Egypt. J. Phytopathol., Vol. 41, No. 2, pp. 145-157 (2013)

Aeroponic Propagation of VA-Mycorrhizal Spores for Soil Inoculation as a Biofertilizer

Fatma M.K. Faramawy

Soil fertility & soil microbiol. Dept., Desert Res. Centre, El Matareya, Cairo, Egypt.

Onion (*Allium cepa*) and Barley (*Hordeum vulgare*) colonized by *Glomus mosseae, G. fasiculatum,* and *G. intraradices* were grown in aeroponic cultures. After 14 weeks, all roots were colonized by the inoculated vesicular-arbuscular mycorrhizal fungi. Abundant vesicles and arbuscules formed in the roots, and profuse sporulation was detected intra- and extra-radically. Within each fungal species, barley contained significantly more roots and spores per plant than onion did, although the percent root colonization was similar for both hosts. Mean percent root colonization and sporulation per centimetre of colonized root generally increased with time, although with some treatments colonization declined by week 14. Spore production ranged from 4 spores per cm of colonized root for *G. fasiculatum* to 51 spores per cm for G. intraradices. Infectivity trials with root inocula resulted in a mean ranged from 28.9 to 51.1% of onion roots colonized by G. *mosseae, G. fasiculatum, and G. intraradices*, respectively, while in aeroponic culture it ranged from 33.8 to 38. However, infectivity studies comparing *G. fasiculatum* spores from soil and aeroponic culture indicated no biological differences between the spore sources. Aeroponically produced *G. mosseae* and *G. fasiculatum* inocula retained their infectivity after cold storage (4°C) in either sterile water or moist vermiculite for at least 4 and 9 months, respectively.

Keywords: Aeroponic culture, *Glomus fasiculatum, G. intraradices* and *G. mosseae*.

Soil-based pot culture is a common method for production of vesicular-arbuscular mycorrhizal (VAM) fungal inoculum (Menge, 1984). Recently, solution culture techniques such as nutrient film (Elmes and Mosse, 1984 and Mosse and Thompson, 1984) and aeroponics (Sylvia and Hubbell, 1986) have been adapted for the production of inocula of VAM fungi. These methods provide an alternative to soil-based pot culture for mass production of clean, soilless VAM inoculum. Clean propagules, especially spores, are not only useful for inoculation, but are also essential for critical physiological and genetic studies. Both solution culture techniques provide well-colonized root inocula (Elmes and Mosse, 1984 and Mosse and Thompson, 1984), but results of sporulation experiments were quite different. In the nutrient film technique, sporulation was sparse, except with full-strength Hoagland nutrient solution, when plants suffered manganese toxicity (Mosse and Thompson, 1984). In contrast, the highly aerated rooting environment of aeroponic culture stimulates rapid and abundant sporulation of the VAM fungi. In an aeroponic culture, inoculation of Bahia grass (*Paspalum notatum* Flugge) with *Glomus mosseae* and *G. intraradices* Schenck & Smith resulted in root colonization and

sporulation superior to that previously reported for a soil-based pot culture (Sylvia and Schenck, 1983). For effective use of the aeroponic culture technique for inoculum production, the following questions must be addressed. (i) Can this technique be applied to other host plants and VAM fungi? (ii) Are aeroponically produced inocula infective? (iii) Is it possible to store inoculum in a viable state for an extended period? The objectives of this study were to investigate host-fungus interactions in aeroponic culture, to test the infectivity of aeroponically produced root and spore inocula, and to determine the viability of these inocula after cold (4°C) storage for different periods.

Materials and Methods

In this study, three VAM fungi, *i.e. Glomus mosseae, G. fasiculatum* and *G. Intraradices,* were compared for their establishment on two host plants, *i.e.* onion and barley.

Precolonization:

Aeroponic cultures were initiated with precolonized seedlings. Spores of *G. mosseae, G. fasiculatum* and *G. intraradices* were wet sieved from 1000g of cold-stored (4°C) soil inoculum and mixed with pasteurized (75°C for 4 h) sandy soil. Surface-disinfected (30% H_20_2, 10 min), then washed with sterile water. Seeds of onion and barley were then placed in the inoculum in shallow (6-cm-deep) trays. Seedlings were grown in a non-shaded greenhouse for 6 to 8 weeks, after which roots were washed and trimmed to a length of 6 cm for onion and 8 cm for barley. Root colonization by VAM fungi was confirmed by the non-destructive auto fluorescence method of Ames *et al*. (1982).

Aeroponic culture:

Colonized seedlings (30 for onion and 20 for barley) were placed into aeroponic chambers in a non-shaded greenhouse with 10 cm spacing between plants for onion and 12 cm spacing for barley. The apparatus of aeroponic chambers was adapted from Zobel *et al*. (1976). The system composed of three parts: the motor, the shaft and the spinner (Fig. 1), the box in which the plants are grown (Fig. 2) is made of glass with 60 cm wide, 120 cm long and 45 cm high. The box is lined with black polyethylene sheeting. The top of the box consists of light screening plastic and then a layer of heavy aluminium foil, and then holes are cut in the plastic foil at intervals at which plants are to be spaced. The motor with its attached spinner is supported on the top centre above the box on a frame made of a horizontal board supported at either end by two vertical boards, in this way the vibration of the motor is not transferred to the plants.

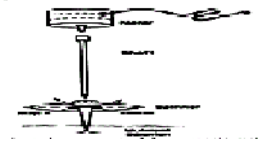

Fig. 1. Mechanical components of the aeroponics system.

Fig. 2. Diagrammatic cut-away view of the aeroponics box for growing plants.

VAM spores:

VAM spores were cultured from April to September 2007 with mean maximum and minimum temperatures of 37 and 25°C. Diluted Hoagland nutrient solution was used in each chamber as described by Sylvia and Hubbell (1986), with pH initially adjusted to 6.50 + 0.05 with 1 N NaOH. Randomly selected roots, of four onion and three barley plants from each chamber, were harvested 14 days after seedlings had been placed in the aeroponic chambers. Successive harvests were continued thereafter at 2 week intervals up to 14 weeks. At each harvest, only roots grown out of the original roots (8 cm for barley and 6 cm for onion) were collected, they were cut into 5-cm segments. These root segments were checked for sporulation under a dissecting microscope and then cleared in 10% KOH and stained with 0.05% trypan blue. The total root length and root length colonized by VAM fungi were estimated by a gridline intersect method (Giovannetti and Mosse, 1980) and the total number of spores produced in each segment was recorded. After each harvest, the final pH of the nutrient solution in the chambers was recorded and the solution was changed. Roots of the remaining seedlings were trimmed to a length of 12 to 15 cm to prevent growth into the nutrient solution.

Viability test:

According to (Schaffer and Peterson, 1993), root segment stained for fungal succinate dehrogenase (SDH) activity using 0.01% (W\V) acid fuchsine in 75% lactic acid and 5% glycerol. The purple formazan pigments formed indicate where respiratory activities have occurred against the pink fuchsine background. In this way the method distinguishes between viable and none-viable mycorrhizal structure.

Infectivity test experiment:

The infectivity test experiment had been done to compare between the infectivity of aeroponic cultured spores and those from soil culture. It had a completely randomized design with 36 treatments and 4 replicates. Spores of the three types of inocula, *i.e. Glomus mosseae, G. fasiculatum* and *G. intraradices* (Fig. 5), collected from soil, were aeroponic cultured, and three rates, *i.e.* 50, 75 and 100 spores, were applied. Pasteurized sandy soil (500 g) was placed in plastic trays. Spore inocula were placed 2 cm below the surface. Trays were seeded with barley and placed in a greenhouse. After 8 weeks incubation at maximum and minimum temperatures of 28 and 16°C, plants were collected to assess VAM development. Roots were cleared, stained and assessed for VAM development as described above.

Storage study:

Aeroponically produced *G. fasiculatum* and *G. mosseae* inoculate grown on barley and onion stored in Mason jars with sterilized distilled water, or sterilized clay or with sterilized moist vermiculite (50 cm^3 of vermiculite and 30 ml of distilled water) at 4°C. Inocula from different fungal and plant species were stored separately. After cold storage for 2, 4, 6 and 9 months, pot experiments were carried out in the greenhouse using barley as a host to detect the viability of the stored spores. They had a completely randomized design with 48 treatments (2 types of inocula grown on 2 hosts, stored on 3 carriers, at 4 periods of storage) and 4 replicates for each treatment. After 8 weeks plants were collected and assess VAM development.

Data analysis:

All data were subjected to analysis of variance, and data from different fungal species were analyzed separately. When significant main effects occurred, treatment means were separated by Tukey's (equal sample size) or Scheffe's (unequal sample size) multiple pair wise comparison at *P* c 0.05 (Neter and Wasserman, 1974). For storage study, treatment means were separated by orthogonal contrast.

Results and Discussion

Aeroponic culture:

By the final harvest, all roots had been colonized by inoculated VAM fungi and contained abundant vesicles and arbuscules (Fig. 3), spores were produced both intra- and extra-radically. The pH of the nutrient solution remained within 0.1 unit of the initial level throughout the experiment. Analysis of variance indicated that no time x position interactions occurred for any variable. At the final harvest (14 weeks), barley inoculated with *G. mosseae* had at least 50% root colonization, whereas plants inoculated with *G. fasiculatum* and *G. intraradices* had 45% and 20% root colonization, respectively, (Fig. 4). This is because barley had a large root system due to the rapid root growth of the graminaceous crop, fibrous root systems are ideal trap for VAM spores (Al-Raddad, 1995). In spite of treatments of *G. intraradices* on barley and onion had the lowest rates of root colonization when planted after aeroponic culture, they had the highest number of spores (Table 1), this is may be due to significant reduced sporulation after repeated propagation cycles (Demir and Fozren, 2009), or due to the portioning of carbon to colonization versus sporulation (Anshula *et al.*, 2012).

Table 1. Mean percent root colonization and sporulation per centimetre of colonized root of six host-fungus combinations

Treatment	Root colonization (%)[a] at following distance (cm) from crown				No. of spores[b]/cm of colonized root at following distance (cm) from crown			
	0-5	5-10	10-15	15-20	0-5	5-10	10-15	15-20
G. mosseae + onion	25^{A}	21^{A}	18^{B}	14^{A}	5^{A}	5^{A}	4^{A}	4^{A}
G. mosseae + barley	30^{A}	26^{A}	25^{A}	19^{A}	4^{A}	4^{A}	5^{A}	3^{A}
G. fasiculatum + onion	39^{A}	31^{AB}	24^{BC}	19^{C}	8^{A}	4^{AB}	2^{B}	2^{B}
G. fasiculatum + barley	34^{A}	27^{AB}	20^{B}	6^{C}	2^{A}	1^{AB}	2^{A}	0^{B}
G. intraradices + onion	21^{A}	17^{AB}	11^{BC}	4^{CD}	16^{A}	15^{A}	7^{A}	6^{A}
G. intraradices + barley	22^{A}	17^{AB}	11^{BC}	3^{C}	23^{A}	21^{A}	24^{A}	19^{A}

[a] The inoculated plants where grown in aeroponic culture for at-least 14 weeks.

[b] Means represent at least 15 replicates.

- Means in the same row followed by the same latter are not significantly different at LSD= 0.05.

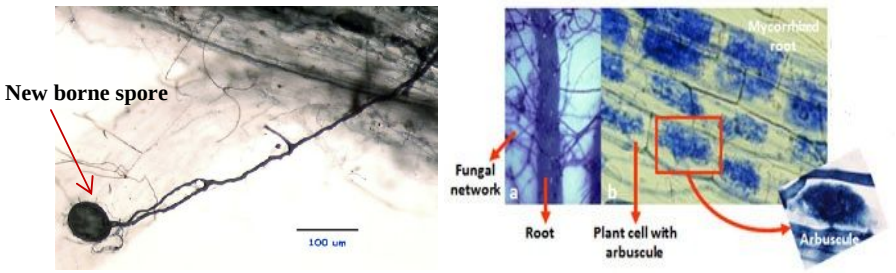

Fig. 3. Stained roots by trypan blue showing VAM colonization and spore releasing from plant roots grown in aeroponic culture.

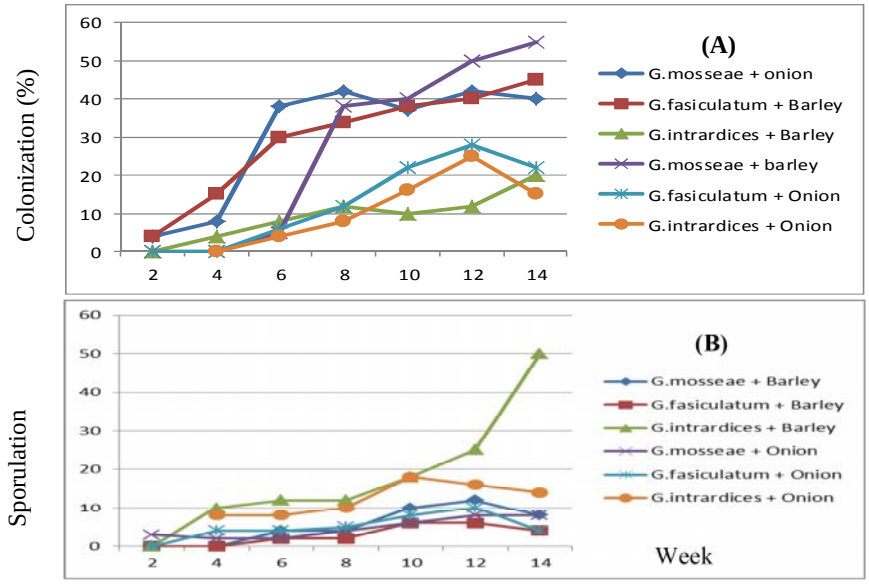

Fig. 4. Mean percent root colonization (A) and sporulation per centimetre of colonize root (B) by *G. mosseae, G. fasiculatum* and *G. intraradices* on barley and onion in aeroponic culture over a 14-week period.

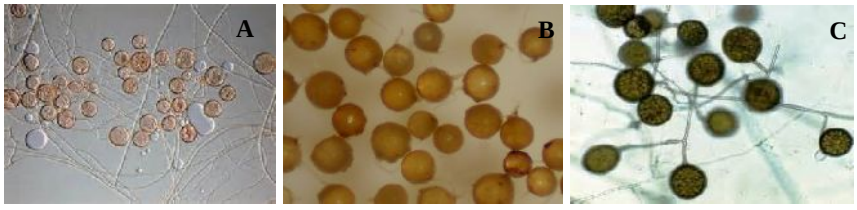

Fig. 5. Spores of *Glomus fasiculatum* (A), *G. mosseae* (B) and *G. intraradices* (C).

The mean percent of root colonization and sporulation per centimetre of colonized root generally increased with time, although with some treatments colonization declined by week 14 (Fig. 4). Percent root colonization decreased toward the apex of the root, except for *G. mosseae* with barley, for which colonization was uniform across the whole root system (Table 1 and Fig. 3). Spore production ranged from 4 spores/cm of colonized root for *G. fasiculatum* to 51 spores/cm for *G. intraradices* (Table 2 and Fig. 5). Sporulation per centimetre of colonized root decreased only on roots inoculated with *G. fasiculatum* (Table 1). The mean percent root colonization in this study was slightly lower than that reported by Sylvia and Hubbell (1986) and Gianinazzi (2004), whereas sporulation per unit length of colonized root was generally higher. Several factors may have contributed to these differences, such as degree of root colonization of precolonized plants before transfer to aeroponic chambers and environmental conditions. Nevertheless, both reports show that root colonization and abundant sporulation occurred in aeroponic culture. This is in contrast to the nutrient film technique (Mosse and Thompson, 1984).

Table 2. Total root length, root colonization and spore production of barley and onion inoculated with three *Glomus* spp.

Treatment	Total root length (cm)[a]	Root colonization (%)[b]	spores[b]	
			No./plant	No./cm of colonized root
G. mosseae + onion	268[C]	39[A]	1055	5.8
G. mosseae + barley	476[B]	54[A]	2062	4.3
G. fasiculatum + onion	202[C]	47[A]	762	3.8
G. fasiculatum + barley	753[A]	44[A]	1218	1.6
G. intraradices + onion	242[C]	17[B]	630	2.6
G. intraradices + barley	492[B]	21[B]	4478	9.1

([a]) and ([b]) as described in footnote of Table 1.

- Means in the same row not followed by the same latter were significantly different at LSD= 0.05.

For aeroponic, the *P* value for barley versus inoculum intensity are 0.2411 (50 spore), 0.1422(100 spores) and 0.1423(75 spores), the P value for onion versus inoculum intensity are 0.0111 (50 spores), 0.0212 (75 spores) and 0.0031 (100 spores). For soil, the *P* values for barley versus inoculum intensity are 0.01522 (50 spores), 0.02111 (75 spores), 0.00211 (100 spore), the *P* values for onion versus inoculum intensity are, 0.0301 (50 spores), 0.00251 (75 spores), 0.0841 (100 spores).

Marleen and Sylvia (2011) reported that some spores of *G. fasiculatum* were produced on bean (*Phaseolus vulgaris*) roots by the nutrient film technique. Thompson (1986) and Kapoor *et al.* (2008) reported abundant external mycelium and ectocarpic spores and sporocarps of *G. mosseae* on nutrient film cultured maize *(Zea mays*) roots. However, neither reported quantitative data on spore production. Bagyaraj and Manjunath (1980) and Tajini *et al.* (2012) demonstrated host plant

effects on inoculum production in soil-based pot culture. From our study, within each fungal species barley produced significantly more roots and spores per plant than did onion, although the percent root colonization was similar (Table 2). Thus, seems to be a preferred candidate for mass production of VAM inoculum in aeroponic culture.

For infectivity of *G. fasiculatum* spores, after 8 weeks, the germination percent of *G. fasiculatum* spores collected from soil was significantly higher than that of spores collected from aeroponic culture at all inoculation rates (58.1%, 59 and 62.1%, respectively). Results of the infectivity bioassay (Table 3) indicated that within each harvest data there were no differences in root colonization due to inoculation rate. Onion inoculated with spores from soil was better colonized than onion inoculated with spores from aeroponic culture (42.7%, 042.4 and 51.1%, respectively). However, spores resulted from barley inoculation had more colonization rate than that did by spore resulted from onion colonization in both aeroponic and soil culture.

For infectivity of *G. mosseae* spores, there were no differences in root colonization due to rate of inoculation. However, spores resulted from barley in soil culture were better than that resulted from aeroponic culture, but in case of using onion as a host plant aeroponic culture gave better results (38.0% ,36.5% and 38.7) than soil culture did.

For infectivity of *G. intraradices* spores, there were no differences in barley root colonization due to source of inoculum or inoculation rate for each VAM fungus treatment (Table 3). Colonization of the plants average ranged from 32.8% to 41.0%.

Table 3. Infectivity percent of different rates of three *Glomus* spp. spores isolated from aeroponic and soil cultures using barley and onion as a host plants

Treatment	Root colonization (%) (spores from aeroponic culture)			Root colonization (%) (spores from soil culture)		
	50 spore/ 500g soil	75 spore/ 500g soil	100 spore /500g soil	50 spore / 500g soil	75 spore/ 500g soil	100 spore /500g soil
G. fasiculatum + barley	50.2	52.4	55.0	58.1	59.0	62.1
G. fasiculatum + onion	33.8	37.1	35.4	42.7	42.4	51.1
G. mosseae + barley	47.0	48.6	48.3	51.1	51.2	50.3
G. mosseae + onion	38.0	36.5	38.7	29.4	28.9	29.7
G. intraradices + barley	37.3	38.2	37.0	39.2	40.4	41.0
G. intraradices + onion	35.4	37.1	37.0	32.8	36.4	36.6

Infectivity and sporulation of VAM spores tend to increase in treatments which were propagated in barley rhizosphere than that produced from onion plants in both soil and aeroponic cultures, this is may be due to the rapid root growth. Castillo *et al.* (2012) demonstrated that barley has higher total mycorrhizal root biomass and those may potentially benefit more VAM spore production.

For *G. mosseae,* the *P* values for onion versus barley are 0.0029 (2 months), 0.009 (4 months), 0.0002 (6 months) and 0.0008 (9 months), the *P* values for water versus vermiculite are 0.2215 (2 months), 0.0654 (4 months), 0.0003 (6 months) and 0.0002 (9 months), for water versus clay 0.2212(2 months), 0.0420 (4 months), 0.0004 (6 months) and 0.0006 (9 months), the *P* values for vermiculite versus clayare.1187 (2 months), 0.0399 (4 months), 0.0005 (6 months) and 0.0004 (9 months), for *G. fasiculatum* the *P* values for onion versus barley are 0.2447 (2 months),0.3672 (4 months),0.8911 (6 months), 0.8391 (9 months), the *P* values for water versus vermiculite are 0.2447 (2 months), 0.9734 (4 months),0.3899 (6 months) and 0.0799 (9 months), the *P* value for water versus clay are 0.1114 (2 months), 0.0311 (4 months), 0.0002 (6 months) and 0.0002 (9 months), the *P* values for vermiculite versus clay are 0.0211 (2 months), 0.0055 (4 months), 0.0001 (6 months) and 0.0006 (9 months).

In case of storage study, in general, cold storage of *G. fasiculatum* root inoculum did not affect its infectivity for up to 9 months, regardless of inoculum carriers and host species (Table 4 a & b). Although a significant difference (*P*= 0.0420) was detected for host species when inocula stored for 2 months was used, this difference might not be biologically significant. The viability studies of the stored *G. fasiculatum* showing that, the viability of the spores were decreased by time, spores grown on barley as a host and stored on vermiculite and clay were remained viable for long period (9 months).

For *G. mosseae* inoculum, onion was a superior host than barley regardless of storage time, there is evidence that the increase of disaccharides such as trehalose inside spores which collected from onion rhizosphere stabilizes mycorrhizal membranes during dehydration stress during storage and keeps the viability of spores, Douds and Schenck (1990). No differences were detected for the inoculum carriers until the inoculum had been stored for 6 months or more. Inocula produced from barley and stored in sterile water lost their infectivity after 6 months of cold storage, whereas others retained their viability (Table 4 a & b).

Owing to different environmental conditions during the various experiments, comparison of root colonization over time is not possible. These different environmental conditions might have resulted in different mean percent root colonization at different storage periods.

Inoculum carriers and storage temperature, humidity, and time are important factors affecting the infectivity of mycorrhizal inoculum after storage. The carrier is the major portion of the inoculants that helps to deliver a suitable amount in good physiological condition (Smith, 1992). A good carrier should therefore possess the following properties: good moisture absorption capacity, easy to process and free of lump-forming materials, easy to sterilize, low cost and availability in adequate amount, and good pH buffering capacity (Keyser *et al.,* 1992). Other characteristics that are affecting the carrier appropriateness are a standardized composition ensuring chemical and physical stability, the possibility of mixing with other compounds, and being composed of biodegradable and non polluting compounds availability and cost are also the main factors affecting the choice of a carrier (Smith, 1992).

Table 4a. Root and viable colonization (%) of onion and barley inoculated with aeroponically produced inoculum stored for 2 and 4 months

Inoculum			Root colonization (%) by using inoculum stored for 2 and 4 months			
			2		4	
Fungus	Host	Carrier	Total colonization	Viable colonization	Total colonization	Viable colonization
G. mosseae	Onion	Water	63	31	59	43
		Vermiculite	62	39	71	46
		Clay	57	29	61	43
	Barley	Water	67	45	65	50
		Vermiculite	73	49	70	54
		Clay	79	45	70	53
G. fasiculatum	Onion	Water	41	25	26	11
		Vermiculite	48	33	34	18
		Clay	50	26	39	25
	Barley	Water	63	43	29	19
		Vermiculite	63	48	43	31
		Clay	58	39	45	27

Table 4b. Root and viable colonization (%) of onion and barley inoculated with aeroponically produced inoculum stored for 6 and 9 months

Inoculum			Root colonization (%) by using inoculums stored for 6 and 9 months			
			6		9	
Fungus	Host	Carrier	Total colonization	Viable colonization	Total colonization	Viable colonization
G. mosseae	Onion	Water	40	28	22	12
		Vermiculite	44	39	28	12
		Clay	45	32	32	26
	Barley	Water	66	41	55	46
		Vermiculite	72	50	58	41
		Clay	74	48	57	39
G. fasiculatum	Onion	Water	8	0	0	0
		Vermiculite	66	50	46	30
		Clay	30	18	11	0
	Barley	Water	31	15	9	0
		Vermiculite	70	50	46	30
		Clay	59	46	46	40

In general, infectivity decreases as storage time and temperature increase (Daft *et al.*, 1987; Hung and Molina, 1986; Mugnier and Mosse, 1984 and Douds *et al.*, 2006). Ectomycorrhizal and plant-pathogenic fungi have been successfully preserved at low temperature in sterile water (Smith and Hubbell, 1986). Recently, Mugnier and Mosse (1984) indicated that spores of *G. mosseae* retained their

viability after cold (4°C) storage in a moist atmosphere for 4 years. Moreover, concerning factors affecting inoculum infectivity after storage, Daft *et al.* (1987) and Abd-Elatif *et al.* (2012) reported that spore infectivity was higher when the spores had been stored under wet or moist conditions. Our results indicate that a cold moist environment maintains inoculum infectivity, although the reason for the loss of infectivity of the *G. mosseae* - barley inoculum after cold storage for 6 months is unclear.

Vermiculite is a common inoculum carrier for vegetative ectomycorrhizal inocula (Marx and Kenney, 1982). Our results indicate that moist vermiculite can also be used as an inoculum carrier for VAM inocula.

This study extends previous findings (Declerck *et al.*, 2009) which demonstrated that VAM fungi colonize roots and sporulate rapidly in aeroponic culture. In addition, we have demonstrated that both colonized roots and spores produced in aeroponic chambers can serve as infective VAM inocula. Moreover, aeroponically produced *G. deserticola* and *G. etunicatum* inocula can be cold stored in either sterile water or moist vermiculite for at least 4 and 9 months, respectively.

Conclusion

Numerous methods have been developed for decades for the large-scale production of VAM fungi, one of the most important method is the hydroponic culture. Aeroponic is a form of hydroponics in which the roots and AM fungus are bathed in a nutrient solution, spraying of Micro-droplets increases the aeration and allow gas exchange. In aeroponic (substrate-free production system) precolonization plants are produced prior to introduction into the systems.

Nowadays, large-scale production of VAM fungi is not possible in the absence of a suitable host, and species cannot be identified in their active live stages (growing mycelium). As a consequence, quality control is often a problem, and tracing the organisms into the field to strictly relate positive effects to the inoculated VAM fungus is nearly impossible. In addition, no clear criteria have been set for the quality control of commercial inoculum (Pringle *et al.*, 2009).

It is expected that in the future new cultivation techniques will emerge, taking into consideration several of these aspects.

References

Al-Raddad, A.M. 1995. Mass production of *Glomus mosseae* spores. *Mycorrhiza*, **5**: 229-231.

Ames, R.N.; Ingham, E.R. and Reid, C.P.P. 1982. Ultraviolet-induced auto fluorescence of arbuscular mycorrhizal root infections: an alternative to clearing and staining methods for assessing infections. *Can. J. Microbiol*., **28**: 351-355.

Anshula, P.; Shanuja, B. and Adholeya, A. 2012. Sporulation pattern in three different Ri T. DNA transformed root hosts in symbiosis with *Glomus intraradices.* Conf. Internat. Res. on Food, Natural Resource.

Bagyaraj, D.J. and Manjunath, A. 1980. Selection of a suitable host for mass production of VA mycorrhizal inoculum. *Plant Soil*, **55**: 495-498.

Daft, M.J.; Spencer, D. and Thomas, G.E. 1987. Infectivity of vesicular arbuscular mycorrhizal inocula after storage under various environmental conditions. *Trans. Br. Mycol. Soc*., **88**: 21-27.

Daniels, B.A. and Skipper, H.D. 1982. Methods for recovery and quantitative estimation of propagules from soil. Pages: 29-35. In: *Methods and Principles of Mycorrhizal Research*. N.C. Schenck (ed.). Amer. Phytopathol. Soc., St. Paul, Minnesota, USA.

Declerck, S.; Jdo, M.; Fernandez, K.; Voets, L. and Providencia, I. 2009. Method and system for in vitro mass production of arbuscular mycorrhizal fungi. WOL\2009\090220.

Demir, S. and Fozren, K. 2009. Effect of whey on the colonization in lentil (*Lens orientalis*) and sporulation of arbuscular mycorrhizal fungi *Glomus intraradices*. *African J. of Biotechnol.,* **8**(10): 1684-5315.

Douds, D.P.; Ogahashi, G.N.; Pfeffer, P.E. and Reider, C. 2006. On farm production of AM fungus inoculum in mixtures of compost and vermiculite. *Bioresource Technology*, **97**(6): 809-818.

Douds, D. and Schenck, N.C. 1990. Cryopreservation of spores of vesicular arbuscular mycorrhizal fungi. *New Phytol*., **115**: 667-674.

Elmes, R.P. and Mosse, B. 1984. Vesicular-arbuscular endomycorrhizal inoculum production. II. Experiments with maize (*Zea mays*) and other hosts in nutrient flow culture. *Can. J. Bot*., **62**: 1531-1536.

Tajini, Fatma; Trabelsi, M. and Drevon, J. 2012. Arbuscular mycorrhizae by contact with mycorrhizae *Stylosanthe sguianensis* enhance P use efficiency for N2 fixation in the common bean . *African J. of Microbiol*., **6** (6): 1297-1305.

Gianinazzi, S. 2004. Inoculum of arbuscular mycorrhizal fungi for production systems . *Canad. J. of Botany*, **82**(8): 1264-1271.

Giovannetti, M. and Mosse, B. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infections in roots. *New Phytol.*, **84**: 489-500.

Hung, L.L. and Molina, R. 1986. Temperature and time in storage influence the efficacy of selected isolates of fungi in commercially produced ectomycorrhizal inoculum. *For. Sci.*, **32**: 534-545.

Keyser, H.H.; Somasegaran, P. and Bohlool, B.B. 1992. Rhizobial ecology and technology. Pages: 205-226. In: *Soil Microbial Ecology: Applications in Agricultural and Environmental Management*. NY, USA: Marcel Dekker.

Marleen, I. and Sylvia, C. 2011. Methods for large scale production of AM fungi: past , present and future, *Mycorrhiza,* **21**: 1-16.

Marx, D. and Kenney, D.S. 1982. Production of ectomycorrhizal fungus inoculum. Pages: 131-146. In: *Methods and Principles of Mycorrhizal Research*. Schenck, N.C. (ed.). Amer. Phytopathol. Soc., St. Paul, Minnesota, USA.

Marx, D.H. and Daniel, W.J. 1976. Maintaining cultures of ectomycorrhizal and plant pathogenic fungi in sterile water cold storage. *Can. J. Microbiol*., **22**: 338-341.

Menge, J.A. 1984. Inoculum production. Pages: 187-203. In: *VA Mycorrhiza*. C.L. Powell and D.J. Bagyaraj (eds.). CRC Press, Inc., Boca Raton, Fla., USA.

Mosse, B. and Thompson, J.P. 1984. Vesicular-arbuscular endomycorrhizal inoculum production. I. Exploratory experiments with beans (*Phaseolus vulgaris*) in nutrient flow culture. *Can. J. Bot*., **62**: 1523-1530.

Mugnier, J. and Mosse, B. 1984. Spore germination and viability of a vesicular-arbuscular mycorrhizal fungus, *Glomus mosseae*. *Trans. Br. Mycol. Soc*., **88**: 411-413.

Neter, J. and Wasserman, W. 1974. *Applied Linear Statistical Models*, Richard D. Irwin, Inc., Homewood, pp. 842.

Kapoor, R.; Sharma, D. and Bhalnuga, A.K. 2008. Arbuscular mycorrhizae in micro propagation on systems and their potential application. *Sci. Hort*., **116**(3): 227-239.

Abd-Elatif, Sausan A.; Abdel Rahman, R.A.; Mazen, M.B.H.; Enany, A.E. and Allam, Nivien. 2012. Biotechnological aspect for VAM aspect mass production. *World Appl. Sci. J.*, **17**(1): 20-28.

Smith, R.S. 1992. Legume inoculant formation and application. *Canad. J. Microbiol*., **38**(6): 485-492.

Smith, D. and Onions, A.H.S. 1983. The preservation and maintenance of living fungi. Commonwealth Mycological Institute, Kew, England, p. 51.

Sylvia, D.M. and Hubbell, D.H. 1986. Growth and sporulation of vesicular-arbuscular mycorrhizal fungi in aeroponic and membrane systems. *Symbiosis*, *1*: 259-267.

Sylvia, D.M. and Schenck, N.C. 1983. Application of super-phosphate to mycorrhizal plants stimulates sporulation of phosphorus-tolerant vesicular-arbuscular mycorrhizal fungi. *New Phytol*., **95**: 655-661.

Sylvia, D.M. and Schenk, N.C. 1983. Application of superphosphate to mycorrhizal plants stimulates sporulation of phosphorus-tolerant vesicular-arbuscular mycorrhizal fungi. *New Phytol*., **95**: 655-661.

Thompson, J.P. 1986. Soilless culture of vesicular-arbuscular mycorrhizae of cereals: effects of nutrient concentration and nitrogen source. *Can. J. Bot*., **64**: 2282-2294.

Zobel, R.W., Del Tredici, P. and Torrey, J.G. 1976. Methods for growing plants aeroponically. *Plant Physiol*., **57**: 344-346.

(Received 10/10/2013;
in revised form 14/11/2013)

إكثار جراثيم الميكوريزا الداخلية فى المزارع الهوائية لاستخدامها فى تلقيح التربة كسمادٍ حيوي

فاطمة محمد كمال فرماوي
قسم خصوبة وميكروبيولوجيا الأراضى - مركز بحوث الصحراء - المطرية - القاهره – مصر.

أجريت تجربة معملية لمعرفة مدى استجابة نباتي البصل و الشعير لإكثار فطريات الميكوريزا الداخلية أجناس *G. mosseae* و *G. fasiculatum* و *G. intraradices* فى المزارع الهوائية. بعد مرور من ١٢ إلى ١٤ اسبوع وجد أن جميع الجذور قد استعمرت بفطريات الميكوريزا الداخلية. أيضاً تكونت الممصات والحويصلات بأعداد كبيرة داخل الجذور وتكونت الجراثيم بأعداد كبيرة داخل وخارج الجذور. أيضاً بالنسبة لكل أجناس الميكوريزا الداخلية وجد أن استجابة نبات الشعير للتلقيح وإنتاج الجراثيم لكل نبات زاد زيادة معنوية عن نبات البصل على الرغم من أن نسبة استعمار الجذور بفطريات الميكوريزا الداخلية كانت واحدة لكلا النباتين. وكانت هناك زيادة فى نسبة إصابة جذور وإنتاج الجراثيم لكل سنتيمترمن الجذور المصابة بفطريات الميكوريزا الداخلية مع زيادة المدة مع الأخذ فى الاعتبار أن هناك نقص فى النسبة فى بعض المعاملات فى الأسبوع الـ ١٤ .تراوح إنتاج الجراثيم من ٤ جراثيم (*G. mosseae*) إلى ٥١ جرثومة لكل سنتيمتر(*G. intraradices*). وكان متوسط نسب الإصابة لجذور نبات البصل٢٨.١ و ٥١.١ لأجناس (*G. mosseae, G. fasiculatum* *G. intraradices*,). وبمقارنة حيوية الجرائيم الناتجة من المزارع الهوائية و النامية فى تربة معقمة فقد أوضحت النتائج أنه لا يوجد فروق معنوية فى بعض الأجناس (*G. intraradices*) وكانت الجراثيم المنماة على التربة أكثر حيوية عن الناتجة من المزارع الهوائية فى بعض الحالات (*G. fasiculatum*).

أما بالنسبة للدراسات الخاصة بحفظ جراثيم فطريات الميكوريزا الداخلية فقد أوضحت النتائج ان جراثيم الميكوريزا الداخلية قد استعادة حيويتها ولم تتأثر عند الحفظ عند درجة حرارة ٤°م حتى ٩ أشهر بغض النظر عن نوع المادة المحملة عليها.