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Effect of arbuscular mycorrhizal fungi on growth and nutrient uptake of some vegetable crops

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ABSTRACT

The present experiment was conducted in the net house of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh with a view to studying the role of Arbuscular Mycorrhizal (AM) fungi on growth and nutrient uptake of some vegetable crops. Root segment of *Cassia tora* along with rhizosphere soil was used as inocula. In inoculated pot, raw inocula were used and in control pot sterilized root and soil inocula were applied to ensure same nutritional condition. Different number of seeds was sown based on seed size of the selected vegetables. A positive growth response to AM was observed in all the selected vegetables such as spinach (*Spinacia oleracea* L.), Indian spinach (*Basella alba* L.) and water spinach (*Ipomoea aquatica*). The seedling emergence, plant height, root length and shoot length of mycorrhiza inoculated vegetables were comparatively higher than that of un-inoculated control. Mycorrhizal root colonization differed among the crops ranging from 18.65 to 44.26%. The mycorrhizal inoculation suppressed root rot, damping off and leaf spot disease of spinach, Indian spinach, and water spinach. Mycorrhizal dependency was ranged 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 and water spinach whereas Zn and Fe uptake was found higher in spinach, respectively. Arbuscular Mycorrhizal fungi showed positive response in case of plant growth parameters and it could be used as a bio-fertilizer.

Introduction

Vegetables constitute a very important group of crops in Bangladesh as well as important component of cropping systems in south and west Asia. Eggplant, radish, cabbage, cauliflower and pumpkin like vegetables gave returns at least three times higher than rice (Ateng, 1998). In addition, the economic returns in terms of domestic resource cost at export parity also indicate that there is a comparative advantage in the production of vegetable in Bangladesh (Shahabuddin & Dorosh, 2002). They are important for their short production time and high nutritive value. Spinach, Indian spinach, and water spinach are mainly cultivated as vegetables in our country. In general, per hectare yield of these crops are low. The country requires a sustainable technology where an agricultural out put can be high as well as minimum residual effects of chemical fertilizer.

Mycorrhiza is the mutualistic association between soil- borne fungi and the roots of plants. Vesicular arbuscular mycorrhizal (VAM) fungi are known to colonize a number of tropical plants including vegetables (Reddy et al., 2006). Mycorrhizal plants are known to have altered nutritional status, increased photosynthetic rates, altered levels of growth regulating substances and altered patterns of root exudation due to changes in membrane permeability. Roots of living plants support the growth of a complex of microbes which create a special habitat that influences growth and survival of the plants (Joshi, 2003). VAM fungi are now-a-days well recognized as bio-fertilizer due to the host plant besides increasing nutrient and water uptake (Bohra et al., 2007). Many reports have indicated that VAM can decrease the severity of diseases caused by root pathogenic fungi, bacteria and nematodes. Reduction of the effects of pathogenic roots is infecting fungi like *Macrophomina phascolina, Rhizoctonia solani* by this fungus, *Glomus mosseae* (Zambolim & 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 & Bagyaraj, 1983). Keeping the above views in mind, the present investigation was undertaken to evaluate the effect of AM fungus on growth, nutrient uptake and disease suppression and yield of some selected vegetables.

Materials and methods

Study area

The experiment was conducted in the net house and in the seed health laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka during the period from May, 2006 to December, 2006.

Plant materials

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

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

Experimental field and implementation of crop

Cassia tora roots were used as inocula which were collected from Agronomy field along with rhizosphere soil. The presence of AM fungi within the root sample was confirmed using the staining procedure of Koske and Gemma (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 ºC at 15 PSI for 15 minutes and used as base materials for control pots. The polythene bags (8" × 12", 2 kg) 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 two-third portion of the seedling bags were filled with substratum. Then a layer of both inoculums i.e. root inoculum 25 g and soil inoculum 100 g were placed in each treated bag. 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. Sixty polythene bags (3 × 2 × 10) were prepared for three crops in this study. For different crops different number of seeds was sown in the bags based on seed size. For spinach 20 seeds/bag, Indian spinach 10 seeds/bag and water spinach 10 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.

Data collection

Data were recorded on seedling emergence (%) (7 days after sowing, 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) and disease incidence. Total nitrogen content in plants samples (shoot) were determined by micro Kjeldahl method (Bremner, 1965). 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. Available other elements like Fe and Zn were determined following ASI method (Hunter, 1984). Nutrient uptake was calculated by using the following formula:

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

Results

The influence of inoculation of AM fungi on seedling emergence of spinach, water spinach and Indian spinach was 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 plant than non-inoculated. For spinach, the per cent seedling emergences increased over control in mycorrhizal treated pots was 16.5, 17.1 and 2.2, for water spinach 10.0, 17.1 and 5.5 and for Indian spinach 15.0, 23.0 and 18.4 at 7, 10 and 15 days after sowing, respectively. The highest seedling emergence was 90% (spinach), 76% (water spinach) and 77% (Indian spinach) in mycorrhiza treated pot at 15 days after sowing and the lowest 56.3%, 50% and 44.3% was counted in control condition at 7days after sowing for spinach, water spinach and Indian spinach.

Table 1. Influence of Arbuscular Mycorrhizal (AMF) inoculation on seedling emergence (%) of spinach, water spinach and indian spinach

Name of the crop	Treatment	Seedling emergence (%) days after seed germination			Seedling emergence (%) increased over control		
		7 DAS	10 DAS	15 DAS	7 DAS	10 DAS	15 DAS
Spinach	Non-inoculated (Control)	56.3 b^{z}	76.0 b	88.0 b	----	----	----
	Inoculated (Mycorrhiza)	65.6 a	89.0 a	90.0 a	16.5	17.1	2.2
Water Spinach	Non-inoculated (Control)	50.0 b	64.0 b	72.0 b	----	----	----
	Inoculated (Mycorrhiza)	55.0 a	75.0 a	76.0 a	10.0	17.1	5.5
Indian Spinach	Non-inoculated (Control)	44.3 b	55.0 b	65.0 b	----	----	----
	Inoculated (Mycorrhiza)	51.0 a	67.6 a	77.0 a	15.0	23.0	18.4

^{z}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, water spinach and indian spinach at different growth stages

Name of the crop	Treatment	Plant height (cm)			Plant height (%) increased over control		
		20 DAS	40 DAS	60 DAS	20 DAS	40 DAS	60 DAS
Spinach	Non-inoculated (Control)	5.3 b[z]	10.3 b	12.6 b	----	----	----
	Inoculated (Mycorrhiza)	7.3 a	12.6 a	14.6 a	37.5	22.6	15.7
Water Spinach	Non-inoculated (Control)	20.1 b	33.3 b	44.1 b	----	----	----
	Inoculated (Mycorrhiza)	21.3 a	36.3 a	48.6 a	6.0	9.1	10.1
Indian Spinach	Non-inoculated (Control)	12.7 b	27.2 b	43.8 b	----	----	----
	Inoculated (Mycorrhiza)	14.4 a	30.8 a	52.5 a	12.1	12.9	20.0

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

Results of table 2 showed the effect of AM fungi on plant height of spinach, water spinach and 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 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 37.5, 22.6 and 15.7 % for spinach, 6.0, 9.1 and 10.1% for water spinach and 12.1, 12.9 and 20.0% for Indian spinach at 20, 40 and 60 days after sowing, respectively. In case of percent growth increment for mycorrhizal and non mycorrhizal water spinach plant, it was observed that the increment was minimum (33.8% in inoculated and 32.6% in non-inoculated) in 3rd 20 days (40 to 60 days) and it was maximum (70.3% in inoculated and 65.4% non-inoculated) in 2nd 20 days (20 to 40 days).

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 101.8, 40.0 (Fresh) and 50.0, 83.3 (dry) for spinach, 20.7 14.5 (Fresh) and 27.1, 14.6 (dry) for water spinach and 83.2, 11.6 (fresh) and 135.2, 35.8 (dry) for Indian spinach after 40 and 60 days sowing, respectively.

Shoot length and shoot weight (fresh and dry) of spinach, water spinach and Indian spinach were presented in Table 4. Mycorrhizal inoculation significantly enhanced plant shoot length in comparison to non inoculated plant. Among the mycorrhizal plant of spinach, the rate of shoot weight increment in 20 days duration (40 to 60 days) was higher (45.9%) in comparison to non mycorrhizal plant (29.1%) for fresh shoot weight. The percent shoot weight increased over control in mycorrhizal plants was 23.4, 39.5 (Fresh) and 46.8, 50.9 (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 (2.3 cm) was found at 40 days after sowing between mycorrhizal and non mycorrhizal plant. Among the water spinach mycorrhizal plants the rate of shoot length increment in 20 days duration (40 to 60 days) was less (36.3%) in comparison to non mycorrhizal plants (48.1%). The percent shoot weight increased over control in mycorrhizal plant was 49.4, 28.9 (fresh) and 25.6, 42.7 (dry) after 40 and 60 days sowing, respectively. For Indian spinach mycorrhizal plant, the rate of shoot weight in 20 days duration (40 to 60 days) was lower (74.9%) in comparison to non mycorrhizal plant (166.9%). The activity of mycorrhiza was reduced due to continuous flooded condition. The percent shoot weight increased over control in mycorrhizal plants was 81.6, 19.0 (fresh) and 100.9, 19.9 (dry) at 40 and 60 days after sowing, respectively. For shoot length maximum variation (8.7 cm) was found at 60 days after sowing between mycorrhizal and non mycorrhizal plant.

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 10.6%, 8.4% and 7.6% in non-inoculated all kind of plant and 2.5% for spinach and 5.0% in water spinach and Indian spinach inoculated mycorrhizal plants. Leaf spot disease was 7.2%, 6.4% and 5.4% in control plants, but in inoculated plant, it was only 3.6%, 3.6% and 3.0% and followed by the root rot disease also. The disease incidence was always significantly higher in non-inoculated control plant in compare to inoculated mycorrhizal spinach, water spinach and Indian spinach plant.

Table 3. Influence of AMF inoculation on root growth of spinach, water spinach and Indian spinach at different growth stages

Name of the crop	Treatment	Root length (cm)		Root weight (g)				Root weight increased over control (%)			
				Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
		40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS
Spinach	Non-inoculated (Control)	5.4 b[z]	8.6 b	0.5 b	0.5 b	0.1 b	0.1 b	----	----	----	----
	Inoculated (Mycorrhiza)	7.6 a	9.7 a	1.1 a	0.7 a	0.1 a	0.2 a	101.8	40.0	50.0	83.3
Water Spinach	Non-inoculated (Control)	21.8 b	29.5 b	9.1 b	10.6 b	1.1 b	2.2 b	----	----	----	----
	Inoculated (Mycorrhiza)	25.5 a	31.4 a	11.0 a	12.2 a	1.0 a	2.5 a	20.7	14.5	27.1	14.6
Indian Spinach	Non-inoculated (Control)	20.0 b	22.5 b	3.8 b	5.7 b	0.5 b	0.7 b	----	----	----	----
	Inoculated (Mycorrhiza)	23.3 a	28.5 a	7.1 a	6.4 a	1.2 a	1.0 a	83.2	11.6	135.2	35.8

[z]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, water spinach and Indian spinach at different growth stages

Name of the crop	Treatment	Shoot length (cm)		Shoot weight (g)				Shoot weight increased over control (%)			
				Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
		40 DAS	60DAS	40 DAS	60DAS	40 DAS	60DAS	40 DAS	60DAS	40 DAS	60DAS
Spinach	Non-inoculated (Control)	10.3 b[z]	12.6 b	3.3 b	4.3 b	0.3 b	0.5 b	----	----	----	----
	Inoculated (Mycorrhiza)	12.6 a	14.6 a	4.1 a	6.0 a	0.4 a	0.7 a	23.4	39.5	46.8	50.9
Water Spinach	Non-inoculated (Control)	33.3 b	44.1 b	8.9 b	14.2 b	1.1 b	1.5 b	----	----	----	----
	Inoculated (Mycorrhiza)	36.3 a	48.6 a	13.4 a	18.3 a	1.4 a	2.1 a	49.4	28.9	25.6	42.7
Indian Spinach	Non-inoculated (Control)	27.2 b	43.8 b	15.1 b	40.3 b	1.0 b	3.5 b	---	----	----	----
	Inoculated (Mycorrhiza)	30.8 a	52.5 a	27.4 a	48.0 a	2.0 a	4.2 a	81.6	19.0	100.9	19.9

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

Table 5. Effect of AMF inoculation of spinach, water spinach and Indian spinach on incidence of different diseases at seedling stage

Name of the crop	Treatment	Infected plant (%)		
		Root rot/ Foot rot	Damping off	Leaf spot
Spinach	Non-inoculated (Control)	6.57 a[z]	10.6 a	7.2 a
	Inoculated (Mycorrhiza)	2.21 b	2.5 b	3.6 b
Water Spinach	Non-inoculated (Control)	4.98 a	8.4 a	6.4 a
	Inoculated (Mycorrhiza)	2.80 b	5.0 b	3.6 b
Indian Spinach	Non-inoculated (Control)	6.00 a	7.6 a	5.4 a
	Inoculated (Mycorrhiza)	4.33 b	5.0 b	3.0 b

[z]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. AM and vegetable crops have an obligate nutritional dependency. The mycorrhizal dependency of spinach, water spinach and Indian spinach were 36.3%, 20.6% and 18.5%, respectively.

Root colonization

The highest percent root colonization (44.2%, 38.6% and 36.1%) of mycorrhiza inoculated plants of spinach, water spinach and Indian spinach was recorded at 60 DAS and lowest (21.8%, 19.8% and 18.6%) at 20 DAS. On the other hand no root colonization was found in untreated control plants of all case.

Inoculation of AM fungi responded to nutrients uptake (N, P, K, Zn and Fe) by spinach, water spinach and Indian spinach are presented in Table 6. It is evident from the study that mycorrrhizal inoculation significantly enhanced nutrient uptake in shoot with comparison to control plant. The percent nutrient uptake of spinach increased over control for N, P, K, Zn and Fe were 36.1%, 31.0%, 13.7% 75.0% and 14.7%, respectively. The highest percent increased was obtained with Zn which was followed by N, P, Fe and the lowest was found with K. In case of water spinach the percent nutrient uptake increased over control for N, P and Zn was 56.2%, 21.6% and 57.8%, respectively. The maximum nutrient uptake increased in inoculated plant was recorded Zn (57.8%) and the minimum was K (0%) and Fe was absent in both mycorrhiza inoculated and non inoculated plant. For Indian spinach the percent nutrient uptake increased over control for N and P is 20.1% and 22.7%, 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 0.2% and 0.8% in treated plant, but not present in control plant.

Table 6. Effect of AMF inoculation on nutrient uptake by Spinach, Water Spinach and Indian Spinach shoots

Name of the crop	Treatment	Nutrient uptake by shoot (%)				
		N	P	K	Zn	Fe
Spinach	Non-inoculated (Control)	2.2 b	0.5 b	2.9 b	0.8 b	0.5 b
	Inoculated (Mycorrhiza)	3.0 a	0.7 a	3.3 a	0.5 a	0.5 a
	%Increased over control	36.1	31.0	13.7	75.0	14.7
Water Spinach	Non-inoculated (Control)	1.1 b	0.8 b	4.4 a	0.9 b	--
	Inoculated (Mycorrhiza)	1.7 a	1.0 a	4.4 a	0.3 a	--
	%Increased over control	56.2	21.6	0.0	57.8	--
Indian Spinach	Non-inoculated (Control)	1.1 b	0.7 b	3.0 b	----	----
	Inoculated (Mycorrhiza)	1.4 a	0.9 a	3.0 a	0.2	0.8
	%Increased over control	20.1	22.7	0.0	----	----

Discussion

There is little information about mycorrhizal colonization of the vegetables. A variety of studies suggest that phosphorus uptake by plant roots can be enhanced when they are infected by AM fungi**.** From the present study all growth parameters under consideration like seedling emergence, height of the plants, length of roots, fresh and dry weight of plants were recorded to be significantly higher in VAM treated plants than the control set. The present findings are in agreement with Vishwakarma and Singh (1996) and Matsubara et al. (1994) who investigated the effects of inoculation with VAM 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. The noticeable plant height was observed in VAM inoculated plant compare to control in this study. The root length and shoot length was increased in positively. Thus is it evidenced from the result that VAM association imparts some beneficial effects on plant growth. AM inoculation significantly increased the plant height of the selected vegetables over un-inoculated control plant (Sasai, 1991). It was reported that the highest percent increased of root growth and shoot growth was recorded in VAM treated plant over control. Inoculated plants had significantly higher root biomass than corresponding non-inoculated plants (Sreeramulu et al, 1996). Tarafdar and Praveen (1996) investigated that shoot biomass was significantly improved in all cases of inoculated plants. Also Giri et al. (2005) reported that root and shoot dry weights were higher in mycorrhizal than non-mycorrhizal plants.

From the result mycorrhizal dependency was found in VAM treated vegetables over control. 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 & Vyas, 1995). The lowest disease severity was observed in VAM treated vegetables than control. Jalali and Thareja (1981) reported that VAM fungi suppress the incidence of wilt caused by *Fusarium oxysporum*. The nutrient uptake (N, P, K, Zn and Fe) was highly influenced by AM inoculation. The finding from Douds and Miller (1999) was that the association of AM fungi increases uptake of immobile nutrients especially phosphorus and micronutrients. The mycorrhizal association is found to be beneficial to the plants in terms of better nutrient uptake and better water potential which lead the plants to become more healthy and productive than non-mycorrhizal plants (Khaleq et al., 2001). Giri et al (2005) investigated that the concentration of P, K, Cu, Zn and Na was significantly higher in AM inoculated seedlings than in non-inoculated seedlings. Srivastava et al (2007) reported that under organic farming management practices, inoculated bioagent i.e. AM fungi increased the yield of vegetables. 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.

Conclusions

Results from the present study indicated that seedling emergence, plant height, root biomass, shoot biomass, incidence of disease, mycorrhizal dependency and nutrient status of different vegetables have been affected significantly by inoculation with VAM fungi. Thus the fungi offer an environmentally sound biological alternative to chemical fertilizer and pesticides for maintaining plant quality and productivity in agriculture in future.

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