculated and 2.53% in inoculated mycorrhizal plants. Leaf spot disease was 7.28% in control plants, but in inoculated plant, it was only 3.67% and followed by the root rot disease also. The disease incidence was always significantly higher in noninoculated control plant in compare to inoculated mycorrhizal plant.

Table 5. Effect of AMF inoculation of Spinach on incidence of different diseases at seedling stage

Treatments	Infected plant (%)		
	Root rot	Damping off	Leaf spot
Noninoculated (Control)	6.57a	10.66a	7.28a
Inoculated (Mycorrhiza)	2.21b	2.53b	3.67b

In a column means followed by uncommon letters are significantly different at the 5% level of significance by T-test.

Number of leaf / shoot

It was observed that the number of leaf per shoot of inoculated mycorrhizal plant is more than the non inoculated control plant.

Mycorrhizal dependency

Mycorrhizal dependency is the degree to which a host relies on the mycorrhizal condition to produce maximum growth at a given level of soil fertility. Arbuscular mycorrhiza and vegetable crops have an obligate nutritional dependency. The mycorrhizal dependency of Spinach was 36.36%.

Root colonization

The highest percent root colonization (44.26%) of mycorrhiza inoculated plants was recorded at 60 DAS and lowest (21.85%) at 20 DAS. On the other hand no root colonization was found in untreated control plants.

Nutrient uptake

Inoculation of arbuscular mycorrhizal fungi responsed to nutrients uptake (N, P, K, Zn and Fe) by Spinach are presented in Table 6. It is evident from the study that mycorrhizal inoculation significantly enhanced nutrient uptake in shoot with comparison to control plant. The percent nutrient uptake increased over control for N, P, K, Zn and Fe were 36.16%, 31.03%, 13.79% 75.00% and 14.75%, respectively. The highest percent increased was obtained with Zn which was followed by N, P, Fe and the lowest was found with K.

Table 6. Effect of AMF inoculation on nutrient uptake by Spinach shoots

Treatments	Nutrient uptake by shoot				
	N (%)	P (%)	K (%)	Zn (%)	Fe (%)
Non-inoculated (Control)	2.24b	0.58b	2.9b	0.0088b	0.0305b
Inoculated (Mycorrhiza)	3.05a	0.76a	3.3a	0.0154a	0.035a
%Increased over control	36.16	31.03	13.79	75.00	14.75

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

A **B**

Fig.1. Influence of Mycorrhizal inoculation on growth of Spinach

A = Mycorrhiza inoculated plants,

B = Non inoculated (control) plants

4.2. WATER SPINACH (*Ipomoea aquatica*)

Seedling emergence

The influence of inoculation of Arbuscular mycorrhizal fungi on seedling emergence of Water Spinach/Kalmishak is presented in Table 7. The seedling emergences were calculated in three different times. With the passing of time the seedling emergence increased in both treatments.Significantly higher seedling emergence was found in case of inoculated water Spinach than non-inoculated. The percent seedling emergence increased over control in mycorrhizal treated pot was 10.00, 17.18 and 5.55 at 7, 10 and 15 days after sowing, respectively. The highest seedling emergence (76%) was in mycorrhiza treated pot at 15 days after sowing and the lowest (50%) was counted in control condition at seven days after sowing.

Plant height

The trend of overall increment of plant height of Water Spinach/Kalmishak among different period of growth for both mycorrhizal and non mycorrhizal plants were more or less similar (Table 8). In both the cases, at first 20 days (20 DAS) and 3rd 20 days (40 to 60 days) after sowing, the growth increment was higher and the rate of growth was also higher at 1st and 3rd 20 days. In case of percent growth increment for mycorrhizal and non mycorrhizal plant, it was observed that the increment was minimum (33.86% in inoculated and 32.64% in non-inoculated) in 3rd 20 days (40 to 60 days) and it was maximum (70.38% in inoculated and 65.42% non inoculated) in 2nd 20 days (20 to 40 days). The percent plant height increased over control in mycorrhiza inoculated pots was 6.01, 9.18 and 10.18 at 20, 40 and 60 days after sowing, respectively.

Table 7. Influence of Arbuscular Mycorrhizal Fungi (AMF) inoculation on seedling emergence (%) of Water Spinach at different periods

Treatments	Seedling emergence (%)			% Increased over control		
	7 DAS	10 DAS	15 DAS	7 DAS	10 DAS	15 DAS
Non-inoculated (Control)	50.00b	64.00b	72.00b	----	----	----
Inoculated (Mycorrhiza)	55.00a	75.00a	76.00a	10.00	17.18	5.55

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Table 8. Influence of AMF inoculation on plant height of Water Spinach at different growth stages

Treatments	Plant height (cm)			% Increased over control		
	20 DAS	40 DAS	60 DAS	20 DAS	40 DAS	60 DAS
Non-inoculated (Control)	20.13b	33.30b	44.17b	----	---	---
Inoculated (Mycorrhiza)	21.34a	36.36a	48.67a	6.01	9.18	10.18

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Root growth

Root length and root weight (Fresh and dry) of Water Spinach is presented in Table 9. The root length of mycorrhizal plants in both harvested period (40 days and 60 days) was higher in comparison to non mycorrhizal plants. In case of AM inoculation, the root weight of Water Spinach was significantly higher than the non inoculated plants. The percent root weight increased over control in mycorrhizal plant was 20.74 14.52 (Fresh) and 27.11, 14.66 (dry) after 40 and 60 days of sowing, respectively.

Shoot growth

Influence of AM inoculation on shoot growth of Water Spinach harvested at different periods has been shown in Table 10. In every growth period the shoot weight and shoot length were always higher in inoculated plants than in non inoculated plants. Among the mycorrhizal plants the rate of shoot length increment in 20 days duration (40 to 60 days) was less (36.38%) in comparison to non mycorrhizal plants (58.18%). The percent shoot weight increased over control in mycorrhizal plant was 49.49, 28.90 (Fresh) and 25.66, 42.76 (dry) after 40 and 60 days sowing, respectively.

Table 9. Influence of AMF inoculation on root growth of Water Spinach at different growth stages

Treatments	Root length (cm)		Root weight (g)				% Root weight increased over control			
			Fresh		Dry		Fresh		Dry	
	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS
Non-inoculated (Control)	21.86b	29.55b	9.11b	10.67b	1.18b	2.25b	---	----	----	----
Inoculated (Mycorrhiza)	25.53a	31.41a	11.00a	12.22a	1.5a	2.58a	20.74	14.52	27.11	14.66

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Table 10. Influence of AMF inoculation on shoot growth of Water Spinach at different growth stages

Treatments	Shoot length (cm)		Shoot weight (g)				%Shoot weight increased over control			
			Fresh		Dry		Fresh		Dry	
	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS
Non-inoculated (Control)	33.30b	44.17b	8.99b	14.22b	1.13b	1.52b	---	---	----	----
Inoculated (Mycorrhiza)	36.36a	48.67a	13.44a	18.33a	1.42a	2.17a	49.49	28.90	25.66	42.76

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Number of leaf / shoot

It was observed that the number of leaf per shoot of inoculated mycorrhizal plant is more than the non inoculated control plant.

Mycorrhizal dependency

Mycorrhizal dependency is the degree to which a host relies on the mycorrhizal condition to produce maximum growth at a given level of soil fertility. Arbuscular mycorrhiza and vegetable crops have an obligate nutritional dependency. The mycorrhizal dependency of Water Spinach was 20.63%.

Root colonization

The highest percent root colonization (38.66%) of mycorrhiza inoculated plants was recorded at 60 DAS and lowest (19.85%) at 20 DAS. On the other hand no root colonization was found in untreated control plants.

Nutrient Uptake

Mycorrhizal inoculation significantly enhanced nutrient uptake (N, P, K, Zn and Fe) compared to control in Water Spinach plants (Table 11). The percent nutrient uptake increased over control for N, P and Zn was 56.25%, 21.69% and 57.89%, respectively. The maximum nutrient uptake increased in inoculated plant was recorded Zn (57.89%) and the minimum was K (o %) and Fe was absent in both mycorrhiza inoculated and non inoculated plant.

Table 11. Effect of AMF inoculation on nutrient uptake by Water Spinach shoots

Treatments	Nutrient uptake by shoot				
	N (%)	P (%)	K (%)	Zn (%)	Fe (%)
Non-inoculated (Control)	1.12b	0.83b	4.4a	0.0019b	--
Inoculated (Mycorrhiza)	1.75a	1.01a	4.4a	0.0030a	--
% Increased over control	56.25	21.69	0.00	57.89	--

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

A **B**

Fig.2. Influence of Mycorrhizal inoculation on growth of Water Spinach

A = Mycorrhiza inoculated plants

B = Untreated (control) plants

4.3. INDIAN SPINACH (*Basella alba L*)

Seedling emergence

The influence of inoculation of Arbuscular mycorrhizal fungi on seedling emergence of Indian Spinach is presented in Table 12. The seedling emergences were recorded in three different times. With the elapse of time the seedling emergence increased in both treatments. But significantly higher seedling emergence was found in case of inoculated Indian Spinach than non-inoculated. The percent seedling emergence increased over control in mycorrhizal treated pot was 15.04, 23.01 and 18.46 at 7, 10 and 15 days after sowing, respectively. The highest seedling emergence was 77% in mycorrhiza treated pot at 15 days after sowing and the lowest (44.33%) was counted in control condition at seven days after sowing.

Plant height

Results of table 13 showed the effect of Arbuscular mycorrhizal fungi on plant height of Indian Spinach. With the increase of growth period, the plant height was increased both mycorrhizal inoculated and non inoculated plants. In both the cases, at 1^{st} 20 days (20 DAS) and 2^{nd} 20 days (20 to 40 DAS) after sowing the growth increment was lower than the 3^{rd} 20 days (40 to 60 DAS) and the rate of growth was also lower in 1^{st} and 2^{nd} 20 days. The percent plant height increased over control in mycorrhiza inoculated pots were 12.11, 12.93 and 20.04 at 20, 40 and 60 days after sowing, respectively

4.3. INDIAN SPINACH (*Basella alba L*)

Seedling emergence

The influence of inoculation of Arbuscular mycorrhizal fungi on seedling emergence of Indian Spinach is presented in Table 12. The seedling emergences were recorded in three different times. With the elapse of time the seedling emergence increased in both treatments. But significantly higher seedling emergence was found in case of inoculated Indian Spinach than non-inoculated. The percent seedling emergence increased over control in mycorrhizal treated pot was 15.04, 23.01 and 18.46 at 7, 10 and 15 days after sowing, respectively. The highest seedling emergence was 77% in mycorrhiza treated pot at 15 days after sowing and the lowest (44.33%) was counted in control condition at seven days after sowing.

Plant height

Results of table 13 showed the effect of Arbuscular mycorrhizal fungi on plant height of Indian Spinach. With the increase of growth period, the plant height was increased both mycorrhizal inoculated and non inoculated plants. In both the cases, at 1st 20 days (20 DAS) and 2nd 20 days (20 to 40 DAS) after sowing the growth increment was lower than the 3rd 20 days (40 to 60 DAS) and the rate of growth was also lower in 1st and 2nd 20 days. The percent plant height increased over control in mycorrhiza inoculated pots were 12.11, 12.93 and 20.04 at 20, 40 and 60 days after sowing, respectively

Table 12. Influence of Arbuscular Mycorrhizal Fungi (AMF) inoculation on seedling emergence (%) of Indian Spinach at different periods

Treatments	Seedling emergence (%)			% Increased over control		
	7 DAS	10 DAS	15 DAS	7 DAS	10 DAS	15 DAS
Non-inoculated (Control)	44.33b	55.00b	65.00b	----	----	----
Inoculated (Mycorrhiza)	51.00a	67.66a	77.00a	15.04	23.01	18.46

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Table 13. Influence of AMF inoculation on plant height of Indian Spinach at different growth stages

Treatments	Plant height (cm)			% Increased over control		
	20 DAS	40 DAS	60 DAS	20 DAS	40 DAS	60 DAS
Non-inoculated (Control)	12.79b	27.28b	43.81b	----	----	----
Inoculated (Mycorrhiza)	14.34a	30.81a	52.59a	12.11	12.93	20.04

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Root growth

Influence of AMF inoculation on root growth is presented in Table 14. The root length of mycorrhizal plants in both harvested period (40 and 60 days) was significantly higher in comparison to non mycorrhizal plants. It is also observed that the rate of root length increment at 40 days after sowing was higher than in 60 days after sowing in both treatments, respectively. With the increase of growth period, the root weight was increased in treated plant and in control plant, but the activity of mycorrhiza is reduced due to the effect of continuous flooded condition. The percent root weight increased over control in mycorrhizal plant was 83.24, 11.61(Fresh) and 135.29, 35.89 (Dry) after 40 and 60 days sowing, respectively.

Shoot growth

Shoot length and shoot weight (Fresh and dry) of Indian Spinach is presented in Table 15. Mycorrhizal inoculation significantly enhanced plant shoot length in comparison to non inoculated plant. Among the mycorrhizal plant, the rate of shoot weight in 20 days duration (40 to 60 days) was lower (74.93%) in comparison to non mycorrhizal plant (166.90%). Because the activity of mycorrhiza is reduced due to continuous flooded condition. The percent shoot weight increased over control in mycorrhizal plants was 81.60, 19.01 (Fresh) and 100.97, 19.94 (Dry) at 40 and 60 days after sowing, respectively. Some variation in shoot length of Indian Spinach always was found in every growth period and maximum variation (8.78 cm) was found at 60 days after sowing between mycorrhizal and non mycorrhizal plant.

Table 14. Influence of AMF inoculation on root growth of Indian Spinach at different growth stages

Treatments	Root length (cm)		Root weight (g)				% Root weight increased over control			
			Fresh		Dry		Fresh		Dry	
	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS
Non-inoculated (Control)	20.00b	22.55b	3.88b	5.77b	0.51b	0.78b	----	----	----	----
Inoculated (Mycorrhiza)	23.33a	28.55a	7.11a	6.44a	1.2a	1.06a	83.24	11.61	135.29	35.89

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Table 15. Influence of AM inoculation on shoot growth of Indian Spinach at different growth stages

reatments	Shoot length (cm)		Shoot weight (g)				%Shoot weight increas over control			
			Fresh		Dry		Fresh		Dry	
	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DA
on-inoculated Control)	27.28b	43.81b	15.11b	40.33b	1.03b	3.56b	----	----	----	----
noculated Mycorrhiza)	30.81a	52.59a	27.44a	48.00a	2.07a	4.27a	81.60	19.01	100.97	19.[illegible]

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Mycorrhizal dependency

Mycorrhizal dependency is the degree to which a host relies on the mycorrhizal condition to produce maximum growth at a given level of soil fertility. Arbuscular mycorrhiza and vegetable crops have an obligate nutritional dependency. The mycorrhizal dependency of Indian Spinach was 18.57%.

Root colonization

The highest percent root colonization (36.13%) of mycorrhiza inoculated plants was recorded at 60 DAS and lowest (18.65%) at 20 DAS. On the other hand no root colonization was found in untreated control plants.

Nutrient uptake

Inoculation of arbuscular mycorrhizal fungi responsed to nutrients uptake (N, P, K, Zn and Fe) by Indian Spinach are presented in Table 16. It is evident from the study that mycorrrhizal inoculation significantly enhanced nutrient uptake in shoot with comparison to control plant.The percent nutrient uptake increased over control for N and P is 20.16% and 22.78%, respectively. The highest percent increased was obtained with P which was followed by N. K is constant in both inoculated and non inoculated plant. The Zn and Fe were present in 0.0112% and 0.0008% in treated plant, but not present in control plant.

Table 16. Effect of AM inoculation on nutrient uptake by Indian Spinach shoots

reatments	Nutrient uptake by shoot				
	N (%)	P (%)	K (%)	Zn (%)	Fe (%)
on-inoculated Control)	1.19b	0.79b	3.0b	----	----
ıoculated Mycorrhizal)	1.43a	0.97a	3.0a	0.0112	0.0008
Increased over ontrol	20.16	22.78	0.00	----	----

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

A **B**

Fig.3. Influence of Mycorrhizal inoculation on growth of Indian Spinach

A = Mycorrhiza inoculated plants

B = Untreated (control) plants

4.4. WHITE GOURD (*Cucurbita vulgaris*)

Seedling emergence

The influence of inoculation of Arbuscular mycorrhizal fungi on seedling emergence of White gourd is presented in Table 17. The seedling emergences were calculated in three different times. With the passing of time the seedling emergence increased in both the treatments. But significantly higher seedling emergence was found in case of inoculated White gourd than non-inoculated. The percent seedling emergence increased over control in mycorrhizal treated pot was 27.71, 18.36 and 11.05 at 7, 10 and 15 days after sowing, respectively. The highest seedling emergence was 63.67% in mycorrhiza treated pot at 15 days after sowing and the lowest seedling (35.00 %) was counted in control condition at seven days after sowing.

Plant height

The trend of overall increment of plant height of White gourd among different period of growth for both mycorrhizal and non mycorrhizal plants was more or less similar (Table 18). In both the cases, at 1^{st} 20 days (20 DAS) and 2^{nd} 20 days (20 to 40 days) after sowing, the growth increment were higher and the rate of growth was also higher at 1^{st} and 2^{nd} 20 days also. In case of percent growth increment for mycorrhizal and non mycorrhizal plant, it was observed that the increase was minimum (10.53% in inoculated and 22.28% in non-inoculated) in 3^{rd} 20 days (40 to 60 days) and it was maximum (230.20% in inoculated and 209.73% non-inoculated) in 2^{nd} 20 days (20 to 40 days). The percent plant height increased over control in mycorrhiza inoculated pot was 17.38, 25.13 and 13.11 at 20, 40 and 60 days after sowing, respectively.

Table 17. Influence of Arbuscular Mycorrhizal Fungi (AMF) inoculation on seedling emergence (%) of White gourd at different periods

Treatments	Seedling emergence (%)			% Increased over control		
	7 DAS	10 DAS	15 DAS	7 DAS	10 DAS	15 DAS
Non-inoculated (Control)	35.00b	49.00b	57.33b	----	----	---
Inoculated (Mycorrhiza)	44.00a	58.00a	63.67a	27.71	18.36	11.05

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Table 18. Influence of AMF inoculation on plant height of White gourd at different growth stages

Treatments	Plant height (cm)			% Increased over control		
	20 DAS	40 DAS	60 DAS	20 DAS	40 DAS	60 DAS
Non-inoculated (Control)	17.66b	54.7b	66.89b	----	---	---
Inoculated (Mycorrhiza)	20.73a	68.45a	75.66a	17.38	25.13	13.11

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Root growth

Root length and root weight (Fresh and dry) of White gourd is presented in Table 19. The root length of mycorrhizal plants in both harvested period (40 days and 60 days) was significantly higher in comparison to non mycorrhizal plants. In case of AM inoculation, the root weight of White gourd was significantly higher than the non inoculated plants. The percent root weight increased over control in mycorrhizal plant was 35.33, 42.85 (Fresh) and 60.00, 54.41 (dry) after 40 and 60 days of sowing, respectively.

Shoot growth

Influence of AM inoculation on shoot growth of White gourd harvested at different periods has been shown in Table 20. In every growth period the shoot weight and shoot length were always higher in inoculated plants than in non inoculated plants. Among the mycorrhizal plants, the rate of shoot length increment in 20 days duration (40 to 60 days) was more (29.66%) in comparison to non mycorrhizal plants (22.00%). The percent shoot weight increased over control in mycorrhizal plant was 39.32, 48.06 (Fresh) and 51.69, 40.30 (dry) after 40 and 60 days sowing, respectively.

Table 19. Influence of AMF inoculation on root growth of White gourd at different growth stages

Treatments	Root length (cm)		Root weight (g)				% Root weight increased over control			
			Fresh		Dry		Fresh		Dry	
	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS
Non-inoculated (Control)	41.66b	50.5b	2.83b	3.5b	0.5b	0.68b	----	----	----	----
Inoculated (Mycorrhiza)	48.59a	56.25a	3.83a	5.00a	0.8a	1.05a	35.33	42.85	60.00	54.41

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Table 20. Influence of AMF inoculation on shoot growth of White gourd at different growth stages

eatments	Shoot length (cm)		Shoot weight (g)				%Shoot weight increased over control			
			Fresh		Dry		Fresh		Dry	
	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS
n- culated ontrol)	54.7b	66.63b	25.00b	30.5b	2.36b	3.25b	----	----	----	----
culated ycorrhiza)	68.45a	75.66a	34.83a	45.16a	3.58a	4.56a	39.32	48.06	51.69	40.30

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Disease suppression

Inoculation of pot grown White gourd plants with VA mycorrhizal plants resulted by the endophyte by the accompany reduction in the incidence of diseases are presented in Table 21. The damping off disease incidence was 11.67% in non inoculated and 4.38% in inoculated mycorrhizal plants. Leaf spot disease incidence was 5.62% in control plants, but in inoculated plant, there was no leaf spot disease in inoculated plant. Root rot disease was 7.57% in control plant and 3.68% in treated plant. The disease incidence was always significantly higher in uninoculated control plant in compare to inoculated mycorrhizal plant.

Table 21. Effect of AMF inoculation of White gourd in suppression of different diseases at seedling stage

Treatments	Infected plant (%)		
	Foot rot	Damping off	Leaf spot
Noninoculated (Control)	7.57a	11.67a	5.62a
Inoculated (Mycorrhiza)	3.68b	4.38b	---

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Mycorrhizal dependency

Mycorrhizal dependency is the degree to which a host relies on the mycorrhizal condition to produce maximum growth at a given level of soil fertility. Arbuscular mycorrhiza and vegetable crops have an obligate nutritional dependency. The mycorrhizal dependency of White gourd was 29.95%.

Root colonization

The highest percent root colonization (53.48%) of mycorrhiza inoculated plants was recorded at 60 DAS and lowest (36.52%) at 20 DAS. On the other hand no root colonization was found in untreated control plants.

Nutrient Uptake

Mycorrhizal inoculation significantly enhanced nutrient uptake (N, P, K, Zn and Fe) compared to control in White gourd plants (Table 22). The percent nutrient uptake increased over control for N, P, K, Zn and Fe were 29.32%, 7.78%, 5.26%, 12.82% and 316.67%, respectively. The maximum nutrient uptake increased in inoculated plant was recorded Fe (316.67%) and the minimum was K (5.26%).

Table 22. Effect of AMF inoculation on nutrient uptake by White gourd shoots

Treatments	Nutrient uptake by shoot				
	N (%)	P (%)	K (%)	Zn (%)	Fe (%)
Non-inoculated (Control)	1.33b	0.90b	3.8b	0.0078b	0.0072b
Inoculated (Mycorrhizal)	1.72a	0.97a	4.0a	00088a	0.03a
%Increased over control	29.32	7.78	5.26	12.82	316.67

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

4.5. CUCUMBER (*Cucumis sativus*)

Seedling emergence

The influence of inoculation of Arbuscular mycorrhizal fungi on seedling emergence of Cucumber is presented in Table 23. The seedling emergences were calculated in three different times. With the elapse of time the seedling emergence increased in both the treatments. But significantly higher seedling emergence was found in case of inoculated Cucumber than non-inoculated. The percent seedling emergence increased over control in mycorrhizal treated pot were 10.00, 9.09 and 12.72 at 7, 10 and 15 days after sowing, respectively. The highest seedling emergence was 62% in mycorrhiza treated pot at 15 days after sowing and the lowest (50.00%) was counted in control condition at seven days after sowing.

Plant height

Results of table 24 showed the effect of Arbuscular mycorrhizal fungi on plant height of Cucumber. With the increase of growth period, the plant height was increased both mycorrhizal inoculated and non inoculated plants. In both the cases, at 1st 20 days (20 DAS) and 3rd 20 days (40 to 60 DAS) after sowing the growth increment was higher than the 2nd 20 days (20 to 40 DAS) and the rate of growth was also higher in 1st and 3rd 20 days. The percent plant height increased over control in mycorrhiza inoculated pots was 11.22, 10.69 and 15.82 at 20, 40 and 60 days after sowing, respectively.

Table 23. Influence of Arbuscular Mycorrhizal Fungi (AMF) inoculation on seedling emergence (%) of Cucumber at different periods

Treatments	Seedling emergence (%)			% Increased over control		
	7 DAS	10 DAS	15 DAS	7 DAS	10 DAS	15 DAS
Non-inoculated (Control)	50.00b	55.00b	55.00b	----	----	----
Inoculated (Mycorrhiza)	55.00a	60.00a	62.00a	10.00	9.09	12.72

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Table 24. Influence of AMF inoculation on plant height of Cucumber at different growth stages

Treatments	Plant height (cm)			% Increased over control		
	20 DAS	40 DAS	60 DAS	20 DAS	40 DAS	60 DAS
Non-inoculated (Control)	29.67b	87.33b	102.31b	----	---	---
Inoculated (Mycorrhiza)	33.00a	96.67a	118.50a	11.22	10.69	15.82

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Root growth

Influence of AM inoculation on root growth is presented in Table 25. The root length of mycorrhizal plants in both harvested period (40 and 60 days) was significantly higher in compassion to non mycorrhizal plants. It was also observed that the rate of root length increment at 60 days after sowing was higher than in 40 days after sowing in both the treatments, respectively. With the increase of growth period, the root weight was increased in treated plant and control plant, but the percent root weight increased over control in mycorrhizal plant was 5.26, 20.75 (Fresh) and 15.38, 25.80 (Dry) after 40 and 60 days sowing, respectively.

Shoot growth

Shoot length and shoot weight (Fresh and dry) of Cucumber is presented in Table 26. Mycorrhizal inoculation significantly enhanced plant shoot length in comparison to non inoculated plant. Among the mycorrhizal plant, the rate of shoot weight increment in 20 days duration (40 to 60 days) was higher (32.732%) in comparison to non mycorrhizal plant (31.17%). The percent shoot weight increased over control in mycorrhizal plants was 9.30, 8.20 (Fresh) and 8.33, 40.00 (Dry) after 40 and 60 days sowing, respectively. Some variation in shoot length of Cucumber always was found in every growth period and maximum variations (21.83 and 14.98 cm) were found at 40 days after sowing between mycorrhizal and non mycorrhizal plant.

Table 25. Influence of AMF inoculation on root growth of Cucumber at different growth stages

Treatments	Root length (cm)		Root weight (g)				% Root weight increased over control			
			Fresh		Dry		Fresh		Dry	
	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS
Non-inoculated (Control)	46.33b	50.66b	9.5b	14.50b	1.3b	3.10b	----	----	----	----
Inoculated (Mycorrhiza)	52.66a	61.35a	10.00a	17.50a	1.50a	3.9a	5.26	20.75	15.38	25.80

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Table 26. Influence of AMF inoculation on shoot growth of Cucumber at different growth stages

Treatments	Shoot length (cm)		Shoot weight (g)				%Shoot weight increase over control			
			Fresh		Dry		Fresh		Dry	
	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS
Non-inoculated (Control)	87.33b	102.31b	43.00b	57.65b	6.00b	7.50b	----	----	----	----
Inoculated (Mycorrhiza)	96.67a	118.50a	47.00a	62.38a	6.5a	10.5a	9.30	8.20	8.33	40.00

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.

Mycorrhizal dependency

Mycorrhizal dependency is the degree to which a host relies on the mycorrhizal condition to produce maximum growth at a given level of soil fertility. Arbuscular mycorrhiza and vegetable crops have an obligate nutritional dependency. The mycorrhizal dependency of Cucumber was 26.38%.

Root colonization

The highest percent root colonization (46.00%) of mycorrhiza inoculated plants was recorded at 60 DAS and lowest (28.00%) at 20 DAS. On the other hand no root colonization was found in untreated control plants.

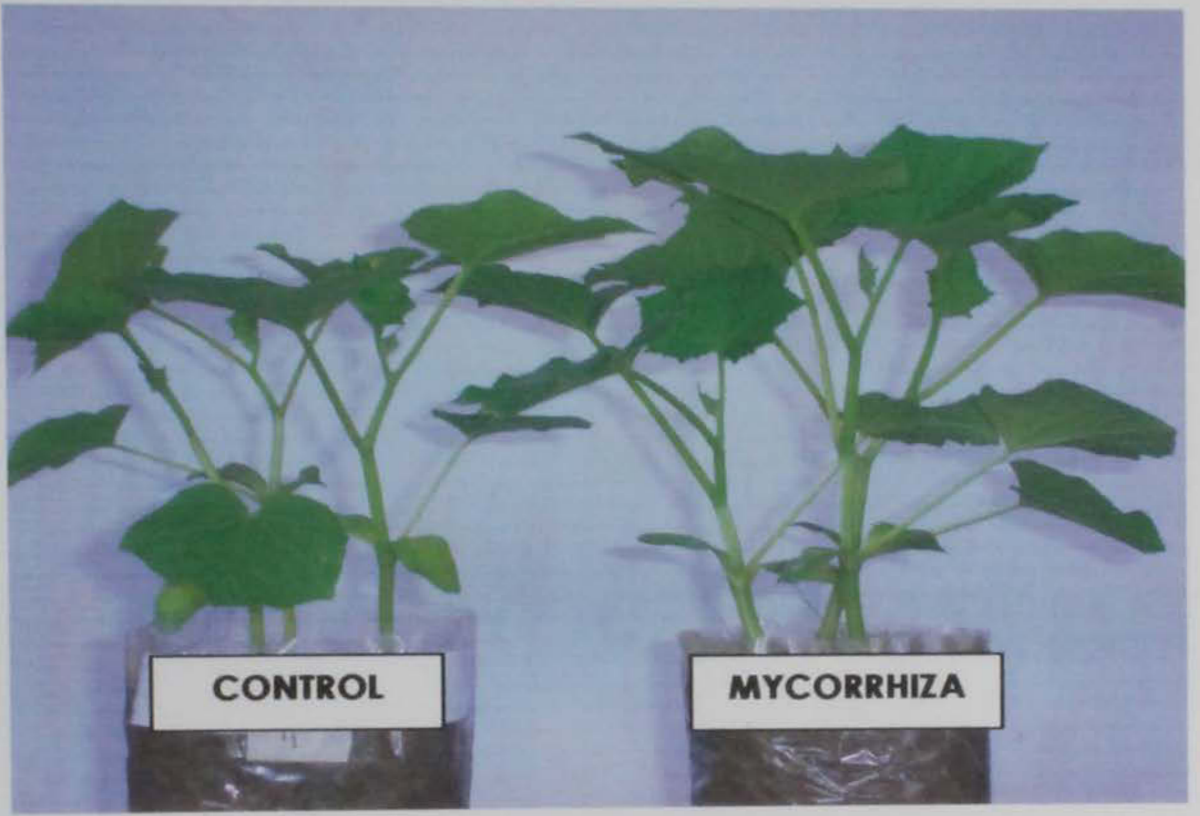
Nutrient uptake

Inoculation of arbuscular mycorrhizal fungi responsed to nutrients uptake (N, P, K, Zn and Fe) by Cucumber is presented in Table 27. It is evident from the study that mycorrhizal inoculation significantly enhanced nutrient uptake in shoot with comparison to control plant.The percent nutrient uptake increased over control for N, P, Zn and Fe were 23.53%, 47.62%, 225.92% and 463.38%, respectively. The percent K uptake was constant in both inoculated and control plant. The highest percent increased was obtained with Fe followed by P, Zn and the lowest was found with N.

Table 27. Effect of AMF inoculation on nutrient uptake by Cucumber shoots

Treatments	Nutrient uptake by shoot				
	N (%)	P (%)	K (%)	Zn (%)	Fe (%)
Non-inoculated (Control)	1.19b	0.69b	3.2a	0.0027b	0.0071b
Inoculated (Mycorrhiza)	1.47a	0.93a	3.2a	0.0088a	0.04a
%Increased over control	23.53	47.62	0.00	225.92	463.38

In a column means followed by uncommon letters are significantly different at 5% level of significance by T-test.



B **A**

Fig.4. Influence of Mycorrhizal inoculation on growth of Cucumber

A = Mycorrhiza inoculated plants

B = Untreated (control) plants

Chapter 5

Discussion

5. DISCUSSION

Bangladesh is one of the most densely populated countries in the world. It is essential to improve crop production for the burgeoning human population and to meet the increasing demands for food. Because of economic and environmental constrains, it is necessary to develop least-expensive and technologically simple methodologies for immediate benefit of crop production. Mycorrhizal technology as a low imputes and nature farming technique can be one of the alternatives to improve crop production, farm profitability and environmental quality (Mridha *et al.*, 1991). The present experiment has been carried out to determine the role of AM fungi on growth and nutrient uptake of five vegetables namely Spinach, Indian Spinach, Water Spinach, White Gourd and Cucumber.

The results of seedling emergence of inoculated pots were increased over control in all the selected vegetable crops. Spinach (*Spinacia oleracea L.*) performed the highest seedling emergence followed by Indian Spinach (*Basella alba L*), Water Spinach (*Impomea aquatica*), White Gourd (*Cucurbita vulgaris*) and the lowest was recorded with Cucumber (*Cucumis sativus*). The high MD value suggests that mycorrhizal inoculation would be useful in producing vigorous seedlings in nursery. The present findings are in agreement 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 VAMF inoculation to Spinach, Water Spinach, Indian Spinach, White Gourd and Cucumber studied in the present investigation. AM fungi promote phosphate uptake in low phosphate soils during the early stages of plant growth. Arbuscular Mycorrhizal inoculation significantly increased the plant height of the selected vegetables over uninoculated control plant (Sasai, 1991). The highest per cent plant height increased over control in mycorrhiza inoculated plants were 37.52% in Spinach (20 DAS), 25.30% in White Gourd (40 DAS) and 20.04% in Indian Spinach (60 DAS). Under nursery conditions, mycorrhizal inoculation improved growth of seedlings (Giri *et al.*, 2005).

Introduced *Glomus caledonium* was effective in improving plant growth and dual inoculation with indigenous VAM fungi was most effective. The tripartite system, indigenous VAM, *G. caledonium* and *Rhizobium phaseoli* improved plant growth and also resulted in increased nodulation and nitrogenase activity (Mathew and Johri, 1989). The growth response of greenhouse cucumber (*Cucumis sativus L.*) to infection by vesicular-arbuscular mycorrhizal (VAM) fungi and phosphorus (P) nutrition was characterized over 38 days of plant establishment. Plants were highly receptive to colonization by *Glomus mosseae*, *G. dimorphicum* and *G. intraradices* and increased growth with selected vegetable crops (Trimble and Knowles, 1995).

Root length, fresh and dry weight of roots was found higher in all the inoculated plants species. This was probably due to uptake of nutrient, which increased vegetative growth. Comparatively higher root lengths were recorded in inoculated treatment over control. Inoculated plants had significantly higher root biomass than corresponding uninoculated plants (Sreeramulu *et al.*, 1996). In inoculated plant, the highest per cent increased of root weight over control were 101.81% in Spinach, 42.85% in White Gourd (fresh weight) and 135.23% in Indian Spinach, 83.33% in Spinach (dry weight) at 40 and 60 days after sowing, respectively. The increased root growth as reported in this study is in agreement with Gaur and Adholeya (2000). They reported the higher growth with some other agricultural crops such as onion, potato and garlic.

Significant increase in shoot weight over control was recorded with all the crops at 40 DAS and 60 DAS. This was probably due to uptake of nutrient, which increased vegetative growth and hence greater translocation of photosynthates from leaf to shoot and thereby enhanced shoot growth and weight. Inoculated plants had significantly higher shoot biomass than corresponding uninoculated plants (Sreeramulu *et al.*, 1996). In inoculated plant, the highest per cent increased of shoot weight over control were 83.24% in Indian Spinach, 48.06% in White Gourd (fresh weight) and 135.29% in Indian Spinach, 50.96% in Spinach (dry weight) at 40 and 60 days after sowing, respectively. It was reported that shoot biomass was significantly improved in all cases of inoculated plants (Tarafdar and Praveen, 1996). Present 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 and seed yields and of these isolates resulted larger increases in 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 shoot and root increased when the plants were inoculated with VAMF (Matsubara *et al.*, 1994). Shoot height, weight and number of shoots produced was greater in bioagents (*Glomus intraradices* and *Trichoderma harzianum)* treated plants than in plants inoculated only with *F. oxysporum* (Arriola, 1997). Root and shoot dry weights were higher in mycorrhizal than non-mycorrhizal plants (Giri *et al.*, 2005).

The different plant diseases like root rot; damping off and leaf spot were recorded in Spinach. The leaf spot disease was completely absent in White Gourd seedlings in mycorrhizal condition. The percent infection of seedlings was 6.57, 10.66 and 7.28 in control condition whereas 2.21, 2.53 and 3.67% in inoculated condition. More or less similar results were reported by the previous investigators. Rosendahl and Rosendahl (1991) reported that VAM inoculation before or simultaneous with the inoculation of the pathogen increased survival of the seedlings. Death rates of biocontrol (*Glomus intraradices* and *Trichoderma harzianum)* treated plants were less than half those of plants inoculated only with *F. oxysporum* (Arriola, 1997). Arbuscular Mycorrhiza decreased plant susceptibility to diseases (Jalali and Chand, 1988). The mycorrhizal fungus reduced the number of sclerotia produced by *Sclerotium rolfsii* (Krishna and Bagyaraj, 1983). Inoculation of *Cicer arietinum* plants with VA mycorrhiza resulted in extensive root colonization by the endophyte and an accompanying reduction in the incidence of wilt caused by *F oxysporum tsp. ciceri* (26.6% in mycorrhizal plants, 80% in non-mycorrhizal). Simultaneous inoculation of *Cicer arietinum* with vesicular arbuscular mycorrhizal fungi (*Glomus mosseae, G. constrictum*) and *Fusrium oxysporum* f.sp. *ciceris* had no effect on wilt incidence in the susceptible JG 62 and resistant WR 315 genotypes (Jalali and Thareja, 1981). On transplantation to the field, the survival rate of mycorrhizal seedlings (75%-90%) was higher than that of non-mycorrhizal seedlings (40%) (Giri *et al.*, 2005).

Mycorrhizal dependency (MD) of the selected vegetables ranged from 18.57 to 36.36 %. The highest mycorrhizal dependency (36.36 %) was observed in Spinach that was superior to all other vegetable crops which was followed by White Gourd (29.95 %), Cucumber (26.38%) and Water Spinach (20.63%) and lowest MD in Indian Spinach (18.57%). Mycorrhizal dependency recorded in the present research programme with the selected vegetable crops is similar with other vegetables and agricultural crops recorder by other investigators. Matsubara *et al.*, (1994) reported that the highest mycorrhizal dependency in the vegetable of Liliaceae. Khalil *et al.* (1994) divulged that Soybean had a higher MD than corn but considerable variation occurred within Soybean cultivars. They also observed that the VAMF colonization of roots ranged from 62 to 87 % for Soybean and 49 to 68 % for corn. *Prosopis cineraria* showed tremendous dependency (226.8%) of *Scutellospora calospora* for biomass production and nutrient uptake (Mathur and Vyas, 1995).

Nutrient uptake was increased for the inoculation of AM fungi in all selected vegetables. The association of AM fungi increases uptake of immobile nutrients especially phosphorus and micronutrients (Douds and Miller, 1999). Potasium uptake was influenced significantly by the inoculation of AM fungi over control in some of the crops but other nutrient was higher amount for other crops. The maximum percent nutrient uptake increased over control plants for P, Zn and Fe in Cucumber and K in Spinach and N in Water Spinach. N, P, K, Zn and Cu concentration was significantly improved in all cases of inoculated plants (Tarafdar and Praveen, 1996). Nutrient uptake by other crops recorded by many other scientists is similar with our study. Khan *et al.* (1995) identified that nitrogen fixation as well as N and P contents in groundnut increased only by dual inoculation with VAM 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). 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. Sivaprasad and Rai (1991) recorded that soil inoculation with *Glomus fasciculatum* and *Rhizobium* sp. increased nodulation by 178%, nitrogenous activity by 185% growth and N and P contents compared with inoculation with *Rhizobium* alone. Krishna and Bagyaraj (1982) reported that the effect of VA mycorrhizal and soluble phosphorus on *Abelmoscus esculentus* (L.). Phosphorus uptake in both shoot and root was significantly higher in inoculated plants over control (Sreeramulu *et al.* 1996). Furland and Cardon (1989) reported the effect of N, P and K on formation of vesicular arbuscular mycorrhiza growth and mineral content of onion. The concentration of P, K, Cu, Zn and Na was significantly higher in AM inoculated seedlings than in non-inoculated seedlings (Giri *et al.*, 2005). The present study indicated that mycorrhizal inoculation increased the growth and nutrient uptake of several vegetable crops inoculated under control conditions and this technology may be useful for growth of vegetable crops in our country. Under organic farming management practices, inoculated bioagent i.e. Arbuscular mycorrhizal fungi increased the yield of vegetables (Srivastava *et al.*, 2007).

Chapter 6

Summary and Conclusion

6. SUMMARY AND CONCLUSION

A pot experiment was conducted to study the effectiveness of AM fungi on growth and nutrient uptake of five vegetables during May to December,2006. The selected vegetables were Spinach, Indian Spinach, Water Spinach/Kalmishak, White Gourd and Cucumber. The studies included the seedling emergence, plant growth and assessment of percent root colonization, Arbuscular mycorrhizal dependency and nutrient uptake of these vegetables.

The seedling emergences were influenced by AM inoculation. Seedling emergences were recorded at 7, 10 and 15 days after sowing and all in recorded period, inoculated Spinach showed higher seedling emergence. The maximum percent seedling emergence increased in Spinach and Indian Spinach were 90.00% and 77.00% at 15 days after sowing over control, respectively, whereas the minimum seedling emergence was recorded in White Gourd (44.00%) at 7 days after sowing.

AM inoculation resulted the highest plant growth in all growth stages for all vegetables. The crops showed the per cent increase of plant height, root weight, shoot weight with AM inoculation over control. The highest plant height was recorded in Spinach with AM inoculation which was 37.52% increased over untreated control followed by 17.38% in White Gourd, 12.11% in Indian Spinach, 11.22% in Cucumber and 6.01% in Water Spinach. The highest percent increased of root weight was recorded in Spinach (101.81%) at 40 DAS and White Gourd (42.85%) at 60 DAS whereas the highest % increased of shoot growth over control was observed in Indian Spinach (83.24%) at 40 DAS and White Gourd (48.04%) at 60 DAS. Effect of AM inoculation on disease development was recorded in Spinach and White Gourd. VA mycorrhiza inoculation resulted an accompanying reduction of incidence of damping off, leaf spot and root rot of all Spinach and

White Gourd. The percent root colonization of mycorrhizal fungi in all vegetables was recorded and it was differed from vegetable to vegetable. The highest root colonization was obtained in White Gourd (53.48%) and the lowest in Indian Spinach (18.65%).

All inoculated vegetables had mycorrhizal colonization percentage (MCP) and increased dry mater values than their uninoculated counterparts. The dependency of different vegetables ranged from 18.57% to 36.36%. The highest mycorrhizal dependency was found in Spinach (36.36%) and the lowest was found in Indian Spinach (18.57%). The nutrient uptake (N, P, K, Zn and Fe) was highly influenced by AM inoculation. The highest N, P and K nutrient uptake was calculated in Spinach and Water Spinach, respectively whereas Zn in Spinach and Fe in Cucumber.

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