doi:10.14720/aas.2019.113.2.13 Original research article / izvirni znanstveni članek

Sunflower response to inoculation with single and mixed species of arbuscular mycorrhizal fungi: Agronomic characteristics

Mazen IBRAHIM [1]

Received January 11, 2018; accepted June 25, 2019.
Delo je prispelo 11. januarja 2018, sprejeto 25. junija 2019.

Sunflower response to inoculation with single and mixed species of arbuscular mycorrhizal fungi: Agronomic characteristics

Abstract: The impact of indigenous arbuscular mycorrhizal fungi (AMF) on agronomic characteristics of sunflower (*Helianthus annuus* L.) was evaluated in a pot experiment. The indigenous AMF, including *Glomus intraradices*, *Glomus mosseae,* and *Glomus viscosum*, were isolated from an agricultural field in which cotton and sunflower plants were grown. The most abundant species (*G. viscosum*) was multiplied in a monospecific culture. Sunflower plants were inoculated with the mixture of three selected AMF species or solely with *G. viscosum*. The number of leaves, shoot length, head diameter, above ground biomass, and seeds mass were significantly higher in the plant inoculated with AMF mixture followed by individual inoculation with *G. viscosum* followed by the control. AMF mixture outperformed the *G. viscosum*by increasing mycorrhizal dependency and mycorrhizal inoculation effect of sunflower. The results indicate that AMF mixture could be considered as a good inoculum for improving growth and yield of sunflower in sustainable agriculture.

Key words: arbuscular mycorrhizal fungi; sunflower; growth; yield

Odziv sončnice na inokulacijo s posameznimi arbuskularnimi mikoriznimi glivami in njihovo mešanico: agronomske lastnosti

Izvleček: V lončnem poskusu je bil ovrednoten vpliv samoniklih arbuskularnih mikoriznih gliv (AMG) na agronomske lastnosti sončnice (*Helianthus annuus* L.). Samonikle AMG, *Glomus intraradices*, *Glomus mosseae,* in *Glomus viscosum*, so bile izolirane iz poljskih tal, kjer sta rastla bombaž in sončnice. Najbolj pogosta vrsta, *G. viscosum,* je bila namnožena v enovrstni kulturi. Sončnice so bile inokulirane z mešanico treh izbranih AMG ali pa samo z vrsto, *G. viscosum*. Vrednosti parametrov kot so število listov, dolžina poganjkov, premer koškov, nadzemna biomasa in masa semen so bile značilno večje, kadar so bile rastline inokulirane z mešanico AMG kot pri inokulaciji samo z vrsto *G. viscosum* in najmanjše pri kontroli. Mešanica mikoriznih gliv se je izkazala boljša kot samo uporaba vrste *G. viscosum* zaradi povečane odvisnosti sončnice od mikorize in povečanega učinka mikorizne inokulacije na sončnico. Rezultati nakazujejo, da bi bila inokulacija sončnice z mešanico mikoriznih gliv dober postopek za izboljšanje njene rasti in pridelka v trajnostnem kmetijstvu.

Ključne besede: arbuskularne mikorizbe glive; sončnica; rast; pridelek

1 Atomic Energy Commission of Syria, Agriculture Department, P.O. Box 6091, Damascus, Syria; Corresponding author, e-mail: ascientific@aec.org.sy

1 INTRODUCTION

Mycorrhiza is a symbiotic association between the roots of higher plants and soil fungi (Smith et al., 2010). Mycorrhizal fungi are widespread in agricultural systems and are particularly relevant for organic agriculture because they can act as natural fertilizers, enhancing plant yield (van der Heijden et al., 2008). Arbuscular mycorrhizal fungi (AMF) influence processes in the root system at different levels (Berta et al., 1995) and reduces fertilizer input into cropping system, without substantial loss in yield (Tripathi et al., 2005). AMF species affect plant growth differently (Zhang et al., 2011). The same species of AMF, in different geographic locations, might vary in their ability to colonize roots and improve the growth of plant (Camprubi and Calvet, 1996). Sunflower as one of the most important edible oilseed crop in the world (Weiss, 2000), can benefit from AMF especially under conditions where nutrient availability is limiting plant growth (van der Heijden et al., 2008). The application of AMF in sunflower increased growth parameters of plant, head diameter, and seed yield (Jalaluddin and Hamid, 2011; Soleimanzadeh, 2010; Kavitha and Nelson, 2014). Mycorrhizal dependency (MD), as a useful trait to quantify plant responsiveness to AMF colonization, was found to be high for sunflower grown at low P treatment (Fernandez et al., 2009). AMF species vary in their ability to acquire resources below ground (Jakobsen et al 1992) and to stimulate host plant growth (van der Heijden, 1998a). Daft (1983) suggested that multiple colonization, i.e. use of a consortium, rather than a single AMF, was probably more beneficial to the plant, because a single AMF may not be able to withstand certain environmental changes.

We hypothesize that sunflower responds differentially to different AMF species and their mixture. To test this hypothesis, sunflower plants were separately inoculated with single AMF species, or mixture of AMF species, to reveal the effect of the two types of inocula on the agronomic characteristics of sunflower in sustainable agriculture.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL SITE

The experiment was conducted in plastic pots (40 x 45 cm) containing 35 kg soil during the summer season (May to September 2015) at Atomic Energy Commission of Syria (AECS) research Station, southeast of Damascus, Syria (33°21’ N, 36°28’ E). Sandy clay loam soil with pH 8.5 and organic matter 8.1 %, were obtained from an agricultural field in which cotton and sunflower were grown. The soil was air dried, sieved to pass a 3 mm screen, and pasteurized at 5 kGy of gamma ray with ^{60}Co source using a gamma irradiator (ROBO, Russia).

2.2 AMF INOCULUM PREPARATION

AMF were obtained from the soil previously isolated (Ibrahim, 2010) from an agricultural field in which cotton and sunflower were grown. For propagating AMF, pot-cultures containing a 1:1 autoclaved soil:sand were established using onion (*Allium cepa* ‘Selmouni Red’) as a trap plant. Pot-cultures were conducted during two successive cycles of 4 months. Plants were fertilized once a week with 20 ml Long Ashton nutrient solution (Hewitt, 1966) containing reduced P concentration. Spores were extracted from soil and trap culture by wet sieving and sucrose density gradient centrifugation (Brundrett et al., 1996) and examined under a stereomicroscope (magnification x 40). AMF species identification based on the spore morphology, was made in Eastern Cereal and Oilseed Research Centre, Canada. Representative spores of each morphotype were mounted on glass slides with polyvinyl lacto-glycerol (PVLG) and Melzer’s reagent (1:1, v/v). Spore morphology was compared with original species descriptions, voucher specimens of the National Mycological Herbarium (DAOM), and internet descriptions of INVAM (http://fungi.invam.wvu.edu/the-fungi/species-descriptions.html, and J. Blaszkowski websites (http://www.agro.ar.szczecin.pl/~jblaszkowski/Mycota/index.html). AMF isolate included *Glomus intraradices* Schenck & Smith, *Glomus viscosum* T.H.Nicolson, and *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe. *G. viscosum* was the most abundant species (based on the number of viable spores in the trap substrate) was multiplied in a monospecific culture which was produced by inoculation onion roots with the spores. After the verification of the monospecificity in the second multiplication, the whole pot’s content was air-dried and the roots were cut into 1-cm pieces and thoroughly mixed with the soil:sand substrate. The inoculum of two AMF types consisting fragments of onion root, mycelium and spores, contained 580 propagules (spores, hyphae and colonized roots) per 100 g substrate as determined using the most probable number assay (Sieverding, 1991).

2.3 PLANTING PROCEDURES AND TREATMENTS

Four seeds of confection-type hybrid sunflower

'Rayal' were sown in each pot (40 x 45 cm). Half of the pots received the AM inoculum (100 g per pot) by layering at 10 cm depth of the pots at the time of sowing. Two types of AMF inocula were used in this experiment: mixed species of AMF that included *G. intraradices*, *G. viscosum*, and *G. mosseae*, and a single species inoculum (*G. viscosum*). Non-AM pots received the same amount of sterilized AMF inoculums which were autoclaved 20 min at 121 °C. Seedlings were thinned to one per pot with keeping the roots of discarded plants in the soil to avoid removing the AMF inoculum. The experiment involved three treatments: control (sterilized AMF inoculum), inoculation with AMF mixture, and inoculation with *G. viscosum*. The pots were arranged in a randomized complete block design with four replicates (4 plants for each treatment). The plants were grown under natural conditions with an average daylength 14h, max/min temperature 36 °C/17 °C, and average relative humidity 40 %. No chemical fertilizers was added, and adequate soil moisture was maintained during the experiment.

2.4 PLANT SAMPLING AND ANALYSIS

Growth parameters, such as shoot length and the leaves number per plant were measured at the maturity stage. The plants were harvested at physiological maturity stage (R9) (Schneiter and Miller, 1981) and used for measuring head diameter, number of seeds per head, hundred seeds mass, above ground dry mass. Mycorrhizal dependency (MD) of sunflower was determined using the equation of Plenchette et al. (1983). Mycorrhizal Inoculation Effect (MIE) as indicator for accessing the growth improvement caused by inoculation with the indigenous AMF, was calculated as the difference between the dry mass of the AM and non-AM plants and was expressed as a percentage of the dry mass of AM plant.

2.5 DETERMINATION OF AMF COLONIZATION

Roots samples were washed thoroughly in tap water and cut into 1 cm fragments. The root segments were then cleared in 10 % (w/v) KOH by heating at 90 °C for 30 to 60 minutes, depending on the degree of lignification of the roots. It was then washed and stained with acid fuchsin in lactoglycerol. The rate of AMF-root colonization was determined under a binocular microscope by the gridline intersect method (Giovannetti and Mosse, 1980).

2.6 STATISTICAL ANALYSIS

The data were analysed by the analysis of variance (ANOVA) using SAS program (SAS Institute Inc., 2004), and means were compared by the least significant difference (Fisher's PLSD) test at a confidence level of 5 %.

3 RESULTS AND DISCUSSION

No AM colonization was noted for roots of non-inoculated plants. AMF structures, *i.e.* arbuscules, vesicles and hyphae were observed in the root of inoculated plants at harvest (Fig.1). However, no significant difference was found for the rate of AMF-root colonization of sunflower plants inoculated with single and mixed species of AMF (Fig. 2). The AMF-root colonization varied between 24.9 and 27.6 %. This result is similar to that of Fernandez et al. (2009) who showed that sunflower had 25.85 % AMF colonization at low P.

The leaves number was significantly greater in the AM plants than the control (Fig.3). The individual inoculation of *Glomus viscosum* had higher number of leaves (27.6) compared to the control (22.3). However, *G. viscosum* had greater effect on leaves number when it was in-

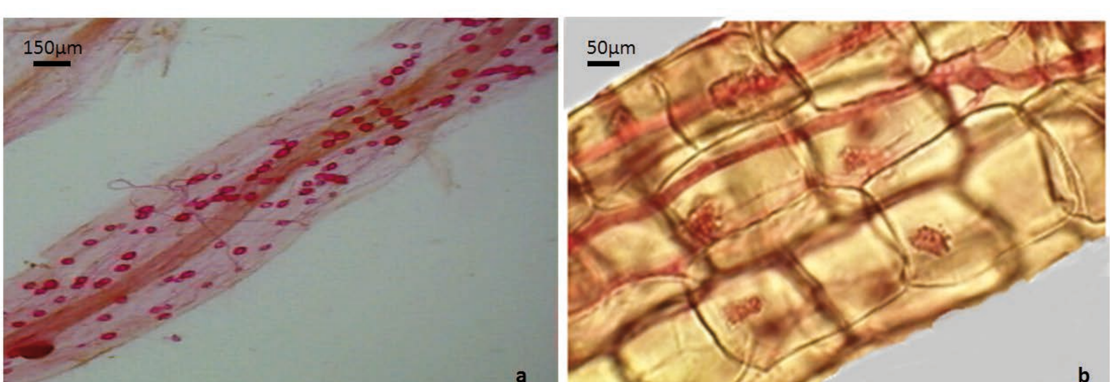

Figure 1: Microscopic images of AMF- root colonization in sunflower: (a) vesicles and hyphae(x40), and (b) arbuscules (x100)

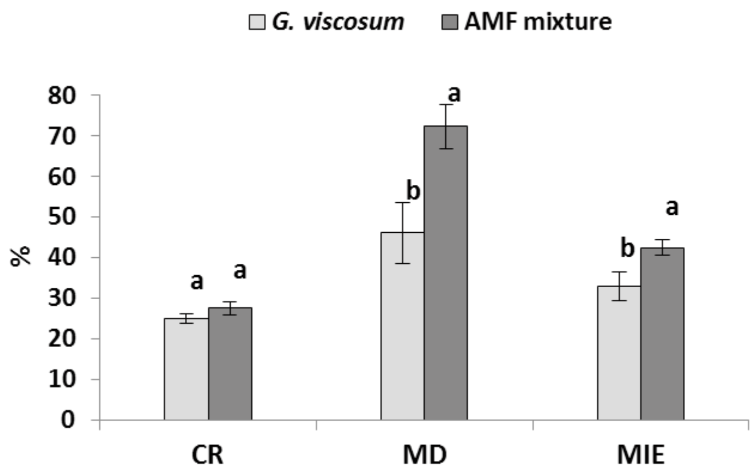

Figure 2: Effect of inoculation with single (*G. viscosum*) and mixed AMF on colonization rate (CR), mycorrhizal dependency (MD), and mycorrhizal inoculation effect (MIE) in sunflower. Vertical bars represent ± SD (n = 4).Different letters (for each parameter) on square bars indicate significant difference ($p < 0.05$).

cluded in AMF mixture. The inoculation of mixed species induced the highest number of leaves (30.4).The increase in number of leaves by AM inoculation was supported by the finding of earlier studies in sesame (Boureima et al., 2008) and sunflower (Kavitha and Nelson, 2014). This difference in leaves number induced by two inocula could be due to the different ability of AMF species to acquire resources below ground (Jakobsen et al 1992) and to stimulate host plant growth (van der Heijden, 1998a).

AMF influenced significantly the shoot length(Fig.3).The individual inoculation with *G. viscosum* increased shoot length compared to the control. The increased shoot length by AMF was supported by the results of Wang and Xia (2009). The shoot length was the highest in plant inoculated with AMF mixture at the physiological maturity stage. These results are in conformity with that of Kavitha and Nelson, 2014who reported that the shoot length of sunflower was found to be higher in plant inoculated with the mixture (*Glomus mosseae, Glomus fasciculatum*, *Acaulospora scrobiculata* Trappe).The results of Ramakrishnan and Selvakumar (2012)showed higher shoot length in tomato plant inoculated with AMF mixture (*G. fasciculatum* (Thaxt.) Gerd. & Trappe and *G. intraradices*) compared to single inoculation and control. Enhanced sunflower growth by AMF can be attributed to stimulation of the production of growth regulating substances which promote the cell division and cell expansion. It is known that AM fungi produced auxin, gibberellin and cytokinin-like substances and stimulated plant growth (Barea and Azcon-Aguilar, 1982; Bass and Kuiper, 1989). However, mixed AMF species which colonized roots are complementary in their

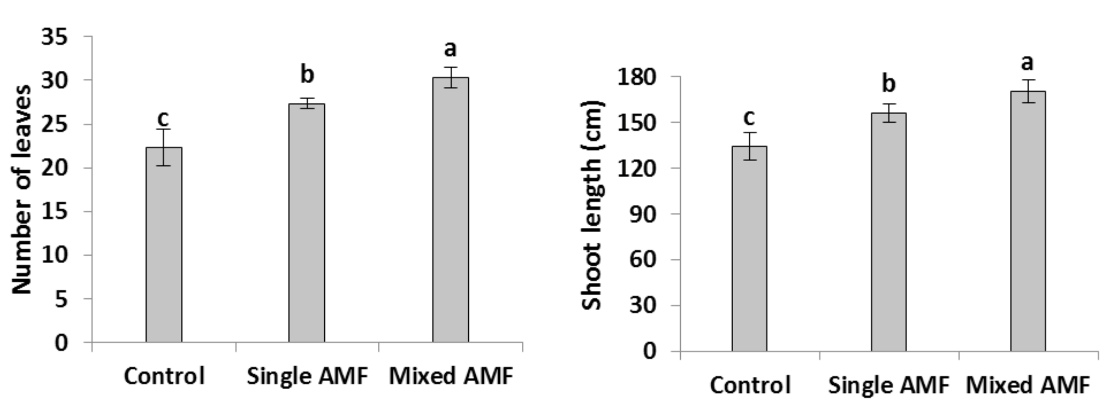

Figure 3:Effect of inoculation with single (*G. viscosum*) and mixed AMF on number of leaves and shoot length of sunflower. Vertical bars represent ± SD (n = 4). Different letters on square bars indicate significant difference ($p < 0.05$).

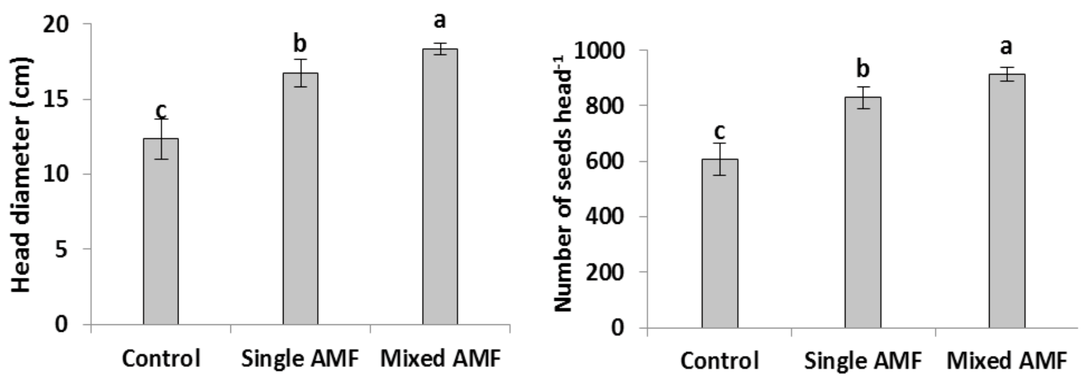

Figure 4: Effect of inoculation with single (*G. viscosum*) and mixed AMF on head diameter and number of seed in sunflower. Vertical bars represent ± SD (n = 4). Different letters on square bars indicate significant difference ($p < 0.05$).

uptake of nutrients from different soil pools, thus, they may be more beneficial for the plant growth as a mixture than any of the species separately (Koide, 2000; Gustafson and Casper, 2006).

In the present study, the head diameter, and number of seeds per head were significantly affected by AM inoculation (Fig.4).The highest head diameter and number of seeds were observed in the plants inoculated with AMF mixture. Also, the hundred seeds mass was significantly increased by AM inoculation (Fig.5). The highest seeds mass was found in the plant inoculated with AMF mixture followed by individual inoculation with *G. viscosum* followed by the control. Results of earlier studies showed higher head diameter, number of seeds per head, and seed yield in AM sunflower plants than in non-AM plants (Soleimanzadeh, 2010; 2012). The inoculation of AMF mixed species increased boll mass and seed yield of cotton (Ibrahim, 2016). The beneficial effect of AMF mixture found in this study, confirms the report of Kavitha and Nelson (2014) which showed that sunflower plants inoculated with AMF mixture had maximum head diameter, number of seeds and seeds mass. It is known that different species of AMF vary in the form of benefits they confer on the growth and development of plants (Howeler et al., 1987).

In this study, above ground dry mass was increased by inoculation of single and mixed species in comparison with non-inoculated control (Fig.5). The positive effect of AMF on growth and biomass yield have been shown by earlier studies with wheat (Rabie, 2005), cotton (Ibrahim and Abu-Rashed, 2017), and sunflower (Kavitha and Nelson, 2014). The application of AMF in sunflower increased growth and yield parameters of plant (Jalaluddin and Hamid, 2011). Soleimanzadeh (2010) had reported that AMF plays an important role in sunflower generative growth. The increase in shoot biomass by AMF mixture could be attributed to the enhancement of nutrients uptake (Motosugi et al., 2002;Jia et al., 2004). Different species of AMF can produce different amounts of extraradical mycelium and can have different efficiencies in phosphate uptake from the soil (Oliveira et al., 2006) and as a result, may contribute to differential growth re-

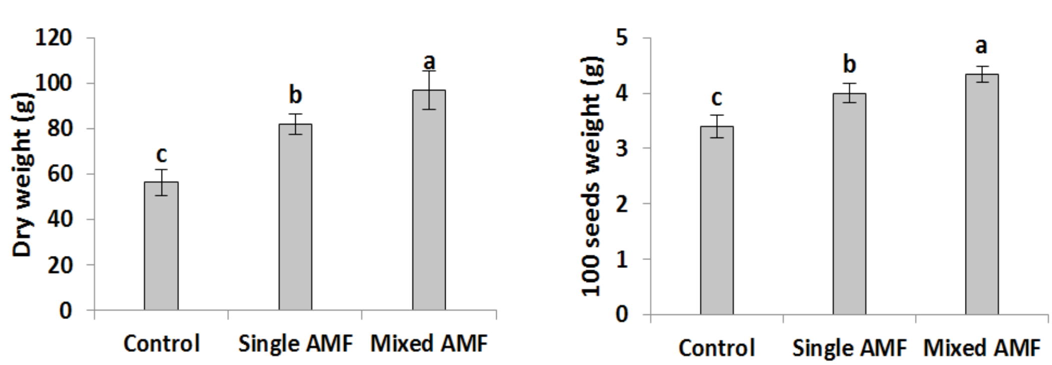

Figure 5: Effect of inoculation with single (*G. viscosum*) and mixed AMF on above ground dry weight and 100 seeds weight in sunflower. Vertical bars represent ± SD (n = 4). Different letters on square bars indicate significant difference ($p < 0.05$).

sponses of plants. Increased biomass produced by AMF was reflected in high values of mycorrhizal dependency (MD) and mycorrhizal inoculation effect (MIE) (Fig. 2). The degree to which sunflower plant relied on AMF for growth was the highest when it was inoculated with AMF mixture (Fig. 2). This result is in accordance with the findings of Ramakrishnan and Selvakumar (2012), who showed that MD of tomato was the highest in the AMF mixture treatment compared to single inoculation. Fernandez et al. (2009) showed that sunflower had 42.21 % MD at low P. The MIE was also high in plants inoculated with AMF mixture with the highest percentage of 42.5 % and followed by *G. viscosum* with 32.9 %.This result indicates that an isolate of several AMF species may cause differences in the growth responses of sunflower plant. Van der Heijden et al. (1998b) reported that plant species responded differently to the mixture AMF inoculation, and that these differential responses were stronger than to those of the single-AMF species.

4 CONCLUSION

The undertaken study shows the importance of indigenous arbuscular mycorrhizal fungi as a biofertilizer for improving growth of non-oil or confection-type sunflower crop grown under semiarid conditions. The combination of indigenous AMF (*G. viscosum, G. intraradices* and *G. mosseae*) gave better result for agronomic characteristics of sunflower compared to the single species (*G. viscosum*). Therefore, AMF mixture can be considered as a good inoculum for improving growth and yield of sunflower in sustainable agriculture.

5 ACKNOWLEDGEMENTS

The author would like to thank the Atomic Energy Commission of Syria for encouragement and technical support.

6 REFERENCES

Barea, J.M., & Aguilar, C. (1982). Production of plant growth regulating substances by the VA mycorrhizal fungus *Glomus mosseae. Applied and Environmental Microbiology, 43,* 810-813

Bass, R., & Kuiper, D. (1989). Effects of vesicular arbuscular mycorrhizal infection and phosphate on *Plantago major* spp. *pleiosperma* in relation to internal cytokine concentration. *Physiologia Plantarum, 76,* 211-215. https://doi.org/10.1111/j.1399-3054.1989.tb05634.x

Berta, G., Trotta, A., Fusconi, A., Hooker, J. E., Munro, M., Atkinson, D., Giovannetti, M., Morini, S., Fortuna, P., Tisserant, B., Gianinazzi-Pearson, V., Gianinazzi, S. (1995). Arbuscular mycorrhizal induced changes to plant growth and root system morphology in *Prunus cerasifera. Tree Physiology, 15*(5), 281-293. https://doi.org/10.1093/treephys/15.5.281

Boureima, S., Diouf, M., Diop, T. A., Diatta, M., Leye, E. M., Ndiaye, F., Seck, D. (2008). Effects of arbuscular mycorrhizal inoculation on the growth and the development of sesame (*Sesamum indicum* L.). *African Journal of Agricultural Research*, *3,* 234-238.

Brundrett, M., Bougher, H., Dell, B., Grove, T., Malajczuk, N. (1996). Working with mycorrhizas in forestry and agriculture. Monograph 32, Australian Centre for International Agricultural Research, Canberra Australia.

Camprubi, A., & Calvet, (1996). Isolation and screening of mycorrhizal fungi from citrus nurseries and orchards and inoculation studies. *Hortscience*, *31,* 366-369. https://doi.org/10.21273/HORTSCI.31.3.366

Daft, M. J. (1983).The influence of mixed inocula on endomycorrhizal development. *Plant Soil*, *71,* 331–337. https://doi.org/10.1007/BF02182672

Fernandez, M., Gutierrez, B. F. H., Rubio, G. (2009). Arbuscular mycorrhizal colonization and mycorrhizal dependency: a comparison among soybean, sunflower and maize. *The Proceedings of the International Plant Nutrition Colloquium XVIU C Davis*. http://escholarship.org/uc/item/0vd8n24g

Giovannetti, M., & Mosse, B. (1980). An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytologist*, *84,* 489-499. https://doi.org/10.1111/j.1469-8137.1980.tb04556.x

Gustafson, D. J. & Casper, B. B. (2006). Differential host plant performance as a function of soil arbuscular mycorrhizal fungal communities: experimentally manipulating cooccurring *Glomus* species. *Plant Ecology, 183,* 257-263. https://doi.org/10.1007/s11258-005-9037-8

van der Heijden, M.G.A., Boller, T., Wiemken, A., Sanders, A. R. (1998b). Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology*, *79*(6), 2082–2091. https://doi.org/10.1890/0012-9658(1998)079[2082:DAMFSA]2.0.CO;2

van der Heijden, M.G.A., Klironomos, J. N., Uršič, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., Sanders, I. R. (1998a). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature, 396,* 69–7210. https://doi.org/10.1038/23932

van der Heijden, M.G.A., Rinaudo, V., Verbruggen, E., Scherrer, C., Bàrberi, P., Giovannetti, M. (2008). *The significance of mycorrhizal fungi for crop productivity and ecosystem sustainability in organic farming systems.* 16th IFOAM Organic World Congress, Modena, Italy, June 16-20.

Hewitte, J. (1966). *Sand and water culture methodsused in the study of plant nutrition*. Tech. Comm. 22 (2nd revised). Commonwealth Agricultural Bureau, London, England. pp. 430–434.

Howeler, R.H., Sieverding, E., Saif, S. (1987). Practical aspects of mycorrhizal technology in some tropical crops and pastures. *Plant and Soil*, *100,* 249–283. https://doi.org/10.1007/BF02370945

Ibrahim, M. (2010). *Influence of arbuscular mycorrhizal fungi (AMF) on the nutrition of cotton (Gossypium hirsutum L.) and its tolerance to water stress.* PhD, Gembloux Agro-BioTech., Belgium.

Ibrahim, M. (2016). Arbuscular mycorrhizal isolate and phosphogypsum effects on growth and nutrients acquisition of cotton (*Gossypium hirsutum* L.). *Advancesin Horticultural Science*, *30*(3), 121-128. https://doi.org/10.13128/ahs-20247

Ibrahim, M. & Abu-Rashed, R. (2017). Effect of Syrian indigenous arbuscular mycorrhizal Fungi in combination with manure on the growth of cotton (*Gossypium hirsutum* L.). *Communications in Soil Science and Plant Analysis, 48,* (17), 2093-2101. https://doi.org/10.1080/00103624.2017.1406499

Jakobsen, I., Abbott, L. K., Robson, A. D. (1992). External hyphae of vesicular–arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. I. Spread of hyphae and phosphorus inflow into roots. *New Phytologist*, *120,* 371–380. https://doi.org/10.1111/j.1469-8137.1992.tb01077.x

Jalaluddin, M. & Hamid, M. (2011). Effect of adding inorganic organic and microbial fertilizers on seed germination and seedling growth of sunflower. *Pakistan Journal of Botany*, *43,* 2807-2809.

Jia, Y., Gray, V. M., Straker, C. J. (2004). The Influence of *Rhizobium* and arbuscular mycorrhizal fungi on nitrogen and phosphorus accumulation by *Vicia faba. Annals of Botany*, *94,* 251-258. https://doi.org/10.1093/aob/mch135

Kavitha, T. & Nelson, R. (2014). Effect of arbuscular mycorrhizal fungi (AMF) on growth and yield of sunflower (*Helianthus annuus* L.). *Journal of Experimental Biology and Agricultural Sciences, 2,* 226-232.

Koide, R. T. (2000). Functional complementarity in the arbuscular mycorrhizal symbiosis. New Phytologist, 147, 233-235. https://doi.org/10.1046/j.1469-8137.2000.00710.x

Motosugi, H., Yamamoto, Y., Naruo, T., Kitabayashi, H., Ishii, T. (2002). Comparison of the growth and leaf mineral concentrations between three grapevine rootstocks and their corresponding tetraploids inoculated with an arbuscular mycorrhizal fungus *Gigaspora margarita. Vitis, 41,* 21–25.

Oliveira, R.S., Castro, P. M. L., Dodd, J. C.,Vosatka, M. (2006). Different native arbuscular mycorrhizal fungi influence the coexistence of two plant species in a highly alkaline anthropogenic sediment. *Plant and Soil,287,*209–221. https://doi.org/10.1007/s11104-006-9067-6

Plenchette, C., Fortin, J. A., 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 and Soil,70,* 199–209. https://doi.org/10.1007/BF02374780

Rabie, G.H. (2005). Contribution of arbuscular mycorrhizal fungus to red kidney and wheat plants tolerance grown in heavy metal polluted soil. *African Journal of Biotechnology, 4,* 332-345. https://doi.org/10.5897/AJB2005.000-3063

Ramakrishnan, K. & Selvakumar, G. (2012). Influence of AM fungi on plant growth and nutrient content of tomato (*Lycopersicum esculentum* Mill.). *International Journal of Research in Botany, 2,* 24-26.

SAS institute Inc. (2004). *SAS user's guide: statistics version 9.1.2.* SAS Institute Inc, Cary, NC.

Schneiter A., Miller J. F. (1981). Description of Sunflower Growth Stages. Crop Science, 11, 635-638.

Sieverding, E. (1991). *Vesicular-arbuscular mycorrhiza management in tropical agrosystems.* Technical cooperation, Germany: Eschborn. pp. 371.

Smith, E.S., Facelli, E., Pope, S., Smith, F.A. (2010). Plant performance in stressful environments: Interpreting new and established knowledge of the roles of arbuscular mycorrhizas. *Plant Soil, 326,* 3-20. https://doi.org/10.1007/s11104-009-9981-5

Soleimanzadeh, H. (2010). Effect of VA Mycorrhiza on growth and yield of sunflower (*Helianthus annuus* L.) at different phosphorus levels. *World Academy of Science, Engineering and Technology, 71,* 414-417.

Soleimanzadeh, H. (2012). Response of sunflower (*Helianthus annuus* L.) to inoculation with mycorrhiza under different phosphorus levels. *American-Eurasian Journal of Agricultural & Environmental Sciences, 12*(3), 337-341.

Tripathi, A., Srivastava, R., Chaturvedi, C., Srivastava, B. K., Singh, H. B. (2005). Response of bitter gourd to mycorrhizal diversity for growth, yield and nutrient uptake. *Indian Journal of Horticulture, 62*(3), 306-307.

Wang, M. & Xia, R. (2009). Effects of arbuscular mycorrhizal fungi on growth and iron uptake of *Poncirus trifoliata* under different pH. *Weishengwu Xuebao, 49,* 1374-1379.

Weiss, E.A. (2000). *Oil seed crops.* Blackwell Sci. Led. London, pp: 364.

Zhang, Q., Tang, J., Chen, X. (2011). Plant mortality varies with arbuscular mycorrhizal fungal species identities in a self-thinning population. *Biology Letters, 23,* 472–474. https://doi.org/10.1098/rsbl.2010.1040