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GROWTH-PROMOTING POTENTIAL OF AEROPONICALLY-PRODUCED MYCORRHIZAL FUNGUS *Glomus* SP. INOCULUM COMPARED WITH TRADITIONAL AM INOCULA.

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ABSTRACT

Aeroponically-produced mycorrhizal fungus *Glomus* sp. was compared for growth-promoting potential, with two traditional sources of mycorrhizal nocula, namely soil-based pot culture of *Glomus* sp. and naturally-mixed AM fungi, in a pot experiment using maize and sorghum as host plants, under green house conditions. Four weeks after sowing, only plants which received infected root segments inocula gave moderate increases in plant growth parameters, compared with non-inoculated ones. However, spores inoculum produced non-significant differences in growth parameters of host plants. Eight weeks after sowing, soil-based pot culture treatment produced more root colonization ratios, shoot dry weights and phosphorus contents than those achieved with aeroponic culture treatment, being most pronounced with plants inoculated with infected root segments. At the final harvest, aeroponic culture of *Glomus* sp. produced root colonization ratios and plant-growth improvements similar to that achieved with soil-based pot culture treatment. However, (naturally mixed AM fungi) treatment gave the least increases in these parameters. These results indicated that, no biological differences were found between the inoculum obtained from aeroponic culture and this collected from soil-based pot culture.

Keywords: Aeroponic culture, AM fungi, *Glomus* sp., growth-promoting potential, maize, soil-based pot culture, sorghum.

INTRODUCTION

Arbuscular-mycorrhizal (AM) fungi form symbiotic association with most economically important crop plants, and have been shown to improve the growth of their host plants (Gianinazzi *et al*., 1989). However, as the fungi are considered obligate symbionts, unable to be grown in pure culture (Hepper, 1984 and Declerck *et al*., 2001), their exploitation is dependent on either producing viable inoculum on plant hosts or manipulating agricultural systems to develop and exploit naturally mixed native A-mycorrhizal populations. However, propagule numbers in natural inocula are generally less than ten propagules per gram of soil (Smith and Smith, 1981).Although some techniques have been developed to produce mycorrhizal inocula such as, nutrient film (Elmes and Mosse , 1984), Hydroponic cultures (Thompson, 1986) and Aeroponic cultures (Hung and Sylvia, 1988; Jarstfer and Sylvia, 1998 and Mikhaeel, 2002), until now, mycorrhizal infected roots and spores from open pot cultures of AM-inoculated plants have been the usual source of A-mycorrhizal inoculum for research purposes (Ferguson and Woodhead, 1982). However, this type of inoculum requires a large space for production

and is prone to contamination even with good phytosanitary care (Ames and Linderman, 1978).

Production of mycorrhizal inoculum using aeroponic culture technique remains one of the most promising ways of obtaining the high quality, pathogen-free and concentrated inoculum available for nursery inoculation and moderate-sized field testing (Jarstfer and Sylvia, 1992). For effective use of the aeroponic culture for A-mycorrhizal inoculum production, precise information is required on the growth-promoting potential of aeroponically produced inoculum.

The objective of this study was to evaluate the efficiency of aeroponically produced AM fungus, *Glomus* sp. inoculum in comparison with two mycorrhizal inoculum sources, namely soil-based pot culture of *Glomus* sp. and naturally mixed mycorrhizal populations using maize and sorghum as host plants.

MATERIALS AND METHODS

A green house experiment in earthenware pots, using maize (*Zea mays*, cv. Giza 2) and sorghum (*Sorghum bicolor,* cv. Giza 15) as a host plants was carried out to evaluate the efficiency of aeroponically produced arbuscular-mycorrhizal inoculum (spores and infected root segments) in comparison with two different sources of mycorrhizal inocula, namely soil-based pot culture of *Glomus* sp. and naturally mixed AM fungal populations.

Preparation of A-mycorrhizal inocula:

1-Aeroponically-produced inoculum:

Root samples were detached from fifteen sorghum plants freshly harvested from a 14-week-old aeroponic culture of a local mycorrhizal isolate *Glomus* sp. (Mikhaeel *et al.,* 2000). The aeroponic culture technique was previously described (Mikhaeel, 2002). Spores were collected on a 67 µm sieve after washing roots with high pressure water over a 500 µm sieve. The remaining roots were cut into 1 cm pieces and mixed thoroughly.

2-Soil-based pot culture inoculum:

Spores and roots of *Glomus* sp. inoculum were also obtained from 14-week-old pot culture by wet sieving (Gerdemann and Nicolson, 1963), while the roots were collected from the pots, washed from soil and cut into 1 cm pieces and mixed thoroughly.

3-Naturally mixed mycorrhizal inoculum:

Rhizospheric soil of onion plants growing in clay loam soil (Agric. Res. Center at Giza) was collected with its onion roots. Mycorrhizal spores were isolated by wet sieving (Gerdemann and Nicolson, 1963), while the onion roots were collected , washed and cut into 1 cm pieces and mixed thoroughly.

Subsamples of the above three inocula were randomly taken to determine the number of spores (Gaur and Adholeya, 1994), and to confirm the mycorrhizal colonization of roots after staining the roots (Phillips and

Hayman, 1970). Pots were filled with sterilized sand-soil mixture (1 : 1 by volume) at the rate of 2 kg per pot. Mycorrhizal treatments were achieved by placing approximately 500 spores or 10 g fresh root segments, 2-4 cm below the soil surface before planting. Non-mycorrhizal pots (controls) received non. At sowing time all pots received ammonium sulphate (20.5% N), rock phosphate (28.5% P2O5) at the rates of 0.29 and 1.2 g. pot^{-1}, respectively. In each pot, 8-10 maize or sorghum seeds were sown and watered immediately and as needed to maintain the moisture content at 60 % of WHC. One week later, maize and sorghum seedlings were thinned to three healthy seedlings per pot. The pots were arranged in a green house of 28 – 35 C٩. A completely randomized block design with 9 replicates for each treatment was applied. There were eight treatments (3 x 2 factorial and 2 non-inoculated controls). The three inoculum sources were aeroponic culture of *Glomus* sp., soil-based pot culture of *Glomus* sp. and naturally mixed AM fungi. The two inoculum formulations were spores and infected root segments.

After 4,8 and 12 weeks of sowing, 3 randomly replicate pots for each treatment were uprooted to determine the dry weights of plant shoots. Phosphorus contents of shoots were estimated according to Chapman and Pratt (1961). Mycorrhizal colonization ratios of both maize and sorghum roots were determined after staining the root samples(Phillips and Hayman, 1970) using the gridline intersect method (Giovannetti and Mosse, 1980). Data were statistically analyzed by an analysis of variance (Snedecor and Cochran, 1980). Least Significant Differences (LSD) were used to separate treatment means.

RESULTS AND DISCUSSION

Mycorrhizal colonization ratios:

Data in Table (1) show that, non-inoculated controls were free of mycorrhizae. Colonization ratios for both maize and sorghum roots increased gradually with time and reached a maximum of 46.2 % for maize and 62.5 % for sorghum with aeroponic culture treatment, at the final harvest. In general, percent colonization was higher with sorghum as host plant than with maize.

Four weeks after sowing, only plants which received infected root segments gave slight colonization ratios. However, those inoculated with mycorrhizal spores gave non. Eight weeks after sowing, all plants which received mycorrhizal root segments were better colonized than those inoculated with AM spores, being most pronounced with (soil-based pot culture) treatment. At the final harvest (12 weeks), there were no differences in colonization ratios due to inoculum formulation. It is interesting to note that, the delay observed before the spore inoculum began to colonize maize and sorghum roots may be related to an innate dormancy of spores. The same findings were reported by Vimard *et al.*(1999). They suggested that, the root segments were readily infected because they contain active hyphae with intraradical vesicles, structures that probably needed no physiological modification and had no nutritional deficiencies. On the other hand, spores may require a dormancy period before they can germinate and produce

infective hyphae. As for inoculum source at the final harvest, both aeroponic and soil-based pot treatments gave the same higher levels of root colonization ratios than those achieved with naturally mixed AM treatment. Gaur *et al.*(1998) reported that, the single isolate *Glomus intraradices* produced higher root colonization percentages than the naturally mixed mycorrhizal populations with both *Capsicum annuum* and *Polianthes tuberose* as host plants.

Table (1): Effect of mycorrhizal inoculation with different sources and formulations of AM inocula on mycorrhizal root colonization percentages of maize and sorghum plants after 4,8 and 12 weeks of sowing.

Inoculum source	Time in weeks					
	4		8		12	
	Inoculum formulation					
	Spore	Root	Spore	Root	Spore	Root
	Maize					
Control.	0.0	0.0	0.0	0.0	0.0	0.0
Aeroponic culture.	0.0	14.1	11.4	36.2	44.5	46.2
Soil-based pot culture.	0.0	25.3	26.4	40.5	43.5	45.5
Naturally mixed AM.	0.0	30.6	20.1	31.3	36.3	38.7
LSD: 0.05	2.81		4.36		3.86	
0.01	3.90		5.98		5.25	
	Sorghum					
Control.	0.0	0.0	0.0	0.0	0.0	0.0
Aeroponic culture.	0.0	22.3	20.4	44.8	58.4	62.5
Soil-based pot culture.	0.0	30.6	36.9	53.2	55.6	59.3
Naturally mixed AM.	0.0	26.5	25.1	30.1	33.5	32.1
LSD: 0.05	3.42		5.11		5.26	
0.01	4.70		6.94		7.23	

Plant growth:

Data of shoot dry weights and phosphorus contents of both maize and sorghum plants are shown in (Tables, 2,3). Generally, growth of maize and sorghum plants responded favorably to mycorrhizal inoculations with any of the three inoculum sources either as spores or infected root segments. However, these improvements were in variable degrees, according to source and formulation of the inoculum, host plant and sampling period. At the first harvest (4 weeks), all plants which received mycorrhizal spores from any of the three inoculum sources, showed non-significant differences in their growth parameters when compared with non-inoculated ones. On the contrary, maize and sorghum plants which received mycorrhizal root segments resulted in variable increases in plant growth and phosphorus uptake, at the same sampling period.

At the second harvest (8 weeks), plants inoculated with spores from soil-based pot culture or aeroponic culture gave significantly increases in shoot dry weights and phosphorus contents of plant shoots, compared with non-inoculated controls. However, these stimulating effects were more pronounced with spores collected from soil-based pot culture than those from aeroponic culture. This trend may due to higher germination percentages of

spores from soil-based pot culture than those from aeroponic culture. This possibility is supported by the higher levels of mycorrhizal colonization ratios observed with spores from the former inoculum source than those from the later (Table 1). Hung and Sylvia (1988) reported that, percent germination of *Glomus etunicatum* spores collected from soil pot culture were significantly higher than that of spores collected from aeroponic culture.

On the other hand, all plants which received naturally mixed AM fungi revealed the least increases in growth parameters compared with control plants. Gaur *et al*. (1998) found that, the host plants showed higher growth and yield when inoculated with a pure culture of *Glomus intraradices* than with mixed indigenous culture containing native mycorrhizal populations. The increases in shoot dry weights of maize plants due to mycorrhizal inoculation with spores were 39.8 and 61.7 % for aeroponic and soil-based pot cultures, respectively. However, sorghum plants gave 95.6 and 164.8 % increases due to the same treatment in the same order. At the same sampling period, maize and sorghum plants which received root segments inoculum gave remarkable improvements in their growth parameters over the levels achieved with spore inoculum. Inoculation of plants with mycorrhizal root segments collected from soil-based pot culture gave the highest growth enhancement as compared with control treatments. However, those received root segments from aeroponic culture, improved plant growth and P-uptake over the level achieved with (naturally mixed AM) treatment but lower than (soil-based pot culture) treatment.

It is obvious from these results that, the use of mycorrhizal root segments to improve plant growth clearly shortened the time needed to achieve a high level of plant growth enhancement, regardless of inoculum source. Similar differences between spores and root segments were previously reported by Warner and Mosse (1980) , Biermann and Lindermann (1983) and Gaur *et al*. (1998). They suggested that, roots infected by *Glomus* spp. can serve as inocula because of the presence of intraradical vesicles and active hyphae, and when used as an inoculum, fragments of roots containing intraradical vesicles produced rapid colonization and response in host plants. This possibility is supported by the higher levels of colonization ratios (Table 1) in plants inoculated with infected root segments than those inoculated with mycorrhizal spores. At the final harvest (12 weeks), non-significant differences in plant growth and phosphorus contents due to formulation of the added inoculum. Since, all plants which received mycorrhizal spores gave growth enhancement similar to that achieved with infected root segments, regardless of inoculum source. On the other hand, with regard to inoculum source, aeroponic culture of *Glomus* sp. produced plant growth improvements similar to that achieved with soil-based pot culture of the same *Glomus* sp. isolate.

The results indicated that, no biological differences between the inoculum obtained from aeroponic culture and this collected from soil-based pot culture. Since, both inoculum sources gave high levels of colonization ratios, dry weight of shoots and phosphorus contents of plant shoots, after 12 weeks of plant growth. Hung and Sylvia (1988) and Jarstfer and Sylvia (1998)

demonstrated that, both colonized roots and spores produced in aeroponic cultures can serve as infective mycorrhizal inocula.

Table (2): Effect of mycorrhizal inoculations with different sources and formulations of AM inocula on dry weight (g . plnt^{-1}) of maize and sorghum plants, after 4,8 and 12 weeks of sowing.

Inoculum sources	Time in weeks					
	4		8		12	
	Inoculum formulation					
	Spore	Root	Spore	Root	Spore	Root
	Maize					
Control.	0.56	0.72	2.01	1.85	4.03	4.16
Aeroponic culture.	0.74	1.12	2.81	3.39	6.51	7.13
Soil-based pot culture.	0.68	1.20	3.25	3.82	6.43	6.85
Naturally mixed AM.	0.54	0.84	2.41	2.87	4.94	5.12
LSD: 0.05	0.34		0.42		0.69	
0.01	0.46		0.57		0.97	
	Sorghum					
Control.	0.43	0.52	0.91	0.85	2.61	2.55
Aeroponic culture.	0.63	0.91	1.78	2.63	4.70	5.05
Soil-based pot culture.	0.58	1.23	2.41	2.96	4.29	4.61
Naturally mixed AM.	0.40	0.82	1.42	1.94	3.22	3.58
LSD: 0.05	0.25		0.45		0.66	
0.01	0.34		0.61		0.89	

Table(3): Effect of mycorrhizal inoculation with different sources and formulations of AM inocula on phosphorus contents (mg . pot^{-1}) of maize and sorghum plants, after 4,8 and 12 weeks of sowing.

Inoculum sources	Time in weeks					
	4		8		12	
	Inoculum formulation					
	Spore	Root	Spore	Root	Spore	Root
	Maize					
Control.	0.61	0.90	2.21	2.22	4.16	4.55
Aeroponic culture.	0.93	1.53	2.83	4.41	7.49	8.91
Soil-based pot culture.	0.75	1.75	3.90	5.35	7.37	9.18
Naturally mixed AM.	0.72	1.09	3.13	3.59	6.13	6.94
LSD: 0.05	0.35		0.51		1.90	
0.01	0.48		0.69		2.62	
	Sorghum					
Control.	0.71	0.77	1.32	1.18	3.32	3.28
Aeroponic culture.	1.10	1.55	2.90	4.58	6.86	7.58
Soil-based pot culture.	0.98	2.24	4.03	5.48	6.48	6.87
Naturally mixed AM.	0.69	1.44	2.19	3.28	4.44	5.05
LSD: 0.05	0.45		0.82		1.78	
0.01	0.62		1.11		2.41	

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كفاءة لقاح الميكوريزا (*Glomus sp.*) والمنتج فى مزارع ال (*Aeroponic*) فى زيادة النمو وذلك بالمقارنه بلقاحات الميكوريزا ألتقليديه.

فتحى توفيق ميخائيل

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تم فى هذه الدراسه مقارنة لقاح فطر الميكوريزا (*Glomus* sp.) والمنتجة فى مزارع ال (Aeroponic) مع أثنين من مصادر لقاحات الميكوريزا ألتقليدية وهما: لقاح منتج فى مزارع الأصص لنفس العزلة (*Glomus* sp.) ولقاح ميكوريزا طبيعى معزول من الأرض الزراعية ويحتوى على خليط من أنواع الميكوريزا , وذلك باستعمال كل من الذرة الشامية والذرة الرفيعه كنباتات عائلة فى تجربة أصص. وقد أعطت ألتجربه النتائج التالية.

١- بعد ٤ أسابيع من الزراعه , فقط النباتات التى تم تلقيحها بلقاحات الميكوريزا المكونه من قطع جذريه معداة بالميكوريزا أعطت زيادات طفيفة فى نمو النبات وذلك بالمقارنه بالنباتات الغير ملقحة , بينما تلك التى تم تلقيحها بجراثيم ميكوريزا فقط لم تعطى أى اختلافات عن نباتات الكنترول.

٢- بعد ٨ أسابيع من ألزراعه ، أظهرت لقاحات الميكوريزا (قطع جذريه معداة أو جراثيم) والمنتجة فى مزارع ألأصص ذياده فى النسب المؤية لأصابة الجذور , ألوزن الجاف للمجموع الخضرى وأيضاً الفوسفور ألممتص بالنبات أكثر من تلك الزيادات المتحصل عليها باستعمال اللقاحات المنتجة فى مزارع ال (Aeroponic) , وفى كل الحالات كانت أستجابة النباتات الناميه للقاح الجذور المعداة أكثر من استجابتها للقاح الجراثيم.

٣- بعد ١٢ أسبوع من الزراعه , حققت لقاحات الميكوريزا للعزلة (*Glomus* sp.) والمنتجة فى مزارع ال (Aeroponic) زيادات فى معدل اصابة الجذور , وتحسن نمو النبات والفوسفور الممتص وهذه تماثل تماماً تلك المتحصل عليها باستعمال اللقاح المنتج فى مزارع الأصص , سواء كان اللقاح جذور معداة بالميكوريزا أو جراثيم.

٤- لقاح الميكوريزا والمعزول طبيعياً من التربه أعطى أقل المستويات فى كل القياسات المدروسة فى النبات بالمقارنة باللقاحات الأخرى.

٥- دلت نتائج التجربة على أنه لايوجد أى أختلافات حيوية بين مصدرى اللقاح للعزلة (*Glomus* sp.) سواء كان اللقاح منتج فى مزارع الأصص أو فى مزارع ال(Aeroponic).