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Biodiversity of Arbuscular Mycorrhizal Fungi Associated with Rhizosphere of Horsegram (*Macrotyloma uniflorum* L.)

[1]Shivani, [2]Hemchander,*[3]ManuVineet,[4]Ritika Singh,[5]Komal Sharma,[6]Manish Chauhan and [7]Om Prakash

1 Research Scholar,Career Point University Hamirpur,176041
[2]Associate Professor, Career Point University Hamirpur,176041
[3]Assistant Professor, Career Point University Hamirpur,176041
[4]Assistant Professor,Abhilashi University Chailchowk Mandi,175045
[5]Assistant Professor,Abhilashi University Chailchowk Mandi,175045
[6]Assistant Professor , Abhilashi University Chailchowk Mandi, 175045
[7]Project Fellow, Abhilashi University Chailchowk Mandi, 175045
Email id: shivanikondal4@gmail.com
Mobile Number: 8544700479
Email id: hem.botany@cpuh.edu.in
Email id:ritika.singh139@gmail.com
Email id: komalsharma002@yahoo.com
Email id: manishchauhanvsc@gmail.com
Email id: omprakasht420@gmail.com
*[3]Corresponding author Email.id.: manuvineet.botany@cpuh.edu.in

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Abstract

Horsegram (*Macrotyloma uniflorum*) (Lam.) is an economically important medicinal plant. The plant has been used for its antiurolithiatic property although it has other medicinal uses. Plant can establish mycorrhizal connections and rhizobial nodules because it belongs to the Leguminosae family. The main objective of the present research was to investigate the biodiversity of Arbuscular Mycorrhizal Fungi that are associated with the Horsegram plant grown in midhill region of Mandi district, Himachal Pradesh. Roots and soil samples were collected from Horsegram plants at the seedling, flowering, and maturity stages for AMF analysis and spore evaluation. The objective of this study was to extract, characterise, and classified vesicular arbuscular mycorrhizal fungal spores from root-adhering soil samples of conventional horsegram crop plants. The research was carried out to study the association of arbuscular mycorrhizal fungal spores with the roots and rhizosphere of the Horsegram plant.Total 33 species of VAM fungal spores were isolated belonging to 6 genera (*Acaulospora*, *Glomus*, *Gigaspora*, *Scutellospora*, *Septoglomus* and *Pacisphora*) from the plant. The dominant genus was *Acaulospora* and *Glomus*. Analysis of root samples under a microscope showed varying levels of arbuscular mycorrhizal fungal colonisation, during this investigation, the qualitative characteristics of the horsegram plant were also examined.

Keywords: Arbuscular mycorrhizal fungi(AMF),colonization, diversity, and rhizosphere.

Introduction

Horsegram (*Macrotyloma uniflorum* L.) is an economically beneficial medicinal plant belongs to the family Leguminosae. It is a native to India, an important food, forage and green manure crop in the peninsular region. Around 5.07 lakh hectares of land are used for horsegram cultivation in India, which produces 2.62 lakh tonnes of product with a productivity of 516 kg/ha. It is mainly cultivated in Karnataka, Andhra Pradesh, Bihar, Chhattisgarh, Odisha, Tamil Nadu, Maharastra, West Bengal, Uttarakhand and Himachal Pradesh. [1,2]. It is an underutilised food legume that is grown in Nepal and Himachal Pradesh at elevations of up to 5,000 feet. It is well known for being able to flourish where other crops cannot, such as in adverse climates and poor soil[3,4]. It may be an ayurvedic medication used to treat kidney stones, piles,oedema,and other conditions. It contains iron that aids in delivering oxygen to cells and forms a portion of haemoglobin in blood, polyphenols with strong antioxidant qualities, and molybdenum that controls calcium intake [5]. Although it has additional medicinal advantages, its antiurolithiatic function is its main application. One common condition that affects people worldwide is kidney stone disease. It can have varying degrees of impact on the renal system. An estimated 10% to 12% of people are known to be impacted. The lack of attention to the crop has resulted in poor global attempts to conserve the horsegram germplasm. It can be utilized as a fodder and green manure crop in many tropical zones. In many parts of India, particularly the southern ones. It has long been utilised as a cover crop and is believed to complement cereals and millets such as finger millet and pearl millet [6,7].

One third of global fungal diversity exists in India.The total number of fungal genera reported from world & India is 7,270 and 2,080 respectively. The fungi colonize the root surface, increase the rate of absorption of water, nutrients from the soil and also acquires their nutrition by absorption from their surrounding [8]. A mutualistic relationship between a plant and a fungus that is found in a root or structures resembling a root is known as mycorrhiza. In 1885, Albert Bernhard Frank came up with the word, which literally translates to "fungus root [9]. Mycorrhiza, or the interaction of fungus with roots, has been studied globally in terms of its mutualistic symbiosis, formation patterns, morphological variability, and ability to colonise around 95% of terrestrial plants, including numerous significant agricultural and forest tree species [10]. The majority of crop and land plants have symbiotic relationships with arbuscular mycorrhizal fungus (AMF).This relationship involves the fungus and plant exchanging minerals and nutrients in both directions. Arbuscular mycorrhizal fungi is known to enhance the transfer of essential mineral nutrients and low-mobility minerals, especially phosphorus, to plants in return for carbon [11,12]. Additionally, they may boost the host's resistance to biotic factors like plant diseases and abiotic pressures like toxic metals [13,14]. The ecosystem services provided by AMF can be enhanced by functional diversity[15]. The beneficial effects of native AM fungus on the nourishment of

agricultural plants depend on the type and quantity of fungi present in the soil. The rhizosphere of many vascular plants contains arbuscular mycorrhizal fungus. To realise the potential for widespread use of AM fungus in agriculture, strains of AM that promote crop growth and are superior to the native soil population of AM fungi must be developed The purpose of the current study was to discover and isolatethenativeAMfungus associatedto horsegram plants cultivated in the Himachal Pradesh midhill region of Mandi District because it is necessary to conduct field research to determine the quantity and kind of these fungi in the crop's rhizosphere.

Materials and Methods

Study Area

Mandi is situated in the foothills of the Shivalik peaks, approximately at the geographical heart of Himachal. It lies next to the left bank of the Beas River. Geographically, the district is 3,950 square kilometres in size. The district can be divided into two primary topographic groups, a) Outer Himalaya region b) Inner Himalaya region. Joginder Nagar, Baldwara, and the Balh Valley are all considered to be in the outer Himalayan zone. The Inner Himalaya region includes parts of the Drang blocks as well as the Chachiot, Karsog, and Seraj areas. There are 10 Blocks in Mandi, District (Balh, SunderNagar, Chachyot, Seraj, Chauntra, Sadar Mandi, Dharmpur, Karsog, Drang and Gopalpur). The district Mandi is predominantly an agricultural one, with over 80% of the people either directly or indirectly dependent on agriculture. The main cereal crops are maize, wheat and paddy. A lot of green peas, tomatoes, ginger, capsicums, and potatoes are grown. As a result, the district's main commercial cash crops have displaced the nutritional traditional crops, which are no longer farmed. To investigate the AMF relationship, seed collections were made from various parts of Mandi district, and trials were set up at Chail Chowk during the kharif season.

Sampling technique for collecting roots and soil samples from the rhizosphere of Horsegram (*Macrotyloma uniflorum* L.) plant. A 70% alcohol-sterilized trowel was used to scrape the soil's surface. The soil surrounding the plant was then collected to adepth of10 to 15 cm. Samples of soil were taken at various depths (10, 15, and 20 cm below the surface) after the top dirt was removed. The samples were gathered and brought to thelab for examination in high-quality, hygienic, airtight plastic bags. For identification purposes, these samples were marked using paper slips. The plant samples were collected at seedling, reproductive, and maturity stages.The soil was cleaned,air-dried, crushed, homogenised, and sieved before being stored in airtight bags for further research [16].

Physicochemical properties of soil

For soil analysis, samples of soil were allowed to air dry before being filtered through a 2 mm sieve and used to examine the physiochemical characteristics.The pH level of soil was determined using (1:2.5) of soil and water suspension respectively [17]. Organic carbon, available nitrogen, phosphorus and potassium was determined by using rapid titration method [18]; alkaline

potassium permanganate method [19]; neutral 1N ammonium acetate method [20], respectively. Whereas, Bulk density (g cm^3) was estimated by the specific gravity method [21,22].

For Morphological Descriptor

Five plants were chosen at random for morphological characterisation, and various observations were made for seven qualitative characteristics: growth habit, inflorescence colour,inflorescence shape, senescence, lower raceme shape, days to flowering and grain colour.

Assessment of VAM infection in roots

The root pieces were carefully washed continuosly with tap water, they were cooked in 10% KOH for one to two hours at 90 degrees Celsius, depending upon the thickness of the roots. The segments were acidified by immersing them in 5N HCL for just a few minutes after being cleaned in distilled water. After that, the root segments were stained with lactophenol and 0.05% cotton blue. The root pieces were divided into 1-cm pieces, placed on slides with a 1:1 v/v acetic acid:glycerol mixture, and had their edges sealed with DPX mountant. The roots were then examined for mycorrhizal connection using a stereomicroscope **[23**,24,25].

Assessment of root colonization by Arbuscular mycorrhizal fungi

The technique used for root staining to analyse AMF colonisation was adapted from Kormanik (1980) and originally used by Phillips and Hayman (1970) for roots [26]. After thoroughly washing the freshly harvested plant roots with tap water, they were cut into 1-cm lengths, cleared in 10% (w/v) KOH for an hour at 90°C, acidified with 1% HCl, and stained with 0.05% trypan blue for an entire night. Finally, they were de-stained with lactic acid-glycerin (1:1 by volume) at room temperature. Slides were made and examined for any mycorrhizal fungal structures, such as hyphae, vesicles, or arbuscules,using a compound microscope [27,28].The following formula was used to assess root colonisation:

$$\% \text{ colonisation} = \frac{Total\ number of root/tissue\ pieces\ examined}{Total\ number of colonized\ root/tissues\ pieces} \times 100$$

Methodology forVAM spores isolation and identification

Isolation of VAM spores:VAM spores can be isolated from soil using a variety of techniques.The current investigation employed the "Wet Sieving and Decanting Technique" [29]. Ten grammes of soil were taken and combined in a large beaker until all of the aggregates had dispersed and the suspension was uniform. The sieves (240µm, 120µm, 100µm, 63µm, and 30µm) were placed in descending order. Large organic materials are removed using a 300um sieve. A sieve was used to decant the contents of the beaker. This procedure was carried out three or four times. Using a level pipe for each sieve, the material gathered on each sieve was meticulously collected in a beaker. Each sieve's collected debris was filtered independently using Whatman No. 1 filter paper. Using micro needles, the filter paper was examined under a stereobinocular dissecting microscope. A

microneedle was used to pick up each spore, and they were then mounted in lactophenol to create a semi-permanent mount.

Diagnostic slide:

The isolated spores were put on a glass slide with a mountant called Polyvinyl Alcohol-Lactic Acid Glycerol (PVLG). After that, the spores were examined under a compound microscope (100–1000X) to look for distinctive morphological characteristics including size, form, wall features, etc.

Spore Identification:

Schenck and Perez's (1990) manual of identification of AM fungus was used to identify the spores [30]. To diagnose the spores, the INVAM worksheet was utilised. The INVAM website (http://invam.caf.wvu.edu/) included a description of more spores that were not listed in themanual. The colour, size, and form of the spores, the number of spore walls, their colour, thickness, and ornamentation, the hyphal attachment, the shape and kind of occlusions, and other features are used to identify the genus.

Results and Discussion

Soil physico-chemical analysis

Samples of soil from the rhizosphere and roots were taken from the Chailchowk area of Mandi District, which is one of the minor millet growing locations.Before the experiment started, composite soil samples were taken from a 15 cm depth. The samples' physical and chemical characteristics were displayed in (Table 1). The samples' soils were acidic, had a low organiccarbon content, medium levels of potassium and phosphate, and a small deficiency or low level of accessible nitrogen.

Morhological Descriptor of Horsegram (*Macrotyloma uniflorum* L.)

The stem of Horesegram was found to be erect, branched, annual and perennial herb about 30-50 cm long. It has long runners. Root system was tap root type. The leaves were alternate , trifoliate, acute and with ovate-elliptical leaflets. The flowers were found to be white or pale yellow, bisexual, bracteate, hypogynous, zygomorphic and complete. The seeds were recorded oblong, round or kidney shaped, and its color varies from pale, dark reddish brown and orange-brown. The crop takes 3 to 4½ months to mature.

AMF root colonization

Horsegram growing in the mid-hill region of Mandi district was found to have AM fungal root association. The colonization potential ranged from 40% to 80 % at seedling, flowering and maturity stage (Table 4). Arbuscular mycorrhizal fungal spores were isolated and morphologically identified from seedling, flowering and maturity stage of plant. Among the soil samples, the maximum spore colonization (80%) was observed in maturity stage, flowering stage (48%) and minimum spores in seedling stage (44%).Uneven distribution of AM fungus species is typical in the natural environment. Numerous investigations have documented significant variances in the spore

species richness of AM fungus across various root and rhizosphere samples (10). During the rabi and kharif seasons of 2019 and 2020, Noreen collected various pulse crop plants and rhizospheric soil inten districts of agro ecological significance (Charsadda, Mardan, Swabi, Chitral, Dir, Bunner, Haripur, Mansehra, Karak, and Abbottabad) to study the AMF diversity [31] . The 17 AM fungal species identified by Wendy [32] were found to be from six genera: *Acaulospora* (9), *Rhizoglomus* (1), *Entrophospora* (1), *Claroideoglomus* (2), *Funneliformis* (1), and *Gigaspora* (3). These species were documented from thethreehabitats. In various environments, different genera weredominant. The *Acaulospora* genus was widespread. A total of 39 VAM fungal spore species from six genera (*Acaulospora, Glomus, Gigaspora, Scutellosora, Pacispora*, and *Septoglomus*) were isolated from Horsegram (*Macrotyloma uniflorum*) root adherent soils at the seedling (9 sp.), flowering (16 sp.), and maturity (15 sp.) stages. The *Acaulospora* genus was represented by fifteen species (*A.denticulate,A.nicosolanii, A. macrocarpum,A.appendiculata, A.bireticulata,A. foveata , A.brasiliensis,A.soloidea, A.mellea, A.tuberculata, A.myriocarpa, A. koska, A. gedensis, and* 5 *Acaulospora sps.* The genus *Glomus* was represented by eight species (*Glomus intraradices, G. fasiculatum, G.pallidium,G.versiforme, G.geosporum, G.mossae, G.botryoides,* and 3 *Glomus sp.).* The genus *Gigaspora* was represented by eight species (*Gigaspora decipens, G. margarita , G. gigentia,* and 5 *Gigaspora species*), and the genus *Scutellospora* was represented by one species (*Scutellospora calospora).*One species of genus *Pacispora* and *Septoglomus* from seedling stage, flowering stage and maturity stage of the plant (Table-5) and (Fig.2). *Acaulospora, Glomus* species were found most dominant genus in present investigation.

The study examined the seasonal variations in VAM fungus in the crops grown in the Karnataka district of Dharwad. The most prevalent genera from *Triticum aestivum* were discovered to be *Glomus* and *Acaulospra*, out of 15 species of AM fungal spores that were recovered from six genera: *Acaulospora, Glomus, Claroideoglomus, Dentiscutata, Scutellospora*, and *Gigaspora* [33,34]. In rice, thecolonisation of fungal associations with dark septate endophytes (DSE) and arbuscular mycorrhizal (AM) was investigated. There was a notable increase in AM colonisation, and nine AM fungal species were found across two locations [35].

Conclusion

The soil samples with the highest spore colonisation (80%), flowering stage (48%) and minimum spores (44%) were those in the maturity stage. For Horsegram (*Macrotyloma uniflorum* L.), A total of 39 species of VAM fungal spores belonging to six genera (*Acaulospora, Glomus, Gigaspora*, *Scutellosora, Pacispora*, and *Septoglomus*) were recovered from soils that adhered to roots at the phases of seedling (9 sp.), flowering (16 sp.), and maturity (15 sp.). Present investigations established a base for future exploitation of VAM fungi for reclamation of wastelands in the form of biofertilizers. The primary goal of studying microbial diversity in conventional crops is to use

these fungi as biofertilizer to grow valuable agricultural plants. The purpose of this study is to inform the near by village farmers about the effectiveness of these indigenous arbuscular mycorrhizal fungi , which can be utilised over extended periods of time and is preferred over chemical fertilisers, resulting in environmentally beneficial organic farming. The fact that these fungi are currently found in rice fields suggests that they may have helped ensure that rice was produced in a way that supported local livelihoods. Additionally, it contributes to producing future inoculums and can be used as fertiliser to produce stronger seedlings and increase their survival in harsh environments.

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References

1. Asha, K.I., Latha, M., Abraham, Z., Jayan, P.K., Nair, M.C. and Mishra, S.K. (2008). Genetic resources. In D.Kumar (Ed.) Horsegram in India. *Scientific Publisher*, Jodhpur, pp 11–28.
2. Rit, R., Choudhury, D., Mukherjee, C. and Dutta, S. (2023). Investigation on microfloral association in the roots of *Macrotyloma uniflorum* (Lam.) Verdc., a medicinally important tropical pulse-crop and their possible applications for crop im-provement: A review. *Plant Science Today.*10(3): 300–311. https://doi.org/10.14719/ pst.2307.
3. Marimuthu,M., and Krishna moorthi,K.(2013).Nutrients and functional properties of horse gram (Macrotyloma uniflorum), an underutilized south Indian food legume. *Journal of Chemical and Pharmaceutical Research*.5(5):390-394. e www.jocpr.com.
4. Suthar, R., Patel, P.H., Kumar, A. andUrmila (2017). Effect of horse gram (*Macrotyloma uniflorum* Lam. Verdec) varieties and different row spacing on yield attributes and yield. *Life sciences international research journal*. 4:1-6.
5. Murthy, S.M., Devaraj, V.R., Anitha,P. andTejavanthi, D.H. (2012).Studies on the activities of antioxidant enzymes under induced drought stress in vivo and in vitro plants of Macrotyloma uniflorum(Lam.)Verdc.*Recent Research in Science and Technology*.4,34-37.http://recent-science.com/.
6. Krishna, K.R.(2010).Legume agroecosystems of south India:nutrient dynamics,ecology and productivity. *Brown Walker Press, Florida, USA*. p. 372-382.
7. Kumar, R.S., Nalliah, D.S., Kannan, V. (2017).Effect of Crop Geometry and FoliarNutrition on Growth and Yield of Irrigated Black gram (*Vigna mungo* L.). *International Journal Current Microbiology Applied Science*.6(11):4084-4094. https://doi.org/10.20546/ijcmas.2017.611.478
8. Frank, A.B. (1885). The mode of nutrition of certain trees through soil fungi based on root symbiosis. *Berichte der Deutschen Botanischen Gesellschaft*.3,128-145.
9. Allen, M.F. (1991). The ecology of mycorrhizae. UK: Cambridge. Cambridge University Press. 8(2), 184. https://www.scirp.org/reference/referencespapers?referenceid=1396065
10. Radhika, K.P., Rodrigues, B.F. (2010). Arbuscular mycorrhizal fungal diversity in some commonly occurring medicinal plants of Western Ghats, Goa region. *Journal of Forestry Research.*21, 45-52p. doi:10.1007/s11676-010-0007-1

11. Smith, S.E. and Read, D.J. (2008). Mycorrhizal symbiosis. Edition 3rd. San Diego, California. *New York: Academic Press.* 73(1),787. doi:10.2136/sssaj2008.0015br
12. Redecker, D., Schüßler, A., Stockinger, H., Stürmer, S.L., Morton, J.B. and Walker, C. (2013). An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). *Mycorrhiza.* 23(7), 515–531.https://doi.org/10.1007/s00572-013-0486-y
13. Wang, F. (2017). Occurrence of arbuscular mycorrhizal fungi in mining-impacted sites and their contribution to ecological restoration: mechanisms and applications. *Critical Reviews In Environmental Science and Technology*. 47(20), 1901–1957. https://doi.org/10.1080/10643389.2017.1400853
14. Wang, F., Adams, C.A., Yang, W., Sun, Y. and Shi, Z. (2020). Benefits of arbuscular mycorrhizal fungi in reducing organic contaminant residues in crops: implications for cleaner agricultural production. *Critical Reviews In Environmental Science and Technology*. 50(15), 1580–1612. doi: 10.1007/s11104-022-05621-z
15. Powell, J.R.and Rillig, M.C. (2018). Biodiversity of arbuscular mycorrhizal fungi and ecosystem function. *New Phytol*. 220(4), 1059–1075.https://doi.org/10.1111/nph.15119
16. Moreno, Walker, C. Duffy, K., Krüger, M., Robinson, C.H. and Pittman, J.K. (2023). Isolation and identification of arbuscular mycorrhizal fungi from an abandoned uranium mine and their role in soil-to-plant transfer of radionuclides and metals. *Science of TheTotal Environment.*876 : 162781. doi: https://doi.org/10.1016/j.scitotenv.2023.162781
17. Jackson, M.L. (1973). Soil chemical analysis. Prentic Hall of India Pvt. Ltd. New Delhi.; 151-153p.http://www.new.dli.ernet.in/handle/2015/135603
18. Walkley, A.J.and Black, I.A. (1934). Estimation of soil organic carbon by chromic acid titration method. *Soil Science.*37(1), 29-38p.
19. Subbiah, H.V. and Asija, G.L. (1956). A rapid procedure for the estimation of available nitrogen in soils. *Current Science.* 25(8),259-60. https://www.scirp.org/reference/referencespapers?referenceid=221144
20. Merwin, H. and Peech, M. (1951). Exchangeability of soil potassium in sand, silt and clay fraction as influenced by the nature of complementary exchangeable cations. *Soil Science Society of America Proceedings.* 15, 125-28p. doi:10.2136/SSSAJ1951.036159950015000C0026X
21. Singh,R.A.(1980).Soil physical analysis. NewDelhi,:*Kalyani Publishers.*165p.
22. Jency,J., Dhivya,K.and Rajesh kumar,S.(2023). Studies on physico-chemical parameters in different soil samples from Erode District, Tamil Nadu, India. *GSC Biological and Pharmaceutical Sciences*. 24(02):029–039. doi:10.30574/gscbps.2023.24.2.0295**DOIurl:**https://doi.org/10.30574/gscbps.2023.24.2.0295
23. Phillips, J.M. and Hayman, D.S. (1970). Improved procedures for clearing roots and staining parasitic andVesicular-Arbuscular Mycorrhizal Fungi for rapid assessment of infection. *Transactions of the British Mycological Society*. 55(1), 158-161p. https://doi.org/10.1016/s0007-1536(70)80110-3
24. Lapanjang I, Keberadaan M. Arbuskular pada Lokasi Pertanaman Jarak Pagar (*Jatropha curcas* L.) di Lembah Palu. In Jurnal Sains Teknologi & Lingkungan 2019; 5(1):3. doi: https://doi.org/10.29303/jstl.v5i1.104
25. Moreno, Walker C., Duffy, K., Krüger, M., Robinson, C.H. and Pittman, J.K. (2023). Isolation and identification of arbuscular mycorrhizal fungi from an abandoned uranium mine and their role in soil-to-plant transfer of radionuclides and metals. Science of TheTotal Environment. 876 : 162781. doi: https://doi.org/10.1016/j.scitotenv.2023.162781
26. Kormanik, P.P., Bryan, W.C. and Schutty, R.C. (1980). Increasing endomycorrhizae fungus inoculums in forest nursery soil with cover crops. Southern J.Applied For. 4, 151-153p.
27. Kusakabe, R. and Yamato, M. (2023). Isolation and identification of an arbuscular mycorrhizal fungus specifically associated with mycoheterotrophic seedlings of *Gentiana zollingeri*(*Gentianaceae*)Mycoscience.28;64(2):5562.doi:10.47371/mycosci.2023.01.001

28. Prasad, K. (2024). Occurrence and interactions of arbuscular mycorrhizal fungi (Rhizophagus fasciculatus) and rhizospheric fungi in *Saccharum officinarum* L. *Tropical Plants*.3: e033. doi: 10.48130/tp-0024-0035
29. Gerdemann, J.W. and Nicolson,T.H. (1963). Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting technique. *Transactions of the British Mycological Society*. 46(2), 235-244p. http://dx.doi.org/10.1016/S0007-1536(63)80079-0
30. Schenck, N.C. and Perez, Y. (1990). A manual for identification of VAM fungi. *University of Florida, Florida, USA*. 1-286.
31. Noreen, S.,Yaseen,T.,Iqbal,J.,Abbasi,B.A.,Elsadek,F.M.,Sayed,M.,Ijaz,E.S.andAli, I. (2023). Morphological and Molecular Characterizations of Arbuscular Mycorrhizal Fungi and their Influence on Soil Physicochemical Properties and Plant Nutrition.*Pubmed*. 8(36), 32468–32482p. doi: 10.1021/acsomega.3c02489.
32. Wendy, F., Xavier, M. and Rodrigues, B.F. (2020). Identification of Dominant Arbuscular Mycorrhizal Fungi in Different Rice Ecosystems. *Agricultural Research*. 9(1),46–55p.doi10.1007/s40003-019-00404-y
33. Lakshman, H.C., Jnchal, R.F. and Mulfa, F.I. (2006). Seasonal fluctuations of arbuscular mycorrhizal fungi on some commonly cultivated crops of Dharwad. *In: Mycorrhiza, Prakash A,Mehrotra VS (eds), Scientific Publishers, India (Jodhpur).*pp 173-179.
34. Sagar, A., Shivani , and Rani, N. (2015). Biodiversity of VAM and Rhizosphere Fungi Associated with Wheat grown in Normal and Disturbed fields. *Plant Archives*. Vol. 15 No. 1, 549-553p.
35. Chakraborty, K., Banik, S., Debnath, A., Das, A.R., Saha, A.K. and Das, P. (2019). Arbuscular mycorrhiza and dark septate endophyte fungal associations of Oryza sativa L. under field condition: colonization features and their occurrence. *Plant Science Today*. 6(1): 63-70. https://doi.org/10.14719/pst.2019.6.1.474

Table1:Physico-chemical properties of soil samples

Sr. No.	Particular	Method of Analysis	Content
1	pH	Glass electrode pH meter(Jackson,1973)	5.65
2	EC (ds/m)	Conductimetric method(Jackson,1973)	0.22
3	N(Kgha-1)	Rapid titration mathod Walkley and Black,1934)	234
4	P(Kgha-1)	Alkaline permagnate merthod (Subbihand Asija,1956)	13
5	K(Kgha-1	Olsen'smethod of extraction with 0.5NNaHCO3 at pH 8.5(Olsen et.al.1965)	203
6	Organic carbon (%)	Ammonium acetate extraction method(Merwina and Peech, 1950)	O.28

Table2.Morhological Descriptor of Horsegram(*Macrotyloma uniflorum* L.)

Sr. No.	Morphological Characteristics of Horsegram(*Macrotyloma uniflorum* L.)	Status
1.	Growth Habit	Erect
2.	Senescence	Actively Growing
3.	Colour of Inflorescence	Light Green
4.	Inflorescence shape	Cylindrical(Racemes more or less a pressed to primary axis)
5.	Shape of lower raceme	Straight not slender
6.	Grain colour	Greenish Brown

Table 3: AM fungal root colonization was recorded forHorsegram (*Macrotyloma uniflorum* L.) growing in the seedling, flowering and maturity stage .

Sr. No.	Name of Crop	Sample of crop plants	No. of root colonized	No.of root segments examined	Rate of colonization (%age)	Average (%age)
1	**Horsegram (*Macrotyloma uniflorum* L.)** At Seedling Stage	Sample1	3	5	60%	64%
		Sample2	4	5	80%	
		Sample3	3	5	60%	
		Sample4	3	5	60%	
		Sample5	3	5	60%	
2	At Flowering Stage	Sample1	4	5	80%	88%
		Sample2	5	5	100%	
		Sample3	4	5	80%	

		Sample4	4	5	80%	
		Sample5	5	5	100%	
3	At Maturity Stage	Sample1	5	5	100%	100%
		Sample2	5	5	100%	
		Sample3	5	5	100%	
		Sample4	5	5	100%	
		Sample5	5	5	100%	

Table 4: Comparison of occurrence of different VAM fungal spores isolated from the rhizosphere soil of Horsegram (*Macrotyloma uniflorum* L.)taken at seedling stage, flowering stage and maturity stage of Plant.

Sr. No.	VAM fungal Spores Isolated	At Seedling stage(From same Field)	At Flowering stage(From same Field)	At Maturity stage (From sameField)
1.	*Acaulospora brasiliensis*	_	+	_
2.	*Acaulospora bireticulata*	+	_	_
3.	*Acaulospora macrocarpum*	+	_	_
4.	*Acaulospora denticulate*	+	+	+
5.	*Acaulospora appendiculata*	+	_	_
6.	*Acaulospora tuberculata*	_	_	+
7.	*Acaulospora nicosolanii*	+	_	_
8.	*Acaulospora foveata*	_	+	_
9.	*Acaulospora soloidea*	_	+	_
10.	*Acaulospora mellea*	_	_	+
11.	*Acaulospora myriocarpa*	_	+	+
12.	*Acaulospora koskei*	_	_	+
13.	*Acaulospora gedansis*	_	_	+
14	*Acaulospora* sp.(5no.)	_	+	+
14.	*Glomus fasiculatum*	+	_	+
15.	*Glomus intradices*	+	_	_
16.	*Glomus botryoides*	_	+	_
17.	*Glomus versiforme*	_	+	_
18.	*Glomus geosporum*	_	+	_
19.	*Glomus pallidium*	_	+	_
20.	*Glomus mossae*	_	+	_
21.	*Glomus sp.*(3no.)	_	+	+
22.	*Gigaspora margarita*	_	+	+
23.	*Gigaspora gigentia*	_	_	+

25.	*Gigaspora decipens*	_	+	_
26.	*Gigaspora species*(5no.)	+	+	+
27.	*Scutellospora calospora*	_	+	_
28.	*Pacisporasp.*	_	_	+
29.	*Septoglomus*	_	_	+

+=Present,-=Absent

PHOTOPLATES

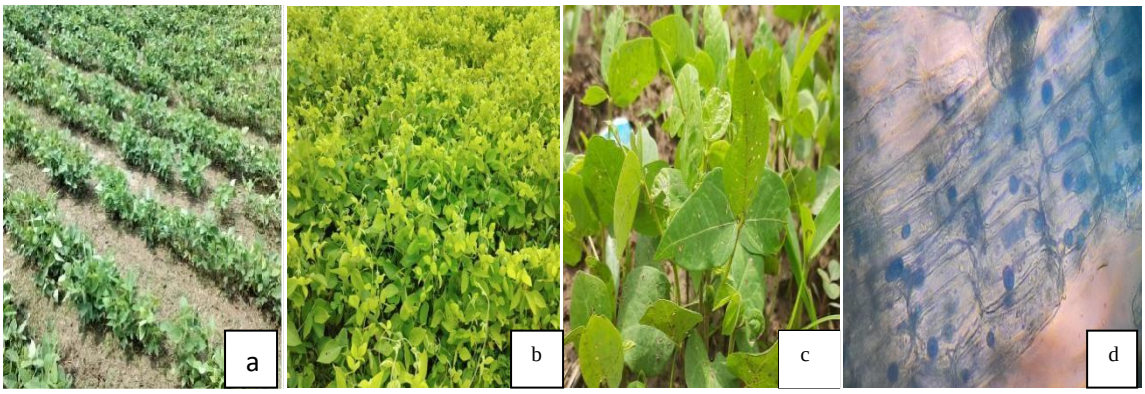
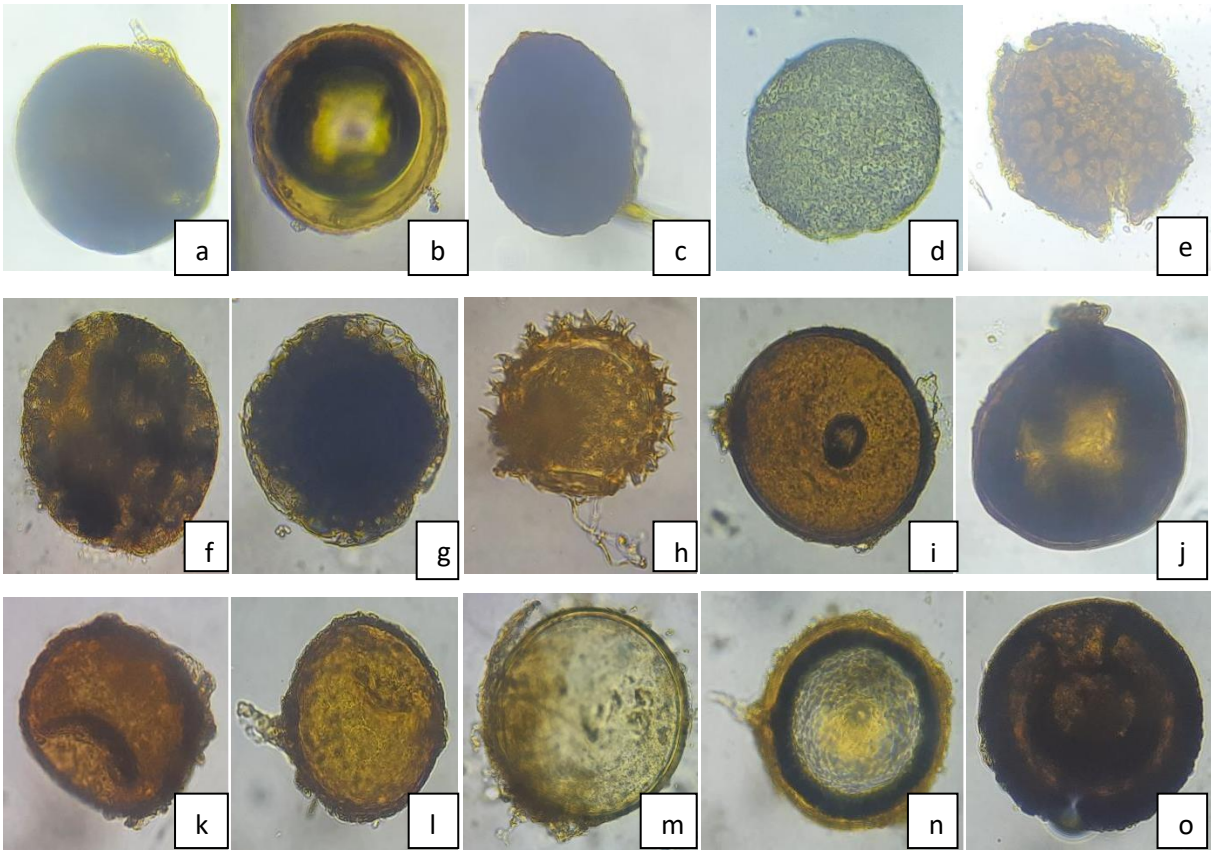


Figure1: Horsegram (*Macrotyloma uniflorum* L.) plant (a,b,c), VAM infection in roots of plant (d)



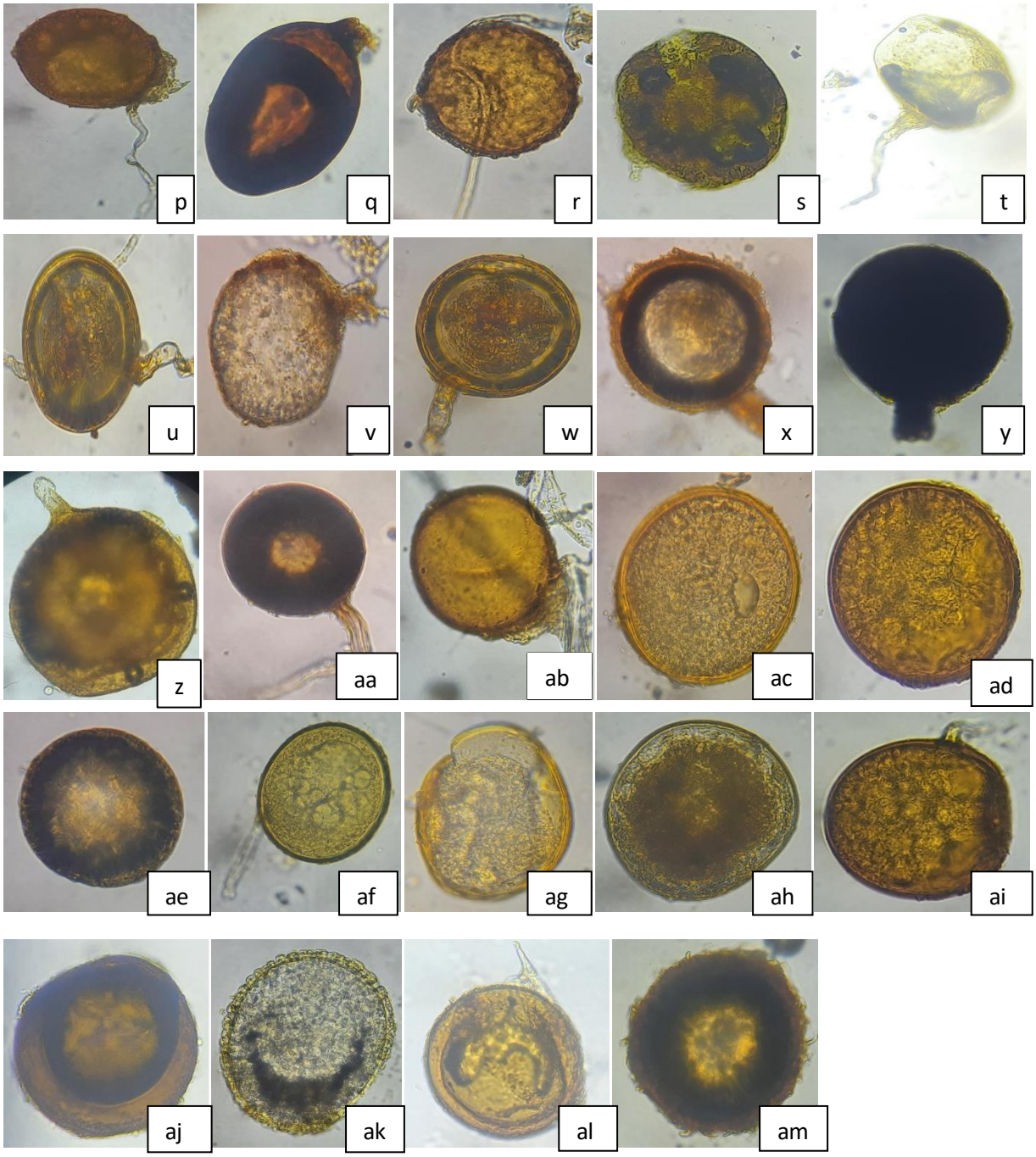


Figure 3: VAM Spores in Horsegram (*Macrotyloma uniflorum* L.) plant *Aaculospra denticulate* (a), *A.nicosolanii* (b), *A. macrocarpum* (c), *A.appendiculata* (d), *A.bireticulata* (e), *A. foveata* (f) , *A.brasiliensis* (g), *A.soloidea* (h), *A.mellea* (i), *A.tuberculata* (j), *A.myriocarpa* (k), *A. koskei* (l), *A. gedensis* (m), *Acaulospora sps* (n-r). *Glomus intraradices* (s), *G. fasiculatum* (t), *G.pallidium* (u),*G.versiforme* (v), *G.geosporum* (w), *G.mossae* (x), *G.botryoides* (y),and *Glomus sp* (z-ab)**,** *Gigaspora decipens* (ac), *G. margarita* (ad) , *G. gigentia* (ae), and 5 *Gigaspora species* (af-aj), *Scutellospora calospora* (ak), *Pacispora* (al), *Septoglomus*(am)