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ARBUSCULAR MYCORRHAIZAE AND YEAST AS BIOFERTILIZERS FOR ORGANIC FARMS

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ABSTRACT

Spores of arbuscular mycorrhaizae (AM) fungi were extracted from rhizosphere soil collected from around the root systems of corn, onion, sorghum and wheat plants (two months old) growing in four localities in Egypt to study their incidence.A green house was used for propagation of AM fungi collected from different sources using corn (*Zea mays*), sorghum (Sorghum *vulgare*), Leek (*Allium porrum*) and Onion (*Allium cepa*). Mycorrhizal spores of corn and sorghum rhizosphere significantly higher than that observed in the case of onion and wheat plants. Sorghum and corn roots are considered to be good plants for localization of AMF. They played an important role in the stimulation of plant growth via increasing uptake of P and other nutrients A mixture of mycorrhizal spores (containing different species belonging to *Glomus fasciculatum, Glomus mosseae, Glomus clarum, Gigaspora, Acaulospora* and *Sclerosystis* as predominant genera) were used as an inoculum. After three months, drip system irrigation was stopped to push the AM fungi to form spores outside the roots. The roots were air dried and cut to small pieces (1 cm in length). The spores were extracted from soil around the root region using special sieves. Clay, perlyte and alginate were used as carriers for spores and roots to produce mycozeen inocula. Results showed that the application of AMF and yeast *(Saccharomyces cerevisiae)* together stimulated sweet pepper growth and yield where yeast cells stimulated AMF to grow well and increased its efficiency to colonize in the root system of plant forming a good symbiotic relationship with plants.

Keywords: AM fungi, propagation, carriers, host plants, extraction, mycorrhizal spores, yeasts, effect on yield.

INTRODUCTION

The mycorrhiza is a symbiotic non-pathogenic relationship occurring between a plant root and fungus. The importance of mycorrhizae in enhancing growth and yield of many crop plants have been well documented (Bagyaraj *et al.,* 1978; and Jeffries, 1987). Dangeared (1900) was the first to name an AM fungus. He described a typical arbuscular mycorrhizal fungi on a popular and named the endophyte *Rhizophagus poepulinum.* Arbuscular mycorrhizae form a loose network of hyphae in the soil surrounding the roots and extensive hyphal growth within the root cortex. The fungi may also grow intercalary but not form a hartig net or a mantle of mycelium around the root. Several distinctly different types of endophytic mycorrhizae have been identified as arbuscular mycorrhizae caused by non-septate fungi , ericoid, orchidaceous caused by septate fungi. In nature, this mycorrhizal association, which is also referred to as vesicular-arbuscular mycorrhiza (VAM) is the most common of all root systems ; the non-mycorrhizal is unusual. However, vesicles are always absent in the genus *Gigaspora* and for this reason it is referred to as arbuscular mycorrhizae (Morton and Benny, 1990). AM fungi are widespread symbiotic root-fungus associations formed by nearly all terrestrial plants. These symbiosis are characterized by bi-directional transfer of nutrients between the plants and associated fungi, where plants provide sugar to the fungi and these help the plants on the acquisition of mineral nutrients from the soil (Smith and Barker, 2002). Additionally, mycorrhizal fungi also aid in soil- water extraction, increasing drought tolerance of the host (Mathur and Vyas, 2000). These associations are also reported to improve the plant's ability to tolerate heavy metal toxicity (Khan, 2001), as well as pathogens (Filion *et al.,* 1999 and Fusconi *et al.,* 1999). As a result of these benefits, mycorrhizal plants are often more competitive and better able to tolerate environmental stress than are non-mycorrhizal plants (Abdel-Fattah and Shabana, 2002 and Paradi *et al.,* 2002). Some mycorrhizal fungi produce phytohormones (indole-acetic acid, gibberellin, Zeatin, Abscisic acid) that have a direct effect on root morphology and plant growth (Ludwing-Muller, 2000; and Liu *et al.,* 2002). One of the most dramatic effects of infection by mycorrhizal

fungi on the host plant is the increase in organic and inorganic phosphorous uptake to the host roots. In addition, mycorrhiza infection results in an increase in the uptake of trace elements like zinc and copper (Oudeh *et al.,* 2002), potassium and magnesium (Sharma *et al.,* 2002). The hyphae associated with mycorrhizal plants can ramify a greater soil volume and provide a greater absorptive surface than root hairs on non-mycorrhizal plants (Al-Karaki and Hammad, 2001). Some mycorrhizal fungi have the capacity to breakdown phenolic compounds in soil that interfere with nutrient uptake (Bending and Read, 1997). Mycorrhizal fungi can increase drought resistance of plants (Abdel-Fattah *et al.,* 2002; Davies *et al.,* 2002) to some extent through enhanced water uptake at low soil moisture levels (Davies *et al.,* 1996). Increased stomatal sensitivity to leaf air vapor pressure deficient and increased root hydraulic conductivity by lowered leaf osmotic potential for greater tugor maintenance (Auge, 2001). Accumulation of some solutes (total soluble sugars) in mycorrhizal plants may serve as osmoregulators (Meddich *et al.,* 2002) and improved host nutrition particularly P (El-Tohamy *et al.,* 1999). In plants forming nodule symbiosis, mycorrhiza formation stimulates nodulation and nitrogen fixation (Gill *et al.,* 2002 and Xavier and Germida, 2002). Mycorrhizal fungi protected horticultural host plants against salinity (Al-Karaki, 2000 and Johnson-Green *et al.,* 2001) through improving plant water relations, increased the osmotic pressure in the cells of mycorrhizal plants by increasing the soluble metabolic materials like carbohydrates and proline. Mycorrhizal fungi may play an important role in increasing heavy metal tolerance (González-Chavez *et al.,* 2002).Mycorrhiza protected roots from heavy metals induced injury by excertion intrinsic metal-chelators or by other defense system (Paradi *et al.,* 2002; Klauberg-Filho *et al.,* 2002 and Schützendübel and Polle, 2002). AM fungi can stimulate plant growth (Gill *et al.,* 2002) and yield production (Xavier and Germida, 2002). This stimulation occurs by increasing the absorption of plant nutrition that is not normally available to non-mycorrhizal plants (Clark, 2002). Mycorrhizal fungi act as a biological control agent for the plant pathogens (Abdalla and Abdel-Fattah, 2000) by reducing the severity of the disease. Modifications in root physiology following mycorrhizal infection may explain the decrease in susceptibility to pathogens. These effects may arise through changes in lignin formation (Dugassa *et al.,* 1996), antibiotic production (Vigo

et al., 2000), induction of new isoforms of the hydrolytic enzymes e.g chitinase, chitosanase and B-1, 3- Glucanase (Dassi *et al.*, 1996) DNA demethylation (increased gene expressed for higher resistance of plant against pathogen) and higher ethylene production. Soil hyphae are likely to have an important role in nutrient cycling by helping to prevent losses from the system especially at times when roots are inactive. In addition, AM fungus hyphae are considered to function primarily by increasing soil volume action (Feng *et al.*, 2002). Hyphae of AM fungi are considered to contribute to soil structure by increasing aggregates and their stability (Caravaca *et al.*, 2002), soil fertility (Eriksson, 2001) and soil reclamation (Barea *et al.*, 1996). Mycorrhizal fungi contribute to carbon storage in soil by altering the quality and quantity of soil organic matter (Pfeffer *et al.*, 1999).

This investigation was aimed to study and evaluate AM fungi in some rhizosphere soil of some Egyptian crops and commercial production of AMF as a biofertilizer (Mycozeen) in pots and green house for biodynamic agriculture in SEKEM farms. The efficiency of this inoculum and yeasts on the growth of sweet pepper were also elucidated.

MATERIALS AND METHODS

Extraction of AM spores:

Spores of arbuscular mycorrhizae were extracted from rhizosphere soil collected from around the root systems of corn, onion, sorghum and wheat plants (two months old) growing in four localities of Elkhaliobia, Elsharkia, Elesmalia and Eldakahlia provinces in different seasons of 2008. The soil texture of these localities were silty loam, clay loam, sandy and sandy loam soils. The wet sieving and decanting technique was used according to Gerdmann and Nicolson (1963) the extracted spores were kept moist by storing at 4°C until used. The extracted spores were used as inoculum sources for the production of indigenous AM fungal isolates. The roots of different plants were gently detached and washed several times using tap water to remove all the remaining soil particles. Then the roots were cut into small segments (0.5 to 1.0 mm) and heated in water bath at 90°C for approximately 2 hrs in 10 % KOH to remove host cell cytoplasm and nuclei. Hence, the root cells become very clear. The roots were neutralized with HCl 1 N then, root segments were washed

by tap water three times and stained with 0.05 % Trypan blue (Sigma) in lacto-phenol for 15 min at 90°C. The excessive stain was removed by washing with tap water and the roots segments were stored in lactic acid until examination. The percentage of mycorrhizal colonization was estimated by the method of Trouvelot *et al.* (1986). Forty stained root pieces were selected randomly, mounted on slides in lacto-glycerol squashed and examined microscopically to estimate the degree of mycorrhizal colonization.

Mycorrhizal colonization assessment of soil samples

A serial dilution of sieved soil (10 fold) of each sample were prepared with pasteurized sand. About 100 gram of each dilution (five replicates) were transferred into plastic tubes with a narrow opening at the base for drainage purpose. Two pre-germinated sorghum seedling were then planted to each of the tubes. All the tubes were incubated at room temperature in green house , watered and fertilized with 15 ml free phosphorus Hoagland solution after every two-three days . After five weeks, the root system from each tube were harvested cleared and stained as mentioned before. The stained roots were then assessed microscopically for the presence of arbuscular mycorrhizal colonization. The MPN value was determined using Cochran's table (Cochran,1950).

Characterization of arbuscular mycorrhizal fungi:

The collected AM fungi were identified according to the key of Scheneck and Perez (1990) using morphological characteristics of hyphae, attached hyphae, chlamydospores and azygospores and sporocarp.

Sources of inocula

1. AM spores extracted from different localities
2. AM colonized roots from SEKEM green house
3. AM spores from Biofertilizers unit, Fac. Agric., Ain Shams Univ., Cairo.
4. AM spores from Dept. Agric. Microbiology, Fac. Agric., Ain Shams Univ., Cairo.
5. AM spores (BioMyc) from Germany.

Host plants

- Corn (Zea *mays*), sorghum (Sorghum *vulgare*), Leek (*Allium porrum*) and Onion (*Allium cepa*) obtained from Agricultural Research Centre, Cairo.

Propagation of AM fungi

The collected spores of AM fungi were propagated by inoculation of pots (35 cm) diameter filled with sandy loam soil amended with compost (72 % sand, 24 % silt and 4 % clay with pH 7.22 and EC 12.7 dSm^{-1} as shown in Table, 1) with 20 gram or 10 ml of AMF inoculum (containing 100- 150 spores) which layered 3 cm below the surface of soil and placed in green house at 25-30°C. Soaked seeds of corn and sorghum (two days) and leek & onion seedlings were planted to each pot (2-3 seeds or 2-3 seedlings / pot) and covered with thin layer of the same soil (5^{th} May 2008). The irrigation was carried out twice / week or more depending on the weather. The pots (80 pots) were classified into four groups *i.e.* 20 pots for each plant. These pots were divided into 4 groups where each group (five pots) were inoculated with the different sources of AM fungi (spores extracted from different localities, *e.g.* Biofertilizers unit, Fac. Agric., Ain Shams Univ.,Dept. of Agric. Microbiology, Fac. Agric., Ain Shams Univ.,BioMyc from Germany. The plants were watered when necessary. During the flowering stage, the irrigation was stopped (after approximately 3 months of planting) for 10 days to encourage mycorhizal sporulation then, the spores were extracted as described previously above. To increase the biomass of extracted spores, A green house (8.5 x 40 m^2) was used for propagation of AM fungi using the same host plants. Different rows of the green house (50 m in length) consisting of the same soil of pots (sandy loam soil) were inoculated with mycorrhizal spores containing a mixture of mycorrhizal spores, representing different species belonging to *Glomus fasciculatum, Glomus mosseae, Glomus clarum, Gigaspora, Acaulospora* and *Sclerosystis* as predominant genera at 2 cm depth under the soaked seeds (one and two rows for corn and another for sorghum). Onion and leek were inoculated in the nursery and planted in two another rows for each plant. The rows were watered with dripping irrigation. After three months, irrigation was stopped to push the AM fungi to form spores outside the roots. The roots were air dried and cut to small pieces (1 cm in length). The spores were

extracted from soil around the root region using special sieves. Clay, perlyte and alginate were used as carriers for spores and roots to produce mycorrhizal inocula (Mycozeen).

Table (1): Soil analysis of green house

Determinations	Values	Determinations	Values
pH (1:2)	7.22	Zn (ppm)	2.04
EC dSm^{-1}(1:2)	12.7	Cu (ppm)	1.5
TN (ppm)	252.4	$CaCO_3$ %	11.6
P (ppm)	388	Sand %	72
K (ppm)	103	Silt %	24
Fe (ppm)	10.6	Clay %	4
Mn (ppm)	250	Texture	Sandy Loam

Gravels 20 %

Effect of AM Fungi and yeasts on sweet pepper

This experiment was conducted in green house (37.5 x 90 m^2) which air conditioned at 25°C at Sekem farms. The actual space for cultivation was 270 m^2. The soil of green house (sandy loam) was treated with compost (15 m^3 /feddan). The area of green house was divided into five treatments, the first part, second part, third part, fourth part and fifth part were treated with AM spores, AM roots, yeasts, AMF+yeast and control respectively. The inoculation was carried out directly under the sweet pepper seedling (10 g of mycorrhizal inocula containing 15 to 20 spores or colonized roots whereas 5 g (containing $5x10^{12}$ yeast cells) were used in the case of yeast. The seedling of sweet pepper (Cabino) were transmitted on 11th of August 2008). The height of plants and fresh weight of fruits were measured periodically.

Chemical analysis

Soil samples collected from different localities were subjected to some analysis including soil reaction (pH), soluble cations and anions, EC, soil texture (Olsen *et al.*, 1954 and Rhichards, 1954).

Measurements

Plant height (in cm), fresh weight and dry weight of test plants were determined at 80°C for 48 h.

RESULTS AND DISCUSSION

Incidence of AM Fungi in rhizosphere soil of tested plants

Arbuscular mycorrhizae (AM) are symbiotic associations formed between plants and soil fungi that benefit both partners. The phytobiont correspond to approximately 80% of plant species and the fungi are classified in the phylum Glomeromycota, including ten genera; *Glomus*, *Paraglomus*, *Sclerocystis*, *Acaulospora*, *Entrophospora*, *Gigaspora*, *Scutellospora*, *Diversispora*, *Geosiphon*, and *Archaeospora* (Schuessler *et al.,* 2001). So, it was found worthy to extract the spores of this mycorrhizae from the most agricultural crops such corn, onion, sorghum and wheat which can get benefit from mycorrhizal association. Results in Fig. (1) showed that the highest numbers of mycorrhizal spores, either total or viable spores, were recorded in rhizoshere soil of sorghum plants being 850 and 800 spores/Kg soil, respectively , followed by corn where the corresponding figures were 730 and 700. Both plants also gave the highest percentage of viable spores. Mycorrhizal spores of corn and sorghum rhizosphere significantly higher than that observed in case of onion and wheat plants. This variation may be due to the cultivated crop, soil type and available phosphorus (Ezawa *et al.,* 2000). Gerdemann and Trappe (1974) reported that plants differ greatly in their need for and response to mycorrhizal infection. The most dominant AM Fungi were *Glomus faseculatum, Glomus mosse, Glomus clarum, Gigaspora, Acaulospora* and *Sclerosystis* as predominant genera (Fig. 2)

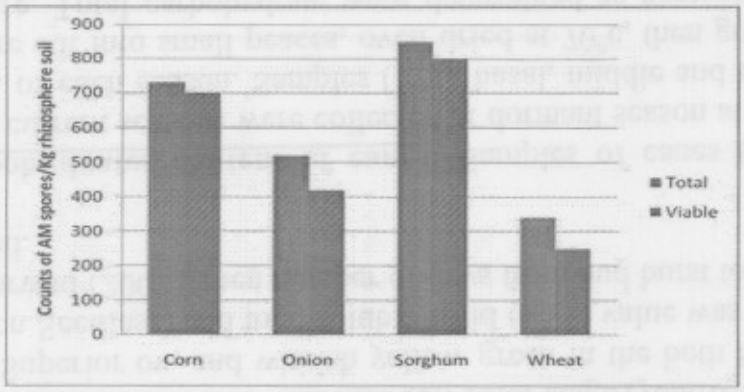

Fig (1): Mycorrhizal counts in rhizosphere soil of selected plants (LSD (0.05) = 41.07)

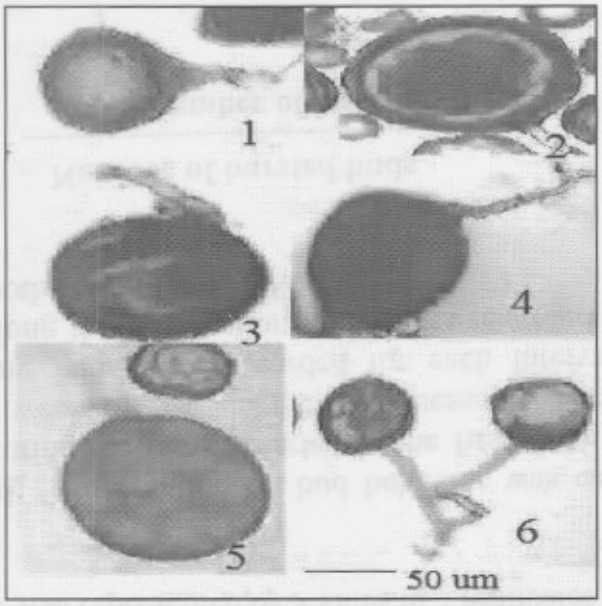

Fig (2): AM spores (1-*Glomus mossae*,2- *Glomus clarum*, 3-*Glomus* sp, 4-*Glomus* sp, 5- *Gigaspora* sp, 6-*Glomus fasceculatum*

Colonization of AM fungi

Results in Table (2) and Fig. (3) Showed that the colonization of AMF ranged from 70 to 95 % in the roots of selected plants. The highest percentage of colonization was recorded in case of sorghum roots followed by corn being 95 and 85 % respectively, whereas wheat roots showed the lowest percentage of 70 % as compared with other plants. It means that the sorghum and corn roots are considered to be good plants for localization of AMF. In this respect, Plenchett *et al.*, (1983) stated that plant species differ in their fertilization requirements, and consequently their dependency on AMF vary considerably from one crop to another.

Table (2): Colonization of AM fungi in the roots of some plants

Plants	Colonization of AMF %	SE ±
Corn	85	3.1
Onion	80	5.5
Sorghum	95	6.3
Wheat	70	3.3

LSD (0.05) = 14.5

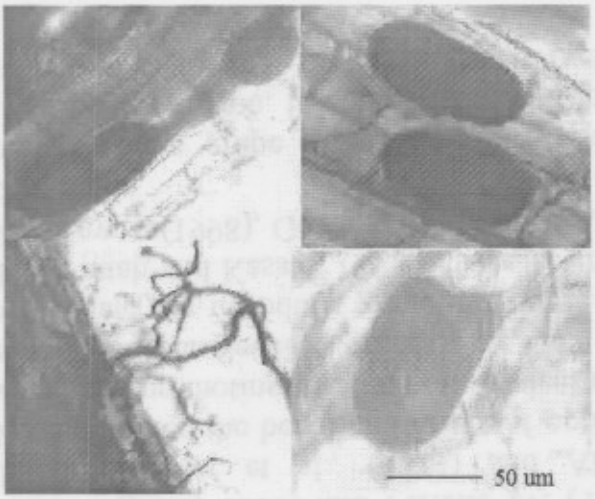

Fig (3) : Colonization of AMF in plant roots

Effect of AMF on the growth of some cereal plants

It is obvious from Table (3) that fresh and dry weights of corn, sorghum and Sudan grass were highly affected with mycorrhizal inoculation. The highest fresh and dry weights was recorded in case of corn being 325.36 and 164 g/plant, respectively. The corresponding figures for the percentage of increase were 21.43 % and 9.32 %. It means that AMF play an important role in the stimulation of plant growth via increasing uptake of P and other nutrients (Clark, 2002). On the other hand , the relative Field Mycorrhizal Dependency (RFMD %) *i.e.* the difference between dry matter of mycorrhizal and non- mycorrhizal plants per 100 unit of dry matter of mycorrhizal plants was 93.4, 91.2 and 89.1 % for corn, sorghum and Sudan grass, respectively.

Table (3): Effect of AMF on fresh and dry weight of plants

Plant	Plant weight (gram)			
	Control		Inoculated	
	Fresh	Dry	Fresh	Dry
Corn	267.94	150.02	325.36	164
Sorghum	123.61	45.33	142.3	72.7
Sudan grass	138.63	44.55	165.78	60.62

LSD (0.05) = 14.1

Effect of AMF on the growth of sweet pepper (Capino)

Results in Fig (4) clearly showed that the height of sweet pepper plants increased gradually during 17 weeks time course of propagation for all treatments and control. The biotreatment with both AMF and yeast showed the highest values among 17 weeks of growth where 125 cm height of plant was recorded, followed by biotreatment with AM spores showing 120 cm plant height as compared with control (96.7 cm height). Bio-treatment with either AM roots or yeast gave low values as compared with other treatments but still higher than control. Table (4) also demonstrated that the fresh weight of sweet pepper fruits exhibited the same trend of plant heights where 446 Kg / row were recorded as compared with other treatments and control.

It could be suggested that the application of AMF and yeast together stimulated sweet pepper growth and yield where yeast cells stimulated AMF to grow well and increased its efficiency to colonize in the root system of plant forming a good symbiotic relationship with plant (Jeffries, 1987). Manfred *et al.*, (2006) concluded that dual inoculation of maize with yeasts and AMF resulted in increased shoot biomass depending on the combination of yeast species and AMF isolate.

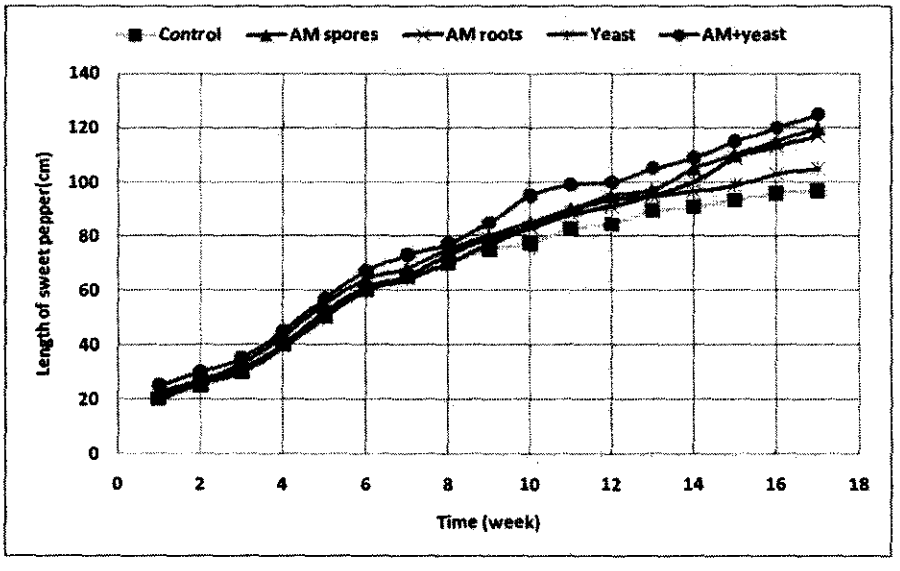

Fig (4): Effect of AMF and yeast on the growth of sweet pepper (Capino)

Table (4): Effect of AMF and yeast on the yield of sweet pepper (Capino)

Time	Fresh weight of sweet pepper fruits (Kg/row)									
(months)	Control	SE	AM fungi	SE	AM roots	SE	Yeast	SE	AM+yeast	SE
1	27	1.48	42	1.03	42	1.1	40	2.1	44	2.5
2	80	2.5	82	2.03	81	2.3	83	2.69	87	3.1
3	68	1.54	78	1.28	77	1.2	80	1.52	90	2.5
4	43	0.41	90	1.93	88	1.53	85	1.64	95	4.4
5	119	7.5	122	6.14	120	6.3	110	10.02	130	9.2
Total	337	1.83	414	1.62	408	2.62	398	2.16	446	4.34

LSD (0.05) =8.7362

Conclusions

Generally, It could be concluded that AM Fungi are widely distributed in soil and their incidence highly varied form plant to another and also from soil to another. The application of arbuscular mycorrhizae and yeasts as a dual biofertilizers was more efficient in increasing the growth and yield of sweet pepper.

REFERENCES

Abdalla, E.M. and Abdel-Fattah, G. M. (2000). Influence of the endomycorrhizal fungus *Glomus mosseae* on the development of peanut pod rot disease in Egypt. Mycorrhiza, 10:29-35

Abdel-Fattah, G.M. and Shabana, Y.M. (2002). Efficacy of arbuscular mycorrhizal fungus *(Glomus clarum)* in protection of cowpea plants from root rot pathogen *Rhizoctonia solani.* J. Plant Dis. Protect., 109:207-215.

Al-Karaki, G. N. (2000). Growth of mycorrhizal tomato and mineral acquisition under salt stress. Mycorrhiza. 10: 51-54.

Al-Karaki, G. N. and Hammad, R. (2001). Mycorrhizal influence on fruit yield and mineral content of tomato grown under salt stress J. Plant Nutr., 24: 1311-1323.

Auge, R. M. (2001). Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza, 11: 3–42.

Bagyaraj, D. J.; Manjunath, A. and Patil, R. B. (1978): Interaction between a vesicular arbuscular mycorrhiza and *Rhizobium* and their effect on soybean in the field. New Phytopathologist, 82: 141-145.

Barea, J. M.; Requena, N. and Jimenez, I. (1996). A revegetation strategy based on the management of arbuscular mycorrhizae. *Rhizobium* and rhizobacterias for the reclamation of desertified Mediterranean shrubland ecosystems. Cahiers Options Mediterr., 20: 75-86.

Bending, G. O. and Read, O. J. (1997). Lignin and soluble phenolic degradation by ectomycorrhizal and ericoid mycorrhizal fungi. Mycol. Res., 101: 1348-1354.

Caravaca, F., Barea, J. M.; Figueroa, D. Roldan, A. (2002). Assessing the effectiveness of mycorrhizal inoculation and soil compost addition for enhancing reafforestation with Plea europaea suhsp sylvestris through changes in soil biological and physical parameters. Applied Soil Ecology, 20: 107- 118.

Clark, R. B. (2002). Differences among mycorrhizal fungi for mineral uptake per root length of switchgrass grown in acidic soil. .J. Plant Nutr., 25: 1753-1772.

Cochran, W.G. (1950). Biometrics 6, 105 – 116.

Dangeared, P.A. (1900). Le *' Rhizophagus populinus'* Dangeared. Botaniste, 7: 285-291.

Dassi, S.; Dumas-Gaudot, E.; Asselin, A.; Richard, C. and Gianinazzi, S. (1996). Chitinase and B- 1,3-glucanase isoforms in pea roots inoculated with' arbuscular mycorrhizal or pathogenic. Europ. J. Plant Pathol., 102: 105-108.

Davies, F.T.; Olalde-portugal, V.; Aguilera-Gomez, L.; Alvarado, M.J.; Ferrera-Cerrato, R.C and Boutton, T. W. (2002). Allevation of drought stress of chile ancho pepper *(Capsicum annuum* L. cv. San Luis) with arbuscular mycorrhiza indigenous to Mexico. Sci. Horticulture, 92:347-359.

Davies, F. T.; Svenson, S. E.; Cole, J. C.; Phavaphutanon, L.; Duray, S. A. and Olalde-Portugal V (1996). Non-nutritional stress acclimation of mycorrhizal woody plants exposed to drought. Tree Physiology, 16: 985-993.

Dugassa, G. O.; Yon Alten, H. and Schonbeck, F. (1996). Effects of arbuscular mycorrhiza (AM) on health of *Linum usitatyssimum* L. infected by fungal pathogens. Plant and Soil, 185: 173-182.

EI- Tohamy, W.; Schnitzler, W. H.; EI-Behairy, U. and EI-Beltagy, M. S. (1999). Effect of VA mycorrhiza on improving drought and chilling tolerance of bean plants (*Phaseolus vulgaris* L.). Angewandte Botanik, 73: 178-183.

Eriksson, A. (2001). Arbuscular mycorrhiza in relation to management history soil nutrients and plant species diversity. Plant Ecology, 155: 129-137.

Ezawa, T., Yamamoto, K., and Yoshida, S. 2000. Species composition and spore density of indigenous vesicular-arbuscular mycorrhizal fungi under different conditions of P-fertility as revealed by soybean trap culture. Soil Sci. Plant Nutr., 46:291-297.

Feng, G.; Yubo, S.; Li, X.; Wang, H.; Zhang, F.; Tang, C.; and Rengel, Z. (2002). Histochemical visualization of phosphatase released by arbuscular mycorrhizal fungi in soil. .J. Plant Nutrition. 25: 969-980.

Filion, M.; St-Arnaud, M. and Fortin, J.A. (1999). Direct interaction between the arbuscular mycorrhizal fungus *Glomus interaradices* / and different rhizosphere microorganisms. New Phytol., 141 :525-533

Fusconi, A.; Gnavi, E.; Trotta, A. and Berta, G. (1999). Apical meristems of tomato roots and their modifications induced by arbuscular mycorrhizal and soil born pathogenic fungi. New Phytol., 142:505-516.

Gerdemann, J.W.and Trappe, J.M. (1974). The Endogonaceae in the Pacific Northwest. Mycologia Memoir, No. 5: 76.

Gerdemann, J. W. and Nicolson, T. H. (1963). Spores of mycorrhizal *Endogamy* species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc., 46: 235.

Gill, T. S.; Singh, R. S. and Jaspal, K. (2002). Comparison of four arbuscular mycorrhizal fungi for root colonization, spore population and plant growth responses in chickpea. Indian Phytopathology, 55: 210-212.

González-Chavez, M. C.; D'Baen, J.; Vangronsveld, J. and Dodd, J. C. (2002). Copper sorption and accumulation by the extraradical mycelium of different *Glomus* spp. (Arbuscular mycorrhizal fungi) isolated from the same polluted soil. Plant and Soil, 240: 287-297.

Jeffries, P. (1987). Use of mycorrhizae in Agriculture. CRC Critical Review in Biotechnology, 5(4): 319-357.

Johnson-Green, P.; Kenkel, N. C. and Booth, T. (2001). Soil salinity and arbuscular mycorrhizal colonization of *Puccinellia nuttalliana.* Mycological Res., 105: 1094-1100.

Khan,A.G. (2001). Relationships between chromium biomagnification ratio, accumulation factor, and mycorrhizae in plants growing on

tannery effluent-polluted soil. Environ. Int., 26:417-423.
Klauberg-Filho, O.; Siqueira, J. O. and Moreira, F. M. (2002). Vesicular arbuscular mycorrhiza fungi in soils polluted with heavy metals. Revista Brasileria de Ciencia do Solo., 26: 125-134.
Liu, R.; Li, M., Meng, X.; Liu, X. and Li, X. (2002). Effects of AM fungi on endogenous hormones in corn and.cotton plants. Mycosystema, 19: 91-96.
Ludwing-Muller, J. (2000). Hormonal balance in plants during colonization by mycorrhizal fungi. In: Arbuscular Mycorrhizas: Physiology and function. Y. Kapulnick and D. D. Douds. Jr. (Eds.). Kluwer Academic Press. pp. 263-286.
Manfred J. G., D. Püschel, J. Rydlová and M. Vosátka (2006). Effect of inoculation with soil yeasts on mycorrhizal symbiosis of maize. Pedobiologia, 50 (4):341-345
Mathur, N. and Vyas, A. (2000). Influence of arbuscular mycorrhizae on biomass production, nutrient uptake and physiological changes in *Ziziphus mauritana* lam. under water stress. J. Arid Environ., 45:191-195.
Meddich, A.; Oihabi, A.; Abbas, Y. and Bizid, E. (2002). Effect of arbuscular mycorrhizal fungi on drought resistance of clover. Agronomic. 20: 283-295
Morton, J. B. and Benny, G. L. (1990). Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order, Glomales, two new suborders, Glomineae and Gigasporinae, and two new families, Acaulosporaceae and Gigasporaceae with an emendation of Glomaceae, Mycotaxon, 37:471-491.
Olsen, S.R.; Cole, C.V.; Watanabe, F.S. and Dean, L.A. (1954).Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Dept. Agric., Circular No. 939,19 P.
Oudeh, M., Khan, M. and Scullion, J. (2002). Plant accumulation of potentially toxic elements in sewage sludge as effected by soil organic matter level and mycorrhizal fungi. Environmental Pollution, 116: 293-300.
Paradi, I.; Bratek, Z.; Berecz, B. and Halasz, K. (2002). Influence of arbuscular mycorrhiza, P limitation and Cd-stress on polyamine contents of plants. Acta Biologica Szegediensis, 46:59-60.
Pfeffer, P. E.; Douds Jr. D.; Becard, G. and Shachar-Hill, Y. (1999). Carbon uptake and the metaholism and transport of lipids in an arbuscular mycorrhiza. Plant Physiology, 120: 587-598.

Plenchette, C., Fortin, J. A., and Furlan, V. 1983. Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. I. Mycorrhizal dependency under field conditions. Plant Soil, 110:199-209.

Richards,L.A. (1954). Diagnosis and imprivment of saline and alkaline soil. Dept. Agric., Handbook No. 60: 17-337. USA.

Schenck, N. C. and Perez, Y. (1990). Manual for the indentification of VA Mycorrhizal fungi 3rd. ed., INVAM. Univ. Florida, Gainesville.

Schuessler, A., Schwarzott, D., and Walker, C. 2001. A new fungal phylum, the Glomeromycota: Phylogeny and evolution. Mycol. Res., 105:1413-1421.

Schützendübel, A. and Polle, A. (2002). Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J. Exp. Bot., 53: 1351–1365.

Sharma, S. O.; Bhutani, V. P. and Awasthi, R. P. (2002). Effect of vesicular-arbuscular mycorrhizae and phosphorus on leaf and soil mineral nutrient status of apple seedling. Indian J. Horticulture, 59: 140-144.

Smith, S. R. and Barker, S. J. (2002). Plant phosphate transporter genes help harness the nutritional benefits of arbuscular mycorrhizal symbiosis-Trends Plant Sci., 75(5): 189-190.

Trouvelot, A.; Kough, J. L. and Gianinazzi-Pearson, V. (1986): Measure dutaux de mycorrhization VA d'un system radiculaire recherché de methods d'estimation ayant une signification functionelle In: Physiological and Genetical Aspects of Mycorrhizae, Gianinazzi-Pearson, V. and Gianinazzi, P. S. (Eds.). INRA, Paris. 217 P.

Vigo, C.; Norman, J. R. and Hooker, J. E. (2000). Biocontrol of the pathogen *Phytophthora parasitica* by arbuscular mycorrhizal fungi is a consequcnce of effects on infection loci. Plant Pathology, 49: 509-514.

Xavier, L. and Germida, J. J. (2002). Response of lentil under controlled conditions to coinoculation with arbuscular mycorrhizal fungi and *Rhizohia* varying in efficacy. Soil Biology and Biochemistry, 34: 181-188.

إستخدام الميكروهيزا الشجيرية والخميرة في التسميد الحيوي بالمزارع العضوية

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** المركز الإقليمي للأغذية والأعلاف – مركز البحوث الزراعية – الجيزة – مصر.

تم فى هذا البحث استخلاص جراثيم فطريات الميكروهيزا الشجيرية (AMF) من ريزوسفير التربة المحيطة لجذور نباتات الأذرة الشامية والرفيعة والبصل والقمح (عمر شهرين) والنامية في 4 مواقع في مصر لدراسة مدي تأثيرها و قد أستخدمت البيوت المحمية لإكثار فطريات AM التي تم تجميعها من مصادر مختلفة بإستخدام الأذرة الشامية والرفيعة وكرات بشوشة والبصل.

لوحظ أن جراثيم الميكروهيزا بمنطقة ريزوسفير الأذرة الشامية والرفيعة كانت تواجدا من تلك الملاحظة في حالة نباتات البصل والقمح.

أثبتت النتائج أن جذور نباتات الأذرة الشامية والرفيعة تعتبر من أفضل النباتات لإكثارAMF، كذلك فقد أشارت النتائج أن AMF تلعب دوراً مهماً في تشجيع نمو النباتات عن طريق زيادة المأخوذ من الفوسفور والمغذيات الأخرى.

أستخدم خليط من جراثيم الميكروهيزا يحتوي علي أنواع مختلفة مناسبة من (*Glomus Fasciulatum, Glomun Mosseae, Glomun Clarum, Gigaspora, Acaulospora and Sclerosystis*) كمثال للأجناس السائدة كاللقاح.

أظهرت النتائج أن التلقيح باستخدام فطريات AM وخميرة (*Sacharomyces Cerevisiae*) كان له تأثيرا ايجابيا علي نمو وإثمار الفلفل الحلو. أيضا فقد تأكد أن استخدام AMF والخميرة معاً تحفز نمو وإثمار الفلفل الحلو. و أن خلايا الخميرة كانت محفزة لنمو AMF و أدت الي زيادة تأثيرها في إصابة جذور النبات مكونة علاقة متبادلة تكافلياً مع النبات.