Current Trends in Biotechnology and Pharmacy
Vol. 18(3) 1841-1849, July 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3.31

Host specific Arbuscular Mycorrhizal Fungi (AMF): A Boost to Growth and Phosphorus Regulation in Cotton (*Gossypium herbaceum*)

Tasleem Sultana and Pavan Kumar Pindi*

Department of Microbiology, Palamuru University, Mahabubnagar-509001, Telangana, India.
* Corresponding Author: pavankumarpindi@gmail.com

Abstract

Cotton holds paramount importance as a natural resource with considerable economic, social, and environmental implications. This study focused on isolating host-specific mycorrhizal spores for the Mahyco cultivar of *Gossypium herbaceum*. A homogenous mixture of soils (HMS) was utilized, incorporating eight widely used cultivars in Mahabubnagar District, and maintained for 60 days. After the designated period, the well-grown Mahyco plant was carefully extracted and transferred to a pot containing sterilized soil, ensuring sterile conditions for 8 weeks. Mycorrhizal colonization was observed in roots, and the soil was sieved to isolate host-specific spores. Abundant spores of *Glomus mosseae* were identified based on the manual by Schenk and Perez (1987). These spores were propagated through a funnel experiment and then transferred to pots for mass cultivation. The efficiency of *Glomus mosseae* was tested with four different soils in Mahabubnagar District. Deep black soil exhibited optimal growth in terms of plant development and phosphorus uptake, followed by shallow black soil. This method demonstrates high host specificity for geographically grown cotton and can be adapted for the sustainable cultivation of cotton.

Keywords: AMF, *Glomus mosseae, Gossypium herbaceum,* HMS.

Introduction

Cotton, a crucial natural fibre and economically significant crop, bestows considerable advantages to humans and stands as a pivotal raw material globally. Despite numerous recent studies affirming the capacity of arbuscular mycorrhizal fungi (AMF) to enhance plant growth (27), yield, quality, and phosphorus acquisition, (5, 11 19) their impact on the economic and agronomic traits of cotton (24) remains largely unexplored. Prior research findings indicate that mycorrhiza-mediated inoculants can potentially reduce the necessity for phosphorus fertilization by at least 25%, and in some cases, up to 50%, without compromising crop yield (7).

Mycorrhizal associations exhibit diverse structures and functions, with the arbuscular mycorrhizal (AM) association being the most prevalent. This association forms between the roots of higher plants and Zygomycete fungi of the order *Glomales* (30). Numerous recent studies affirm that arbuscular mycorrhizal fungi (AMF) enhance plant growth (19), yield, quality, and phosphorus acquisition (24). Fungi play pivotal roles in microbiological and ecological processes, influencing soil fertility (17), decomposition, mineral and organic matter cycling (14), as well as plant health and nutrition (29). A significant effect on host plants is the mobilization of nutrients like N and P from structural and other polymers otherwise inaccessible to plant roots.

Mycorrhizal fungi develop on plants through three main methods: forming spores (4), colonized root fragments, or vegetative hyphae. The latter two are known as propagules, structures containing mycorrhizal fungi capable of initiating new associations. Propagules in mycorrhizae encompass spores, hyphal fragments, or other structures produced by the fungi. Two types of fungus colonize plants: endomycorrhiza or ectomycorrhiza. Endomycorrhiza form 90% of relationships with all plant species (35), while ectomycorrhizal fungi colonize from outside the root cells, forming relationships with approximately 10% of all plant species.

Arbuscular mycorrhizal fungi (AMF) establish symbiotic relationships with plant roots and can enhance the adaptability of host plants (3), particularly by providing additional phosphorus (P), (1), nitrogen (N), and zinc (31). The symbiosis with AMF leads to the extension of root systems, increasing the root surface area utilized for nutrient uptake by more than 100-fold. A diverse range of essential agricultural crops, including wheat, rice, corn, potato, tomato, onion, pulses, cotton, and soybean, can engage in symbiotic associations with AMF, highlighting their significance in global agriculture.

Numerous studies have reported isolating mycorrhizal fungi from plant roots, demonstrating their specificity to particular plants due to their presence in the rhizosphere vicinity. However, confirming that plant growth is solely due to the presence of specific fungi remains challenging. The present study primarily focuses on isolating host-specific mycorrhizae using the HMS (Homogenized Mixture of Soil) method, where the plant (*Gossypium herbaceum*) actively takes up spores beneficial for its growth. This approach empowers the plant with the ability to select the most suitable fungi for optimal development.

Materials and Methods

A uniform blend of soils: Rhizosphere soils associated with cotton plants were gathered from six forest locations, namely Amrabad-Nagarkurnool (Ngkl), Bhadrachalam-Khammam (Khm), Eturunagaram-Warangal (Wrngl), Jannaram-Adilabad (Adb), Kataram-Karimnagar (Krmn), and Narsapur-Medak (Mdk). The soil samples were carefully enclosed in sterile zip-lock bags and transported to the laboratory.

Preparation of soil samples for homogenous mixture of soils (hms)

Elimination of undesired materials such as roots, gravel, stones, and pebbles was conducted upon receiving the soil samples collected from different locations in the laboratory. Subsequently, the bulk soil was reduced to the required quantity using the quartering technique (28), serving as a source for the isolation of efficient mycorrhizae specifically for the Mahyco cultivar of *Gossypium herbaceum*. Before sowing, the seeds underwent washing 2 to 3 times to eliminate adhering impurities and miscellaneous bacteria from the seed surface.

Preparation of Sterile Soil

A sterile soil mixture was created by combining sand and red soil in a 1:1 ratio, followed by autoclaving at 121°C under 15 lbs pressure.

Determination of percentage of mycorrhizal root infection

Roots were cautiously removed and rinsed thoroughly with water to eliminate any adhering soil particles. Subsequently, they were fixed in FAA (Formalin acetic acid alcohol solution). The roots were submerged in a KOH solution and autoclaved for 15-20 minutes, followed by cleaning with distilled water. Acidification was carried out in 5N HCl, and the staining process was performed using lactophenol trypan blue. The clarified roots underwent staining according to the technique outlined in (23). The percentage of infection was determined using the formula provided by (13).

Funnel experiment

Sowing of Mahyco cotton cultivar was conducted using *Glomus mosseae* spores in funnels filled with sterilized soil supplemented with Hoagland's Solution (16).

Spore isolation and identification

A total of 100 grams of soil underwent the process of submersion in 1 litre of water within a beaker. Thorough mixing was accomplished using a glass rod, and the mixture was allowed to settle for a duration of 4 to 5 hours. The suspension was meticulously poured through a series of sieves with mesh sizes of 400, 100, 70, and 50 µm. The residues retained on these sieves were then rinsed onto Whatman filter paper. Subsequently, spores were carefully selected using a needle under a stereo binocular research microscope and arranged on a glass slide, with or without the application of Melzer's reagent for enhanced identification. The identification process relied on a thorough analysis of morphological and subcellular characteristics, with spores being cross-referenced against the original description provided by (26).

Sampling diversity

Soil samples were gathered from various cotton fields in Mahabubnagar District, including shallow black soil from Malleboinpally, red soil from Makthal, deep black soil from Kalwakurthy, and sandy soil from Narayanpet.

Physico-chemical profile of soils in cotton farming environments

Soil's available nitrogen content was assessed using the alkaline potassium permanganate method, as described by (32). The determination of available phosphorous was conducted following the method outlined by Bray and Kurtz in 1945, while potassium levels were determined through flame photometry, following the protocol established by (18).

Results and Discussion

The growth of cotton plants is significantly influenced by Arbuscular Mycorrhizal Fungi (AMF), as observed in the study by (33). Plants that undergo AMF inoculation demonstrate increased adaptability to adverse conditions, as reported by (34). In this study, particular emphasis is placed on the host-specific mycorrhizal spores for the Mahyco cotton cultivar. Eight Cotton cultivars were planted in an HMS mixture (Table-1), and optimal conditions were maintained to facilitate seed germination, allowing the plants to grow for a period of 60 days.

Table-1: Growth parameters of Eight cotton cultivars grown in HMS (aged 50 days)

Cultivar	Mycorrhizal Colonization (%)	Height of the plant (cm)		Plant fresh weight (g)		Plant dry weight (g)	
		Shoot	Root	Shoot	Root	Shoot	Root
Mahyco	82	35.03 ± 0.76	14.5 ± 0.4	10.43 ± 0.35	6.33 ± 0.32	1.91 ± 0.02	1.16 ± 0.02
Rashi	75	29.4 ± 0.29	12.56 ± 0.28	9.76 ± 0.3	4.9 ± 0.26	1.44 ± 0.03	0.86 ± 0.2
kaveri	71	25.63 ± 0.2	11.93 ± 0.25	9.66 ± 0.15	4.53 ± 0.25	1.31 ± 0.02	1 ± 0.04
Marvel	70	24.7 ± 0.19	12.06 ± 0.11	9.13 ± 0.25	4.3 ± 0.2	1.29 ± 0.01	0.93 ± 0
Obama	70	24.23 ± 0.15	11.16 ± 0.25	9.23 ± 0.15	3.9 ± 0.2	1.26 ± 0.01	0.89 ± 0.01
Nusun	68	21.73 ± 0.11	10.6 ± 0.2	8.73 ± 0.2	3.6 ± 0.2	1.21 ± 0.02	0.82 ± 0.02
Raj seeds	62	20.7 ± 0.3	10.23 ± 0.15	8.63 ± 0.11	3.36 ± 0.15	1.12 ± 0	0.79 ± 0.01
Sunny	57	20.56 ± 0.15	9.7 ± 0.2	8.36 ± 0.15	3.33 ± 0.11	1.06 ± 0.01	0.78 ± 0.02

After 50 days of cultivation, the Mahyco cultivar exhibited a peak mycorrhizal colonization rate of 82%, showcasing significant growth in both plant height and weight. Remarkably, it demonstrated maximal shoot and root lengths. Subsequent to the 50-day growth period, the Mahyco plant underwent transplantation into

sterile soil. During this transfer, the root zone underwent three thorough washes with sterile water and received a mercuric chloride treatment to eliminate any adhered particles. The seedling was carefully situated in a pot filled with sterile soil, and stringent laboratory conditions were maintained to foster its robust growth over the ensuing five (5) weeks.

Following a 5-week growth period (Table-2), the roots were carefully extracted to assess mycorrhizal colonization, revealing a recorded rate of 62% based on the methodology outlined by (13). Additionally, 8-10 spores were isolated using the Wet Sieving and Decanting method and subsequently mounted on coverslips for identification, referencing the Schenk and Perez manual from 1987, leading to their classification as *Glomus mosseae*. These spores were then propagated through a funnel experiment, a technique described by (33), spanning a period of 7-14 days.

Throughout this germination phase, the seeds had direct contact with the mycorrhizal spores, minimizing the likelihood of spore unavailability under controlled conditions. Following germination, the setup was transferred to a sterile pot environment for the extensive cultivation of spores.

Table-2: Growth parameters of Mahyco cultivar in sterile soil aged 5 weeks.

Cultivar	Mycorrhizal Colonization (%)	Height of the plant (cm)		Plant fresh weight (g)		Plant dry weight (g)		Isolated Spore
		Shoot	Root	Shoot	Root	Shoot	Root	
Mahyco	85%	44.9±0.19	15.4±0.2	12.46±0.25	7.53±0.23	2.05±0.04	1.26±0.02	Glomus mosseae

Efficacy of Glomus mosseae in four diverse soil samples

Based on the findings outlined in Table-4, it is apparent that the presence of *Glomus mosseae* arbuscular mycorrhizal fungi (AMF) spores significantly contribute to promoting plant growth. In an effort to address poor plant growth conditions, as elaborated in the 'Materials and Methods' section, four distinct soil samples were subjected to NPK and trace element analyses (refer to Table-3). These soils underwent treatment with *Glomus mosseae* AMF spores, with an uninoculated sample serving as a control. The Mahyco cultivar was cultivated in these diverse soils over a 50-day period to assess the efficacy of AMF under natural conditions.

Table-3: Physico chemical characteristics of four diverse cotton soils.

Location	Soil type	pH	N	P	K	S	Fe	Mn	Zn	Cu
Malleboinpally	Shallow black soil	8.0	220.14	91.72	121.84	8.6	2.32	23.24	0.31	0.23
Chitteboinpally	Red soil	7.0	189.68	85.15	102.69	9.8	4.57	25.31	1.02	0.22
Kalwakurthy	Deep black	8.0	235.23	102.58	138.12	8.0	2.12	31.21	0.32	0.52
Divtipally	Sandy soil	8.0	175.87	76.92	91.75	9.8	0.12	12.25	0.18	0.04

Across all soil types, Mahyco exhibited favourable growth parameters and phosphorus content. The most substantial growth and phosphorous content were observed in deep black soil, followed by shallow black soil. Conversely, sandy soil displayed comparatively less growth, and red soil exhibited the least growth and phosphorous content, as detailed in Table-4.

Table-4: Growth parameters of Mahyco inoculated with *Glomus mosseae* (G.m) in four different soils of Mahbubnagar district. (aged 50 days).

Soil Type	Location	Combination	Height of the plant (cm)		Plant fresh weight (g)		Plant dry weight (g)		P content (%)	Mycorrhizal Colonization (%)
			Shoot	Root	Shoot	Root	Shoot	Root		
Sandy Soil	Narayanpet	Control	18.53±0.15	10.76±0.15	2.06±0.15	1.2±0.19	0.55±0.01	0.36±0.02	0.13±0.01	-
		M+G.m	41.7±0.19	20.53±0.15	7.73±0.11	3.5±0.2	1.96±0.02	1.16±0.01	0.17±0.02	57
Deep black Soil	Kalwakurthy	Control	20.63±0.2	12.23±0.15	3.56±0.15	1.7±0.1	1.46±0.02	0.76±0.02	0.2±0.01	-
		M+G.m	50.26±0.11	24.33±0.2	9.23±0.15	4.66±0.15	3.49±0.02	2.13±0.01	0.33±0.02	68
Shallow black Soil	Jadcherla	Control	19.9±0.2	11.13±0.11	2.93±0.15	1.03±0.15	0.96±0.01	0.55±0.01	0.17±0.02	-
		M+G.m	48.53±0.15	23.53±0.15	8.5±0.19	4.16±0.15	3.16±0.01	1.8±0.01	0.27±0.01	65
Red Soil	Makthal	Control	19.33±0.11	11.13±0.15	2.63±0.11	1.33±0.15	0.65±0.01	0.43±0.02	0.16±0.01	-
		M+G.m	44.5±0.2	22.6±0.2	8.03±0.15	3.86±0.15	2.86±0.01	1.55±0.01	0.23±0.02	61

Cotton, being a mycotrophic plant, typically experiences enhanced growth and nutrient uptake through arbuscular mycorrhizal (AM) colonization. The symbiotic relationship between terrestrial plants and AM fungi (AMF) is well-established, and this association commonly results in improved nutritional status and overall fitness for the host plant (8, 27). Nonetheless, the role of slow AM colonization in cotton growth disorders remains uncertain, and it is unclear whether it is a symptom or the underlying cause of reduced plant growth (23).

To address nutrient deficiencies in the soil, mycorrhizal fungi play a crucial role in facilitating the uptake of mineral nutrients, thereby promoting plant growth (22). The symbiotic association of arbuscular mycorrhizal (AM) fungi is particularly impactful in supplying less mobile nutrients like phosphorus to the host plant through the roots, thereby mitigating the adverse effects of salt stress (35).

As highlighted by (23), arbuscular mycorrhizal (AM) fungi are recognized as bio ameliorators of salinity stress due to their adaptability to contaminated soils and their involvement in modulating biochemical processes. The pivotal role of AM fungi in supplying phosphorus to the plant is of significant importance, with the ability to solubilize phosphorus from the surrounding areas and make it available to the roots (28). In the case of the Mahyco variety, inoculation with AMF demonstrated the most substantial impact on both plant growth and nutrient uptake, accompanied by a noticeable increase in mycorrhizal root colonization.

Some authors assert that the most significant growth stimulation in plants by beneficial bacteria and fungi occurs when the plants face stressful conditions. In contrast, non-treated plants under similar conditions tend to exhibit poor performance (12, 23)

Research findings indicate that the initial colonization of arbuscular mycorrhizal (AM) fungi in cotton roots can commence within 2-4 weeks after planting, as noted by (2). During

seed germination in HMS, a symbiotic relationship forms with specific fungi in the rhizosphere, significantly promoting the growth of the cultivar and exhibiting high specificity for that particular cultivar. The fungi, as part of their reproductive cycle, produce spores. These spores may be released into the soil when conditions are suitable for dispersal and germination, as reported by (38) and (37). However, the precise timing of spore release from root-associated fungi may not follow a strict schedule or predictable timeline. This variability is influenced by diverse environmental and biological factors, including plant age, growth stage, soil temperature, moisture levels, and nutrient availability, all of which impact both the growth of mycorrhizal fungi and the propensity of cotton roots to establish symbiotic relationships. The fifty-day mark post-sowing is a critical juncture for assessing arbuscular mycorrhizal fungi (AMF) colonization; hence, agricultural traits were examined during this period. Within 36 days, over 80% of cotton roots situated 25 cm below the soil surface had developed mycorrhizal associations at the inoculation point, with subsequent secondary spread occurring in a span of 10–13 days (13, 36).

The HMS Host-Specific Selection method emerges as a promising solution to address challenges posed by climate change and the obstacles associated with developing efficient bioinoculants, including cost, time, and the complex study of genetic factors influencing specificity. This innovative approach ensures the long-term sustainability of agricultural productivity. The method, as demonstrated, successfully identifies and isolates geographically specific arbuscular mycorrhizal fungi (AMF) spores tailored to the host plant, such as the Mahyco cultivar and *Gossypium herbaceum,* emphasizing high plant specificity within forest soil HMS. Given its reliance on the availability of various spores under specific climatic conditions and its adaptability to interactions between the plant and soil, this method holds global applicability for developing efficient bio-inoculants tailored to specific plants. The overarching aim is to contribute to environmentally friendly and sustainable agricultural practices worldwide.

Unlike traditional random selection for mycorrhizal spore, this method distinguishes itself by referring to and exploring natural combinations. This technique could be the most effective for isolating novel, efficient mycorrhizal spore. This method demonstrates that the isolated spores are specific to the Cotton cultivar Mahyco.

The efficiency of *Glomus mosseae* was assessed across four distinct cotton soil samples, revealing varying degrees of colonization, plant growth, and phosphorous content. Among these, deep black soil exhibited the highest levels, followed by shallow black soil, showcasing maximum colonization, plant growth, and phosphorous content. In contrast, sandy soil demonstrated the least colonization, plant growth, and phosphorous content, with red soil exhibiting values intermediate between sandy and black soils.

Conclusion

Successfully identifying and isolating geographically specific organisms for *Gossypium herbaceum*, particularly the highly plant-specific mycorrhizal spores from forest soil HMS, demonstrates the efficacy of our method. The approach relies on the availability of various mycorrhizal spores to the cotton plant under specific climatic conditions. The selection process is intricately determined by the interaction between the plant and mycorrhizae. As a result, this method holds universal applicability for developing efficient mycorrhizal spores tailored to the specific needs of cotton plants worldwide. The versatility of this method proves advantageous for fostering environmentally friendly and sustainable agricultural practices.

References

1. Abbott, L. K. and Robson, A. D. (1991). Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. Agric Eco-

syst Environ, 35: 121–150.

2. Abdul Qayyum (2013). Impact of Mycorrhizal Fungi on Cotton Yield and Nutrient Uptake in Calcareous Soil. Pakistan Journal of Botany.

3. Allen, M. F. (1996). The ecology of arbuscular mycorrhizas: a lookback into the 20th century and a peek into the 21st. Mycol Res, 100: 769–782.

4. Babu, K. S. and Manoharachary, C. (2003). Occurrence of Arbuscular Mycorrhizal fungi in Rhizosphere soils of some medicinal plants. Indian Phytopathology, 56: 223-227.

5. Bona, E. et al. (2017). Arbuscular mycorrhizal fungi and plant growth-promoting pseudomonads improve yield, quality and nutritional value of tomato: a field study. Mycorrhiza, 27: 1–11.

6. Bray, R. H. and Kurtz, L. T. (1945). Determination of total, organic, and available forms of phosphorus in soils. Soil Science, 59: 39-45.

7. Ceballos, I. et al. (2013). The in vitro mass-produced model mycorrhizal fungus, rhizophagus irregularis, significantly increases yields of the globally important food security crop cassava. PLoS One 8, e70633.

8. Daniella Hoffmann., Vierheilig H., Riegler P., and Schausberger, P. (2009). Arbuscular mycorrhizal symbiosis increases host plant acceptance and population growth rates of the two-spotted spider mite Tetranychus urticae, Oecologia, 158: 663–671.

9. David, B. Nehl., Allen, S. J. and Brown, J. F. (1998). Slow arbuscular mycorrhizal colonisation of field-grown cotton caused by environmental conditions in the soil, Mycorrhiza 8:159–167.

10. David, J. Read. (1998). Mycorrhiza - the state of the art. In: Varma A., Hock B. (eds) Mycorrhiza, Springer, Berlin, 3–34.

11. Delavaux, C. S. et al. (2017). Nutrient enrichment effects on mycorrhizal fungi in an Andean tropical montane Forest. Mycorrhiza, 27: 311–319.

12. Dilfuza Egamberdieva. (2013). Response of Maize to Bacterial Inoculants under Different Soil Conditions, Turkey International Plant Nutrition Colloquium, XVII: 583-585.

13. Feng, G., Zhang, FS., Li, X. L., Tian, C. Y., Tang, C. X., Rengel, Z. (2002). Uptake of nitrogen from indigenous soil pool by cotton plant inoculated with arbuscular mycorrhizal fungi. Communications In Soil Science and Plant Analysis, 33: 3825-3836.

14. Giovannetti, M. and Mosse, B. C. (1980). An evaluation of techniques for the measuring VAM infection in roots. New Phytologist, 84: 489-500.

15. Gosling, P. et.al., (2006). Arbuscular mycorrhizal fungi and organic farming. Agriculture, Ecosystem and Environment, 133: 17-35.

16. Gu Feng., Zhang, F.S., Li, X. l., Tian, C.Y., Tang C., and Rengel, Z. (2002). Improved tolerance of maize plants to salt stress by Arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots, Mycorrhiza, 12: 185–190.

17. Hoagland and Arnon. (1938). *The water-culture method for growing plants without soil* (Circular (California Agricultural Experiment Station), 347. ed.), Berkeley, Calif.: University of California, College of Agriculture, Agricultural Experiment Station. OCLC 12406778.

18. Irene, M., Cardoso., Thomas., and Kuyper, W. (2006). Mycorrhizas and tropical soil fertility. Agriculture, Ecosystem and Environment. 116: 72-84.

19. Jackson, M. L. (1973). Soil chemical analysis, prentice hall of India Pvt. Ltd., New Delhi, India, 498: 151-154.

20. Majewska, M. L., Rola, K. & Zubek, S. (2016). The growth and phosphorus acquisition of invasive plants Rudbeckia laciniata and Solidago gigantea are enhanced by arbuscular mycorrhizal fungi. Mycorrhiza, 27: 83–94.

21. McGee, P. A., Torrisi, V. & Pattinson, G. S. (1999). The relationship between density of *Glomus mosseae* propagules and the initiation and spread of arbuscular mycorrhizas in cotton roots. Mycorrhiza, 9: 221–225.

22. Mozafar Sharifi., Ghorbanli, M., and Ebrahimzadeh, H. (2007). Improved growth of salinity-stressed soybean after inoculation with salt pre-treated mycorrhizal fungi. Plant Physiol, 164:1144–1151.

23. Neera Garg, Baher, N. (2013). Role of arbuscular mycorrhizal symbiosis in proline biosynthesis and metabolism of Cicer arietinum L. (Chickpea) Genotypes Under Salt Stress. Plant Growth Regul, 32: 767–778.

24. Nehl, D. B., Allen, S.J. and Brown, J.F. (1998). Slow arbuscular mycorrhizal colonisation of field grown cotton caused by environmental conditions in the soil. Mycorrhiza, 8(3):159-167.

25. Phillips, J.M., (1970). Hayman's improved procedure for clearing and staining parasitic and vesicular-Arbuscular mycorrhizal fungi for assessment of infection. Transactions of the British Mycological Society, 55: 158.

26. Rahimzadeh, S. & Pirzad. (2017). Arbuscular mycorrhizal fungi and Pseudomonas in reduce drought stress damage in flax (Linum usitatissimum L.)-a field study. Mycorrhiza, 27:s 537–552.

27. Read, D. J., Ducket, T. J. G., Francis, R., Ligrone, R. and Russell, A. (2000). Symbiotic fungal associations in lower land plants. Royal Society, Phil Trans R Soc, 815-831.

28. Rishi, K., Behl., Sharma, H., Kumar, V., and Narula, N. (2003). Interactions amongst mycorrhiza, Azotobacter chroococcum, and root characteristics of wheat varieties. Agronomy and Crop Science, 189:151–155.

29. Schenck, N. C., and Perez, Y. (1987). Manual for the identification of Vesicular Arbuscular Mycorrhizal INVAM. Florida University, Gainseville, USA. Pp. 245.

30. Schübler, A., Krüger, C. & Urgiles, N. (2016). Phylogenetically diverse AM fungi from Ecuador strongly improve seedling growth of native potential crop trees. Mycorrhiza, 26: 199–207.

31. Schumacher, B. A., Shines, K. C., Burton, J. V., and Papp, M. L. (1990). Comparison of three methods for soil homogenization. Soil Science Society of America Journal, 54(4): 1187-1190.

32. Smith Sally, E., and Andrew Smith, F. (2011). Roles of Arbuscular Mycorrhizas in Plant Nutrition and Growth: New Paradigms from Cellular to Ecosystem Scales. Annual Review of Plant Biology, 62: 227-250.

33. Smith, S. E. and Read, D. J. (2008). Mycorrhizal symbiosis. Q Rev Biol, 3: 273–281.

34. Smith, S.E., and Read, D.J. (2008). Mycorrhizal symbiosis. 3rd edn. Academic press. Spring Harbor Laboratory Press, New York.

35. Subbiah, B.V., Asija, G.L. (1956) A rapid procedure for the estimation of available nitrogen in soils. Curr Sci, 25: 259-260.

36. Tasleem Sultana., and Pavan Kumar Pin-

di. (2012). Role of Arbuscular Mycorrhizae (AM) Fungi and Multi Bioinoculants in Cotton Plant Growth. British Microbiology Research Journal, 2(3): 123-130.

37. Tian, C.Y., G. Feng., Li, X.L. and Zhang, F.F. (2004). Different effects of arbuscular mycorrhizal fungal isolates from saline or non-saline soil on salinity tolerance of plants. Appl Soil Ecol, 26: 143-148.

38. Vazquez, M., Cesar, S., Azcon, R., Barea, J.M. (2000). Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (Azospirillum, Pseudomonas, Trichoderma) and their effects on microbial populations and enzyme activities in the rhizosphere of Maize plants. Appl Soil Ecol, 15(3): 261-272.

39. Xinpeng, Gao., Huihui Guo., Qiang Zhang., Haixia Guo., Li Zhang., Changyu Zhang., Zhongyuan, Gou., Yan Liu., Junmei Wei., Aiyun chen., Zhaohui chu. and Fanchang Zeng. (2020). Arbuscular mycorrhizal fungi (AMF) enhanced the growth, yield, fiber quality and phosphorus regulation in upland cotton (*Gossypium hirsutum L*.). Scientific Reports, 10: 2084. https://doi.org/10.1038/s41598-020-59180-3.

40. Xiu-Hua Chen., Feng-Ling Wang., Rui Zhang., Ling-Ling Ji., Zheng-un-Yang., Hui Lin. and Bin Zhao. (2016). Evidences of inhibited arbuscular mycorrhizal fungal development and colonization in multiple lines of *Bt* cotton. Agriculture Ecosystems & Environment, 230: 169-176.

41. Zak, J.C., McMichael, B., Dhillion, S., Friese, C. (1998). Arbuscular-mycorrhizal colonization dynamics of cotton (*Gossypium hirsutum L*) growing under several production system on the southern high plains, Texas. Agriculture Ecosystem and Environment, 68(3): 245-254.