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Isolation of Arbuscular Mycorrhizal (AM) Fungi and Their Effect on Growth Performance of Kalmegh (Andrographis Paniculata (Burm.F.) Nees)

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Abstract- ***Arbuscular mycorrhizal (AM) fungi were symbiotically associated in roots of the Andrographis paniculata in mutual relationships that help in enhancing plant growth performance. The present study was carried out to study the effect of arbuscular mycorrhizal (AM) fungi on the growth parameters of Kalmegh (Andrographis paniculata). The rhizospheric soil and plant samples were collected from the diverse regions of Washim district and were used in the pot experiment. The pots were filled with a mixture of sterilized rhizospheric soil and Farm Yard Manure (FYM) and labeled experimental pots were sown with seeds of Andrographis paniculata along with control. The experimental pots were inoculated using three species of isolated AM fungi like Glomus fasciculatum, Glomus intraradices, and Glomus mosseae. The effect of these isolated AM fungi was evaluated in growth performance comprising microscopic characterization of isolated AM fungal species, plant height, root length, fresh and dry weight, and total number of roots and leaves of pot grown Andrographis paniculata. In results, the AM fungi were posing a noteworthy effect on plant height (76.4±4.9 cm of G. mosseae treated plants), root length (16.8±1.3 cm of G. mosseae treated plants), total number of average roots (26±1.6) and leaves (41±3.3) per plant, and fresh weight (50.3 ± 4.6 g) and dry weight (8.9 ± 1.0 g) in G. mosseae treated plants. Such improvement in above tested growth parameters revealed that AM fungi can significantly contribute to enhance the growth performance of Kalmegh.***

Keywords- ***Andrographis Paniculata, Kalmegh, AM Fungi, Colonization, Growth Analysis***

I. INTRODUCTION

Kalmegh (Andrographis paniculata (Burm.f.) Nees) is a commonly wild and cultivated crop for its potential medicinal properties (Hossain et al., 2014; Wanichananan et al., 2025). It is an herbaceous annual plant belongs to the family Acanthaceae (Suharno et al., 2024). It is an erect and branched annual herbaceous plant of height 0.3 0.9 meters with sharply quadrangular branches, lanceolate leaves, flowers are small, white in colour, and solitary with yellowish brown coloured seeds (Valdiani et al., 2012; Wanichananan et al., 2025). The fresh and dried leaves of Andrographis paniculata and juice extracted from the whole herb is a certified drug of Indian pharmacopoeia (Chiramel et al., 2006). The leaves extract contains several diterpenoid compounds among which andrographolide is very essential diterpenoid compound. The drug andrographolide diterpene is utilized for common weakness, certain types of dyspepsia, chronic malaria, dysentry and jaundice (Mishra et al., 2007; Chun et al., 2010). Numerous researchers have investigated that andrographolide diterpene has the potential to be incorporated in the mixed vaccine in the treatment of HIV-AIDS through desirable quality of its antagonistic effect with HIV-II virus (Rajani et al., 2000; Dumrongsak et al., 2009; Talei, 2019; Chutimanukul et al., 2022). This plant was previously being used in the treatment of tumors as it associated with cell differentiation events in tumour cells. Hence, the leaves of Andrographis paniculata contain highest quantity of andrographolide, however the entire plant is utilized for isolating the other bioactive metabolites (Chiramel et al., 2006; Chutimanukul et al., 2022).

AM fungi are associated with white growing plant roots and establish symbiotic mutual relationships (Johnson et al., 2004; Powell et al., 2009). About 90% of terrestrial plants are with associated colonization arbuscular mycorrhizal fungi and these AM fungal species are grow in plant rhizosphere soil that assist in the augmented absorption of immobile or fixed ions or elements, nitrogen, phosphorus, potassium and water (Pena Venegas et al., 2021; Camenzind et al., 2024). Such improved uptake or assimilation of useful macro- and micronutrients assists in the overall performance of the plant system. AM fungi assist in rising plant growth and health, stress tolerance, and improved accumulation of medicinally important secondary metabolites (Rajeshkumar et al., 2015; Wahab et al., 2023). Moreover, the symbiotic association of these AM fungi reduced the additional irrigation and fertilizers requirements of plants (Afshar et al., 2014; Liu et al., 2018). Isolation and identification of different AM fungal species have already been studied by several scientists from the rhizospheric soil of different medicinal and crop plants like Asparagus sp., Pisum sativum, rice crop, Rhizophagus sp., Smilax sp., Citrus sp., Withania sp., and Zea mays (Yaseen et al. 2016; Trejo-Aguilar and Banuelos, 2020; Johny et al., 2021; Manjula et al., 2022). Therefore, keeping these advantageous aspects of isolated AM fungi association with plant roots, the aim of present investigation was to study the isolation of AM fungi and their effect on some of the growth parameters of Kalmegh (Andrographis paniculata).

II. MATERIAL AND METHODS

2.1. Isolation of AM fungi

For isolation of AM fungi, 50 g of soil samples were collected from the rhizosphere of Andrographis paniculata plants were mixed in 100 ml of distilled water to achieve a consistent suspension and incubate for five minutes so that debris floated on the water surface (Manjula et al., 2022). The prepared soil suspension was passed through a sequence of sieves of different pore sizes such as 240, 120, 100, 60, and 30 µm. The final decanted suspension of sieving was passed through Whatman filter paper No. 1. The filter papers were examined under the stereo-microscope for the observation of isolated AM fungal spores. The fungal spores were observed under the microscope were picked up with the help of a needle and transferred onto a surface sterilized glass slide for identification.

2.2. Inoculation of AM fungi

The experimental pots were inoculated with the isolated AM fungi at the time of seed sowing according to the method modified by Manjula et al. (2022). The pots with inoculated five isolated AM fungi served as treatments and the non-inoculated pots were served as controls. A total of 3 pots were inoculated with AM fungi (Glomus fasciculatum, Glomus intraradices, and Glomus mosseae) for each treatment and control pots were maintained as non-inoculated. The AM fungi treated Andrographis paniculata plants were analyzed at 90 days post inoculation (DPI).

2.3. Colonization of AM fungi

About five different root regions were randomly selected to evaluate the range of AM fungal colonization from each treated plants separately along with control ones. The frequency of roots were colonized with AM fungi and the abundance of arbuscules percentage were determined according to the method described by Maltz and Treseder (2015) after staining of roots with 1.0% methyl blue in lactic acid.

2.4. Analysis of plant growth parameters

To determine the effect of isolated AM fungi namely Glomus fasciculatum, Glomus intraradices, and Glomus mosseae on plant height, root length, and total number of roots and leaves per plant were assessed. The plant height and root length were examined and results were expressed in centimeters (cm). The total number of leaves and roots were counted by visual observation and results were expressed in number counts.

2.5. Fresh and dry weight of Andrographis paniculata

The well grown AM fungi treated and control plants of 90 days after inoculation were selected randomly and harvested from culture pots and immediately subjected to taking fresh weight (FW) using digital balance (Contech, India) to avoiding water loss. The FW was recorded of individual plant in grams and

weighed plants were transferred into hot air oven (Steelmet, India) at 40°C temperature for drying. After 24 h the oven dried individual plant were subjected to taking dry weight (DW) till the constant weight was recorded. The DW was recorded in grams of same individual AM treated and control plants. The results of FW and DW of AM fungi treated and control plants were expressed in grams (g).

2.6. Experimental design and statistical analysis

All experiments were arranged in a completely randomized design (CRD) and conducted at least three times with minimum 14 replicates per treatment. Observations were recorded time to time wherever is necessary. Statistical methods were used for comparison of treatment means during optimization of tested parameters. Data was analyzed by analysis of variance (ANOVA) to detect significant differences between means. Means differing significantly were compared using Duncan's Multiple Range Test (DMRT) (Duncan, 1995) at the $p<0.05$ level of significance. Variability in data has been expressed as mean ± standard error (mean ± SE) and significant differences between means were indicated by using different alphabets.

III. RESULTS

3.1. Morphology of Andrographis paniculata

It is an erect annual herbaceous plant reaching a height of 30–100 cm. The stem part is acutely quadrangular, dark green, often with longitudinal furrows and intensely branched (Li et al., 2007). The leaves are simple, glabrous surface, typically lanceolate to elliptic-lanceolate in shape, 2–9 cm long, 1–2.5 cm wide, opposite, decussate, and acute to acuminate apex (Suharno et al., 2024). The flowers are small, solitary, and arranged in terminal or upper axillary raceme inflorescence (Jarukamjorn and Nemoto, 2008). The calyx has five linear, hairy lobes while corolla is tubular and bi-labiate, usually white or pale pink with purple or brownish spots. The upper lip is entire, and the lower lip is tri-lobed. The fruit is a linear, oblong, capsule type that is acute at both ends and size about 1.5–3 cm long. The capsule contain numerous, sub-quadrate to oblong seeds that are yellowish-brown to golden brown colored at maturity (Akba, 2011; Adishesha and Murthy, 2022).

Fig. 1: Morphology of Andrographis paniculata. a: Habitat of plant, and b: Mature habit of plant with flowers and capsules

3.2. Colonization of AM fungi

AM fungi colonization was found significantly higher in the roots of AMF-inoculated Andrographis paniculata plants. Whereas the AM fungi colonization in roots was found significantly lower in non-inoculated (control) plants. The isolated vesicles from Glomus fasciculatum, Glomus intraradices, and Glomus mosseae were found them to be infective and the remaining hyphal fraction within the roots was observed. The AM fungi colonization in root was not affected by fertilization, water supply and nor by their interaction.

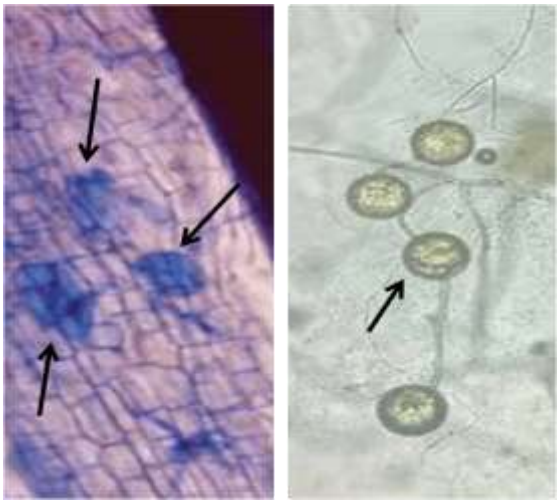

Fig. 2: Light microscopic images of arbuscular mycorrhizal (AM) fungal colonization in the roots of Andrographis paniculata

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3.3. Identification of AM fungal species

The isolated AM fungal spores were identified using standard literature. Total three species of AM fungi were isolated and identified through microscopic characterization, expert mycologists, and using fungal literature and monographs namely as Glomus fasciculatum, Glomus intraradices, and Glomus mosseae and selected to carry out present research work.

3.4. Microscopic characteristics of AM fungi

The identification of isolated AM fungal species was carried out based microscopic observations such as spore diameter, shape, wall width, presence, or absence of hyphae and spore colour. Microscopic characteristics were revealed that the spores of Glomus fasciculatum were pale yellow in colour with presence of subtending hyphae, whereas spores of Glomus intraradices were cream to yellowish brown with presence of hyphae. Likewise, the pale yellow to golden yellow coloured spores was recorded in case of Glomus mosseae. The diameter of spores was found 60–175, 28-78, and 80-280 µm in Glomus fasciculatum, G. intraradices, and G. mosseae respectively. The wall diameter of all three tested AM fungal species was found diverse in Glomus fasciculatum (1.0-1.8 µm), G. intraradices (1.5-2.5 µm), and G. mosseae (0.5-0.8 µm). The detailed microscopic observations and measurement parameters of isolated AM fungi were examined (Table 1).

Table 1: Microscopic characteristics for identification of AM fungi isolated from rhizosphere of Andrographis paniculata

AMF species	Diameter (µm)	Wall width (µm)	Hyphae	Shape	Colour
Glomus fasciculatum	60 - 175	1.0 - 1.8	Present	Globose to sub-globose	Pale yellow
Glomus intraradices	28 - 78	1.5 - 2.5	Present	Irregularly shaped and usually elliptical	Cream to yellowish brown
Glomus mosseae	80 - 280	0.5 - 0.8	Present	Globose to sub-globose	Pale yellow to golden yellow

3.5. Growth parameters of AM fungi inoculated Andrographis paniculata

Effect of AM fungi on growth parameters of Andrographis paniculata were recorded on 90 days after seed sowing under pot experiment. Analysis of growth performance results were exhibited that these inoculated AM fungi facilitates significant effects on plant height, root length, total number of roots and leaves, and fresh and dry weights.

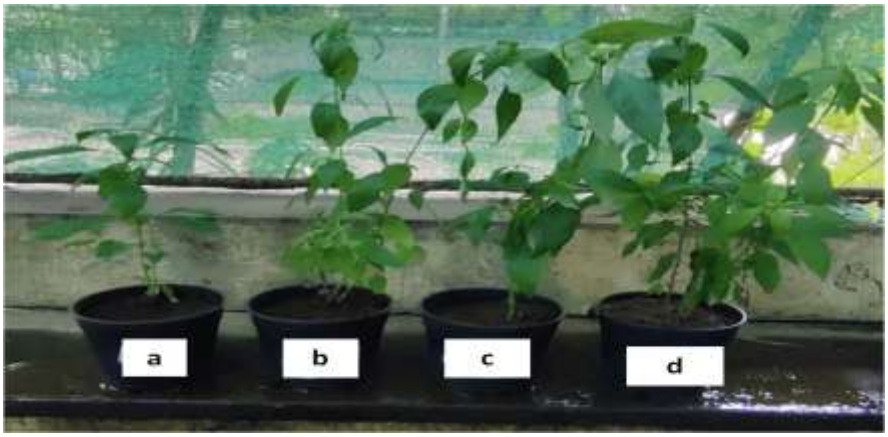

Fig. 3: Effect of three different species of Glomus on growth parameters of Andrographis paniculata. a: control plant; b: G. fasciculatum treated plant; c: G. intraradices treated plant; and d: G. mosseae treated plant

3.5.1. Plant height

The effect of all five tested Glomus sp. on plant height was found significantly higher as compared to the control plants of Andrographis paniculata. The significantly higher plant height (76.4±4.9 cm) was measured on 90 days after sowing in Andrographis paniculata plants inoculated with Glomus mosseae, followed by Glomus fasciculatum (68.6±4.4 cm), and G. intraradices (65.1±5.9 cm) when compared to control (40.2±4.3 cm). These results were revealed that the significantly higher effect of these tested Glomus sp. fungi on the plant height of Andrographis paniculata plants.

3.5.2. Root length

Inoculation of Glomus sp. of tested AM fungi exhibited the variations in root length was recorded in AM fungi inoculated Andrographis paniculata plants as compared to controls. All these tested five AM fungi did not exhibited the similar effects on the root length. This effect was found significantly positive in Andrographis paniculata plants inoculated with Glomus fasciculatum and Glomus mosseae. The significantly increased root length was recorded to be 16.8±1.3 cm in kalmegh plants inoculated with G. mosseae on 90 days after sowing in pots. Similarly, kalmegh plants were inoculated with G. fasciculatum showed root length of 13.3±1.6 whereas G. intraradices showed root length of 12.6±1.1 cm on 90 days after sowing in pots. The significantly lower effect on root length (9.1±0.4 cm) was recorded in control plants of kalmegh. The results of root length was revealed that the AM fungi treated plants showed significantly higher response for root length as compared to control (9.1±0.4 cm) plants.

Table 2: Effect of isolated AM fungal species on plant height and root length of Andrographis paniculata plant at 90 days post inoculation under pot culture experiment

AM fungal species	Growth parameters (mean ± SE)	
	Plant height (cm)	Root length (cm)
Control	40.2±4.3	9.1±0.4
Glomus fasciculatum	68.6±4.4	13.3±1.6
Glomus intraradices	65.1±5.9	12.6±1.1
Glomus mosseae	76.4±4.9	16.8±1.3

Data are mean ± SE scored and followed by same letter are not significantly different at $P \leq 0.05$ level by Duncan's multiple range test.

3.5.3. Total number of roots

Total number of roots was found increased in G. intraradices, G. fasciculatum, and G. mosseae treated plants of Andrographis paniculata in the present investigation. The total number of roots per plant in kalmegh plants inoculated with G. mosseae was recorded to be 26±1.6 after 90 days of sowing. Correspondingly, the total number of roots per plant in kalmegh plants was recorded to be 21±1.4 on 90 days after sowing when inoculated with G. fasciculatum. Likewise, the total number of roots per plant in kalmegh plants was recorded to be 20±1.9 on 90 days after sowing when inoculated with G. intraradices. The significantly lower effect on the total number of roots per plant was recorded (11±0.7 roots) in control Andrographis paniculata plants.

3.5.4. Total number of leaves

The total number of leaves per plant was examined in the AM fungi inoculated Andrographis paniculata plants under pot experiment. The results revealed that inoculation of AM fungi in the roots of kalmegh plants has significant effect on the total number of leaves per plant. The total number of leaves per plant was found to be significantly higher in kalmegh plants inoculated with G. mosseae (41±3.3), followed by the total number of leaves per plant (38±2.6) was found in kalmegh plants inoculated with G. fasciculatum after 90 days after sowing. The significant variation in number of leaves per plant was recorded in AM fungi-treated plants than control (24±1.8).

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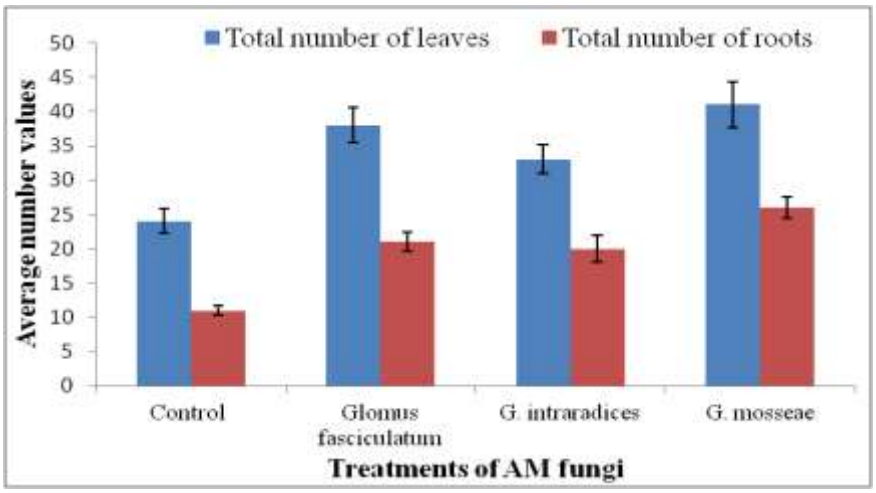

Fig. 4: Effect of AM fungi on total number of roots and leaves in Andrographis paniculata. Data are mean ± SE scored and followed by same letter are not significantly different at P ≤ 0.05 level by Duncan's multiple range test.

3.5.5. Fresh and dry weights of Andrographis paniculata

After harvesting of AM fungi treated and control plants were screened for fresh weight (FW) and dry weight (DW) (after oven drying at 40°C). The G. fasciculata, G. intraradices, and G. mosseae treatments resulted into significant increased in FW and DW was recorded when compared to untreated plants. The FW of G. mosseae (50.3 ± 4.6 g) and G. fasciculatum (46.7 ± 4.1 g) was found significantly higher than control untreated plants (15.6 ± 0.9 g). Whereas, the similar trend was observed for DW of G. mossae (8.9 ± 1.0 g) and G. fasciculatum (8.2 ± 0.9 g) treated plants and control untreated plants (4.1 ± 0.3 g) biomass. There was positive correlation was found to be occur in FW and DW of AM fungi treated plants and control untreated plants. Therefore, the significant increased in biomass of AM fungi treated plants than controls that could be results of due to the increased size of somatic cells due to growth performance and increased number of leaves and plant height.

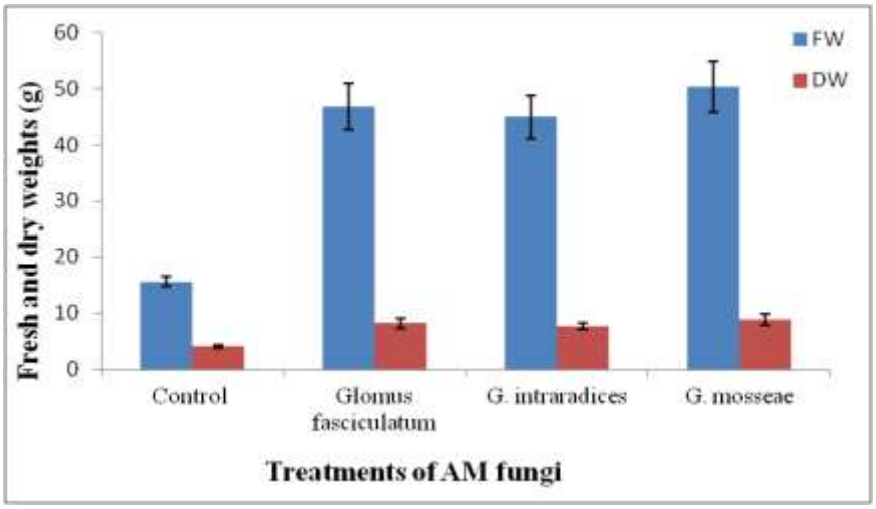

Fig. 5: Effect of AM fungi on fresh and dry weights of Andrographis paniculata. Data are mean ± SE scored and followed by same letter are not significantly different at P ≤ 0.05 level by Duncan's multiple range test.

IV. DISCUSSION

The present investigated revealed that the inoculation of different isolated AM fungi facilitates significant effects on the growth parameters of Andrographis paniculata. Total three AM fungi species comprise Glomus fasciculatum, Glomus intraradices, and Glomus mosseae were used in the present investigation were isolated as principal AM fungi from rhizosphere of Andrographis paniculata. As per previous literature, the maximum numbers of AM fungi species were isolated during month of November. The present results are in agreement with the investigations of previous literature was carried out by several researchers (Yang et al., 2014; Hijei, 2016; Manjula et al., 2022; Ajkar et al., 2024) where they stated that G. mosseae and G. fasciculatum exhibited the potential effects on the rhizosphere of Andrographis paniculata and other medicinal plants. Goswami et al. (2018) reported the morphological and molecular identification of arbuscular mycorrhizal (AM) fungi. According to previous literature, the similar investigations were reported the variable percentage of colonization of AM fungi was associated with roots and rhizosphere of other several plant species (Ismail et al., 2013; Manjula et al., 2022; Ajkar et al., 2024).

The variability in the incidence of AM fungi might be due to the soil properties, physico-chemical parameters, growing season, plant growth phase, and availability of soil moisture (Cong et al. 2010; Manjula et al., 2022). All the tested AM fungal species possess the potential to facilitate significant effects on various growth parameters of Andrographis paniculata. The modulations in growth parameters of AMF-inoculated plants were achieved compared to control are possibly due to the changes in physiological and biochemical parameters (Abdelaal et al. 2020). In Lettuce and tomato (Lycopersicon esculentum) crop plants, two AM fungi like Rhizophagus irregularis and Glomus intraradices facilitates enhanced biomass production (Ruiz-Lozano et al., 2016). The existence of AM fungi in the rhizosphere is reported to split complex nutrient forms into simpler ones and may facilitate and improve their absorption in the roots. Such phenomenon could be the possible reason to obtain significant enhance in plant height, root length, number of roots and leaves, and fresh and dry weight. A similar study was carried out on the influence of different AM fungi inoculums like Acaulospora kentinensis, G. mossae, and G. etunicatum on cowpea (Vigna unguiculata) roots colonization and improvement in soil profile was achieved (Surayya et al., 2021). Effect of Glomus deserticola inoculation on Snapdragon (Antirrhinum majus) crop plant results in enhanced morphological traits such as leaf area, shoot length, root and shoot diameter, and number of leaves per plant (Asrar et al., 2012). According to Wu and Xia (2004) investigated that arbuscular mycorrhizal (AM) fungi application could increase the plant growth like plant height, diameter of stem, leaf area, and root and shoot dry weight. Influence of Glomus mosseae, Glomus fasciculatum, and Gigaspora decipiens on Wheat (Triticum aestivum) crop facilitates the improved plant growth parameters (Pal and Pandey, 2016). Inoculation of AM fungi affects various growth parameters of different crop plants at various growth stages and these AMF inoculated plants can effectively fight against multiple environmental stresses like salinity stress, alkali stress, cold stress, drought stress, and nutrient stress (Begum et al., 2019). Likewise, the effect of G. intraradices AM fungi on plant height of common bean (Phaseolus vulgaris) was carried out (Olivera et al., 2004). Inoculation of Funneliformis mosseae and Rhizophagus intraradices AM fungi in roots of pea (Pisum sativum) plant results in the improvement of dry biomass (Yang et al., 2014). Application of Glomus sp. in the roots of Lolium perenne facilitate significantly increased root vigor along with augmented photosynthetic carbon assimilation potential (Zhang et al. 2018). According to Boyer et al. (2016) the inoculation of F. mosseae BEG25, and F. geosporus BEG11 AM fungal isolates exhibited increased root and shoot fresh weights in Strawberry (Fragaria x ananassa) crop.

The findings of the current investigation revealed that AM fungi possess the potential use as biofertilizers. Regardless of such valuable properties, the potential of these AM fungi is still unexploited as a feasible biofertilizer for the sustainable plant-based medicinal productivity of Andrographis paniculata. Nowadays, encouragement in the application of AM fungi in

plant roots is very useful for the pharmaceutical industries for the enhanced production of secondary metabolites for their reliable sustainability.

V. CONCLUSION

AM fungi interact directly with microorganisms present in the rhizospheric soil or indirectly by the modulation of morphological, physiological, and biochemical parameters of the host plant that affects the growth performance of the plant Andrographis paniculata. Inoculation of these isolated AM fungi during an early stage of plant growth has confirmed to be an effective method for improving plant growth performance. In the current investigation, the screening of AM fungal association has not only confirmed to improve the growth of Andrographis paniculata but also synthesized numerous different bioactive metabolites. Therefore, the AM fungi can be utilized effectively for enhancing the growth performance of medicinally important plant Kalmegh (Andrographis paniculata) in a relatively shorter duration and with cost-effective eco-friendly way.

VI. ACKNOWLEDGEMENTS

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