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Research Article Arbuscular Mycorrhizal Fungi (AMF) Status and Diversity Of weedy Plants in Degraded Land

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Abstract

Background and Objectives: Study area of Naldurg is located at 17.82°N 76.3°E in Osmanabad district of Marathwada region in Maharashtra state. This area lacks natural resources and is prone to drought, rocky and dry with low and uncertain rainfall, therefore the objectives of this study was to ascertain the status of AM fungi in weeds growing on barren and degraded land and their use in agriculture during both rabi and kharif season. Methodology: Degraded land fields weedy plant species of roots were collected in rabi and kharif season during 2015-2016. Three each plant species were selected for assessment of AMF root colonization, spore density and spore diversity was calculated from every sample collected of weedy plants species. Results: Altogether 21 species belonging to 11 families and 21 genera in rabi season and 20 weedy plant species belonging to 9 families and 20 genera in kharif season were collected from degraded field and examined diversity and Arbuscular Mycorrhizal Fungi (AMF) status. Percent AMF root colonization ranged from 40.62-84.37%. The highest colonization was recorded in *Phyllanthus niruri* (84.37%) and least in *Cocculus hirsutus* (40.62%) in rabi season. In case of kharif, percent root colonization ranged from 43.75-81%. The highest colonization was recorded in Dichanthium caricosum (81%) and least in Adenostemum alavenia (40.62%). Vesicular, arbuscular and hyphal types of root colonization was recorded in both season. Seven of the 11 common plant species showed higher root colonization in rabi season as compared to kharif. AM fungal spore density varied in all weedy plant species and ranged from 112-1168 spores/100 g soil in rabi while 237-702/100g soil kharif season. A total of three AM genera viz., Acaulospora, Glomus and Scutellospora were recorded in both the season. In seasonal variation, AM spore density was greater in the rabi season than kharif. Conclusion: A positive correlation was found between AM root colonization in weed plants. There were significant differences between the organic and conventional systems in the density and biomass of both non-competitive weeds and competitive weeds.

Key words: Naldurg study area, degraded land, weeds, seasonal variation, AMF status

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Data Availability: All relevant data are within the paper and its supporting information files.



Environmental degradation is a major threat confronting the world, the uncontrolled use of chemical fertilizers contributes largely to the deterioration of the environment through depletion of fossil fuels, generation of carbon dioxide (CO₂) and contamination of water resources. It leads to loss of soil fertility due to imbalanced use of fertilizers that has adversely impacted agricultural productivity and soil quality and has caused soil degradation¹. Now there is growing realization that adoption of ecological and sustainable farming practices can only reverse the refuse trend in the global productivity and environment protection². The distribution of certain vesicular arbuscular mycorrhizal (VAM) fungal species has been related to soil pH, phosphorus level, salinity, soil disturbance³, vegetation⁴ or hydrologic condition of the soil⁵.

Mycorrhizal symbiosis is a highly evolved mutually beneficial relationship found between AM fungi and vascular plants. The benefit of AM fungi to plants is mainly attributed to their ability to increase plant uptake of phosphorus (P) and other non-mobile soil nutrients. Agronomic practices such as crop rotation, fertilization and tillage affect the extent of AM colonization and nutrient uptake of crops. Proper management of AM fungi has the potential to improve the profitability and sustainability of agricultural systems. These fungi are very important constituent of plant-soil-microbe system and can adapt to a wide range of environments. They are found in soils with very different water establishment including very arid habitats. Mycorrhizae establish symbiotic relationships with plants and play an essential role in plant growth, disease protection and overall soil quality⁶. The main challenges faced in the reclamation of severely degraded lands are in the management of the systems and finding plant species that will grow under the harsh conditions common in degraded soils. The P deficiency is widespread in tropical soils in existing soils and under such conditions the AM contributes towards P uptake⁷. Plantation of seedlings inoculated with AM fungi provides favorable soil conditions for naturally growing vegetation in the overburden spoil. The extra-radical mycelium of AM fungi act as an extension of the host root system, absorbing and providing nutrients (especially P) to the plant and receives photo synthetically assimilated carbon8. It was suggested that plant growth in wastelands could be effectively improved by incorporating AM fungi9. Weeds are an important variable in organic crop production, both economically and ecologically and weeds may serve to maintain diversity and agronomically beneficial taxa of AM fungi¹⁰. It was observed that the number of AM fungal spores increased significantly with increasing weed species number¹¹.

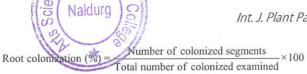
Therefore, the present investigation was made to potential of arbuscular mycorrhizal fungal (AMF) diversity in weedy plant species growing in degraded land during rabi and kharif season.

MATERIALS AND METHODS

Study area: Naldurg is situated at National Highway NH-9 Vijayawada to Pune. Degraded (non-agricultural) land investigation was conducted during 2015-16. Total agricultural and non- agricultural land area of Naldurg is 2787 ha is occupied. Out of which 2367 ha is using for agriculture and remaining land is non-agricultural (barren), it is considered as degraded or deficient land. It has an average elevation of 566 m (1856 feet). The study site is located at 17.49°N Latitude, 76.16°E Longitude, with an altitude of 509 m. Temperature ranges from 10.1-43.1°C. Average rainfall per year is 760 mm.

Assessment of arbuscular mycorrhizal status

Root colonization: Degraded fields weedy plant species of roots were collected in rabi and kharip season during 2015-2016. Three each plant species were selected for assessment of AMF root colonization. The plant roots i.e., primary and secondary fine roots were washed in water to remove soil debris and then preserved in Formalin-Acetic-Alcohol (FAA) (Ethyl alcohol 50 mL, Glacial acetic acid 5 mL, Formaldehyde (37-40%) 10 mL and distilled water 35 mL) in specimen bottles. At the time assessment of root colonization, preserved roots of weedy species were washed in water to remove traces of FAA. About 20-30 root segments cut in 2-3 cm length were added in 50 mL beaker half filled with 10% KOH to facilitate stain penetration in cortex tissue. Beaker was placed in oven for 2 h at 70°C. Roots were heated till depigmentation. In some cases microwave oven (30 sec) was used for 10% KOH treatment. The root segments were rinsed till no brown precipitate in water to dilute KOH residue and then immersed in 15 mL of Hydrochloric acid (5%) for 3 min at room temperature to improve the root staining efficiency. The acidified root segments were washed in water for 4 to 5 times and deeped in trypan blue (0.05%) for overnight period. To remove excess stain from root tissues using water for destaining¹². Observed the root segments under the binocular compound microscope (LOBAMED Vision 2000) and photographed with a Sony digital camera (DSC-W310/BCE37). In root having hyphae, vesicles, arbuscules were present was considered as mycorrhizal infection. The percentage of root colonization was calculated¹³ according to the following formula:



Isolation and quantification of AMF spores: The rhizospheric soil of weedy plant species were collected from study fields. The 500 g of rhizospheric soil was taken at a depth up to 10-15 cm of the plants of each species in separate polythene ziplock bags. Soil was dried at room temperature for 48 h. These soil samples were store at 4°C until processing. In soil sample AM fungal spores were isolated by using wet sieving and decanting method¹⁴ and identification of AM fungal spores was carried out based on morphotaxonomic criteria using INVAM International Collection of Vesicular Arbuscular Mycorrhizal (http://invam.wvu.edu/the-fungi) and available manuals 15,16. Spore density and spore diversity was calculated from every sample collected of weedy plants species. The voucher specimens of AM fungi were deposited in Department of Botany, Arts, Science and Commerce College Naldurg, Maharashtra, India.

Statistical analysis: All data were statistically analyzed and the significance of differences was determined by using book¹⁷.

RESULTS

Root colonization: Twenty one weedy plant species were assessed for AM colonization. Percent colonization ranged from 40.62-84.37%. The highest colonization was recorded in Phyllanthus niruri (84.37%) and least in Cocculus hirsutus (40.62%). Poaceae members were found to show higher colonization levels followed by Asteraceae, Fabaceae, Amaranthaceae and Euphorbiaceae. Seven weedy species viz., Lantana camara, Ocimum basilicum, Parthenium hysterophorus, Pulicaria wighitiana, Dinebera retroflexa, Spermacoce ramaii and Acanthospermum hispidum showed above 70% root in rabi season. Twenty weedy plant species were assessed for AM colonization. Percent colonization ranged from 43.75-81%. The highest colonization was recorded in Dichanthium caricosum (81%) followed by Dinebera retroflexa (78.44%) and least in Adenostemum alavenia (40.62%). Poaceae members were found to show higher colonization levels followed by Asteraceae and Fabaceae. Weedy species viz., Indigofera cordifolia, Indigofera tinctoria, Indigofer alinnaei, Ocimum basilicum, Lantaa camara, Celosia argentea showed above 60% colonization in *kharif* season. Colonization Vesicular, arbuscular and hyphal colonization were recorded in both seasons (Table 1 and 2, Fig. 1).

Spore density: The AM fungal spore density varied in different weedy species in rabi season. It was ranged from 112-1133 spores/100 g soil. Highest spore density was recorded in *S. ramaii* (1168/100 g soil) followed by *H. suaveolens, D. retroflexa* and *Dichanthium caricosum,* while it was least in *P. niruri* (112/100 g soil). AM fungal spore density was also found more in some plants even when the colonization was less. It was ranged from 237-702 spores/100 g soil. Highest spore density was recorded in *Cassia tora* (702/100 g soil) while least was recorded in *Tribulis terristeris* (237/100 g soil) in kharif season. Three AM genera viz., *Acaulospora, Glomus* and *Scutellospora* were recorded in weedy plants growing in degraded land during both season.

Seasonal variation among common weedy plants species:

A total 11 weedy species belonging to 6 families and 11 genera were collected and examined from degraded field during rabi and kharif season for percent AM root colonization, spore density and AM richness (Table 3). Here, we investigated the effect of AM fungi on the growth of individual weedy species and on weedy-crop interactions. Eleven weedy plants were found common in both season and were assessed for AM root colonization. Of the eleven plant species, seven species i.e., Ocimum basilicum, Parthenium hysterophorus, Lantana camara, Tridax procumbens, Cassia tora, Spermacoce ramaii and Acanthospermum hispidum were recorded higher percent AM colonization in rabi season as compared to kharif. During the kharif season, highest root colonization was recorded only in Heteropogan contortus compared to colonization in the rabi season. Three plant species i.e., Dichanthium caricosum, Dinebera retroflexa and Celosia argentea were observed similar percent root colonization in both seasons. The AM fungal spore density showed variation in both seasons. In almost all weedy plants, AM spore density was greater in the rabi season than in the kharif with the exception of O. basilicum, P. hysterophorus and T. procumbens.

DISCUSSION

Our findings are discussed the Kharif and rabi weeds plant species were collected from degraded field and extensive observation was made for diversity and richness of Arbuscular

| Name of the weeds | Family | RC (%) | Types of RC | Spore density/100 g | AM fungal genera |
|--|-----------------|------------------|-------------|---------------------|------------------------------------|
| Ocimum basilicum (L.) | Lamiaceae | 71.42±2.11 | HAV | 136±1.22 | Acaulospora, Glomus |
| Parthenium hysterophorus (L.) | Asteraceae | 78.12±3.44 | HAV | 282±3.33 | |
| Tephrosia purpurea (L.) Pers. | Fabaceae | 65.62士4.11 | AV | 325土12.11 | Acaulospora, Glomus |
| Lantana camara (L) | Verbenaceae | 81.25±8.22 | HAV | 666土11.22 | ALCOHOL: |
| Tridax procumbens (L.) | Asteraceae | 63.25 ± 3.33 | H | 146±21.11 | Acaulospora |
| Cassia tora(L.) | Caesalpiniaceae | 71.67±2.66 | HAV | 398±7.88 | Acaulospora, Glomus, Scutellospora |
| Malvastrum tricuspidatum (Kuntze) Stuck. | Malvaceae | 68.75±1.22 | HA | 270±12.33 | Acaulospora, Glomus, Scutellospora |
| Phyllantus niruri(L) | Euphorbiaceae | 84.37 土 4.11 | HAV | 112±2.11 | Acaulospora, Glomus, Scutellospora |
| Phyllanthus retiulatus (Poir) | Euphorbiaceae | 59.37 ± 0.11 | H | 559±11.03 | Glomus |
| Pulicaria wighiitana (DC) C.B. Clarke | Asteraceae | 75.11±6.22 | ⋖ | 520土12.11 | Acaulospora |
| Cynodon dactylon (L.) Pers | Poaceae | 59.25 ± 3.33 | AH | 499±32.11 | Acaulospora, Glomus |
| Heteropogan contortus(L.) P. Beauv. ex Roem. and Schult. | Poaceae | 46.87 ± 1.22 | HAV | 831±33.14 | Acaulospora, Glomus, Scutellospora |
| Dichanthium caricosum (L.) A. Camus | Poaceae | 81.00土4.32 | HAV | 912±3011 | Glomus, Scutellospora |
| Dineberaretroflexa (Vahl) Panz. | Poaceae | 78.44±2.31 | HAV | 1003±37.12 | Acaulospora, Scutellospora |
| Spermacoce ramanii Sivar. and R.V. Nair | Rubiaceae | 78.12±11.01 | HAV | 1168±39.11 | Glomus |
| Acanthospermum hispidum (D.C.) | Asteraceae | 75.33±2.11 | HV | 859土11.45 | Acaulospora, Glomus |
| Hyptis suaveolens (L.) Poit | Lamiaceae | 59.33 ± 6.34 | A | 1133±34.11 | Acaulospora, Glomus |
| Indigofera tinctoria (L.) | Fabaceae | 69.12 ± 4.33 | HAV | 431±12.11 | Acaulospora, Glomus |
| Indigofera cordifolia (L.) | Fabaceae | 75.77±2.11 | HAV | 740±3.12 | Acaulospora, Glomus |
| Cocculus hirsutus (L.) Diels | Minispermeceae | 40.62±3.22 | ΗΛ | 322 ± 2.44 | Glomus, Scutellospora |
| Celosia argentea (L.) | Amaranthaceae | 6567+444 | \VI | 560+1011 | |

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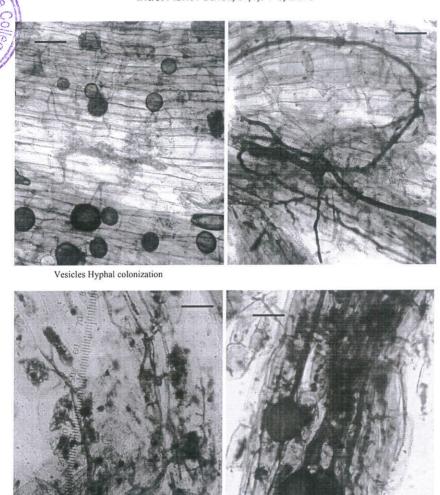
| Name of the weeds | Family | RC (%) | Types of RC | Spore density/100 g soil | Types of AMF species |
|--|-----------------|--------------|-------------|--------------------------|------------------------------------|
| Cassia tora (L.) | Caesalpiniaceae | 46.87±2.11 | HAV | 702±14.23 | Acaulospora |
| Acanthospermum hispidium (DC) | Asteraceae | 56.25±3.33 | HAV | 318±2.11 | Acaulospora |
| Adenostemma lavenia. R. Forst. and G. Forst. | Asteraceae | 43.75±11.00 | HAV | 370±22.11 | Acaulospora, Glomus, Scutellospora |
| Spermacoce stachydea (D.C) | Rubiaceae | 50.00土10.11 | HAV | 380土11.45 | Acaulospora, Glomus |
| Indegofera australis Willd. | Fabaceae | 53.12±4.44 | HAV | 491±13.22 | Glomus |
| Indigofera cordifolia (L.) | Fabaceae | 65.62±5.11 | HAV | 304土11.23 | Acaulospora, Glomus |
| Indigofera tinctoria(L.) | Fabaceae | 68.75±2.11 | HAV | 299±2.11 | Acaulospora, Glomus, Scutellospora |
| Catharanthus pusillus (Murray) G. Don | Apocynaceae | 58.33±7.11 | HAV | 286±3.67 | Acaulospora, Glomus, Scutellospora |
| Indigofera linnaei(L.) | Fabaceae | 68.75±3.87 | HAV | 299±5.32 | Glomus, Scutellospora |
| Glossocardia bosvallea (Cass.) | Asteraceae | 58.33±1.11 | HAV | 286±7.12 | Acaulospora, Scutellospora |
| Parthenium hysterophorus (L.) | Asteraceae | 59.37±1.11 | HAV | 369±6.77 | Acaulospora, Glomus |
| Ocimum basilicum (L.) | Lamiaceae | 62.50±8.99 | HAV | 242±2.11 | Acaulospora, Glomus |
| Tridax procumbens (L.) | Asteraceae | 59.37 ± 4.33 | HAV | 369±12.11 | Acaulospora, Glomus |
| Lantana camara (L.) | Verbenaceae | 62.50±11.11 | HAV | 242±14.23 | Acaulospora, Glomus |
| Tribulis terristeris (L.) | Zygophyllaceae | 62.05±10.11 | HAV | 237±2.33 | Glomus |
| Celosia argentea (L.) | Amaranthaceae | 66.66±2.11 | HAV | 647±14.11 | Acaulospora, Glomus |
| Heteropogan contortus(L.) P. Beauv. ex Roem. and Schult. | Poaceae | 51.51±2.11 | HAV | 425±21.11 | Acaulospora, Glomus, Scutellospora |
| Digitaria sanjuinalis (L.) Scop. | Poaceae | 48.26土4.12 | HA | 310±3.44 | Glomus |
| Dichanthium caricosum (L.) A. Camus | Poaceae | 81.00±6.55 | Α | 268±11.21 | Acaulospora |
| Dinebera retroflexa (Vahl) Panz. | Poaceae | 78.44±12.13 | HAV | 798+711 | Glomus Scutellospora |

Values are means of three replicates, RC: Root colonization, A.: Arbuscular, V. Vesicular, H.: Hyphal, ±standard error

Values are means of three replicates, RC: Root colonization, A: Arbuscular, V: Vesicular, H: Hyphal, ±standard error

| Name of the weeds | Family | Seasons | RC (%) | Types of RC | Spore density/100 g soil | Types of genera |
|---|-----------------|---------|------------------|-------------|--------------------------|------------------------------------|
| Ocimum basilicum (L.) | Lamiaceae | Rabi | 71.42±2.11 | HAV | 136±1.22 | Acaulospora, Glomus |
| | | Kharif | 62.50±8.99 | HAV | 242±2.11 | Acaulospora, Glomus |
| Parthenium hysterophorus (L.) | Asteraceae | Rabi | 78.12±3.44 | HAV | 282±3.33 | Glomus |
| | | Kharif | 59.37土1.11 | HAV | 369±6.77 | Acaulospora, Glomus |
| Lantana camara (L.) | Verbenaceae | Rabi | 81.25 ± 8.22 | HAV | 666土11.22 | Acaulospora |
| | | Kharif | 62.50±11.11 | HAV | 242±14.23 | Acaulospora, Glomus |
| Tridax procumbens (L.) | Asteraceae | Rabi | 63.25±3.33 | ¥ | 146土21.11 | Acaulospora |
| | | Kharif | 59.37 ± 4.33 | HAV | 369±12.11 | Acaulospora, Glomus |
| Cassia tora(L.) | Caesalpiniaceae | Rabi | 71.67±2.66 | HAV | 398土7.88 | Acaulospora, Glomus, Scutellospora |
| | | Kharif | 46.87±2.11 | HAV | 702±14.23 | Acaulospora |
| Heteropogan contortus(L.) P.Beauv. ex Roem. and Schult. | Poaceae | Rabi | 46.87 ± 1.22 | HAV | 831±33.14 | Acaulospora, Glomus, Scutellospora |
| | | Kharif | 51.51±2.11 | HAV | 425±21.11 | Acaulospora, Glomus, Scutellospora |
| Dichanthium caricosum (L.) A. Camus | Poaceae | Rabi | 81.00 ± 4.32 | HAV | 912±3011 | Glomus, Scutellospora |
| | | Kharif | 81.00±6.55 | ¥ | 268±11.21 | Acaulospora |
| Dinebera retroflexa (Vahl) Panz. | Poaceae | Rabi | 78.44±2.31 | HAV | 1003±37.12 | Acaulospora, Scutellospora |
| | | Kharif | 78.44±12.13 | HAV | 298±2.11 | Glomus, Scutellospora |
| Spermacoce ramaii Sivar. and R.V. Nair | Rubiaceae | Rabi | 78.12±11.01 | HAV | 1168±39.11 | Glomus |
| | | Kharif | 50.00土10.11 | HAV | 380土11.45 | Acaulospora, Glomus |
| Acanthospermum hispidum (D.C.) | Asteraceae | Rabi | 75.33 ± 2.11 | HA | 859土11.45 | Acaulospora, Glomus |
| | | Kharif | 56.25 ± 3.33 | HAV | 318±2.11 | Acaulospora |
| Celosia argentea (L.) | Amaranthaceae | Rabi | 65.62 ± 4.44 | HAV | 569土10.11 | Acaulospora, Glomus |
| | | Kharif | 66.66±2.11 | HAV | 647±14.11 | Acaulospora, Glomus |





Vesicles Hyphal colonization

Fig. 1: AMF root colonization in weedy plants from degraded land (400X) $_{\mbox{Scale}}$ bars =100 μm

Mycorrhizal Fungi (AMF) status. Percent AMF root colonization found more in rabi while optimum in kharif season. Vesicular, arbuscular and hyphal types of root colonization was recorded in both seasons. AM fungal spore density varied in all weedy plant species but rabi season is favorable for richness. Three dominant AM genera viz., *Acaulospora*, *Glomus* and *Scutellospora* were recorded in both the season.

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Previous studies have indicated that inoculation with AM fungi appeared to improve drought tolerance of host plants ¹⁸ and there is evidence to suggest the presence of mycorrhizal weed hosts maintains a diverse AMF population and promotes highly effective symbiosis with the crop plant. It was found that the AMF benefits to maize yield from maintaining a diverse weed cover crop outweighed any yield penalty due to competition ¹⁹. Indigenous AMF host weed species may

provide an effective bridge for AMF in between cropping periods²⁰. It was reported that with well-developed root systems, competitive weeds had a strong ability to acquire available N from soil and grew very fast, resulting in high plant biomass and plant nitrogen¹¹. The present studies enlist the prevalence of AM fungal colonization in amaranthaceae and isolate interesting AM fungal spores from twenty three plants²¹ and altogether thirty five indigenous AM fungal spores are recovered from this study and variation in the mycorrhizal colonization and spore number. Some studies have reported increased number of arbuscular mycorrhizal fungal spores when studied in association to large number of weeds species and enhanced positive effects of AM fungi on the growth and existence of *Vincetoxicum rossicum* species²². Weed species differed in the response to AM root

colonization, the highest AM root colonization was found for *Lactuca serriola*, *Picris echioides*, Plantago lanceolata and *Gallium aparine* and in addition, *Avena sterilis*, *Fumaria officinalis* and *Stellaria media* had the lowest AM root colonization²³. It was reported fourteen weed species and belonging to eight angiospermic families were studied for arbuscular mycorrhizal association, the infection was maximum on *Sonchu saspera* L. (81.2%), followed by *Cynodon dactylon* (70.1%), *Oxalis corniculata* (69.3%), *Malvastrum coromandelianum* (68.2%) and *Phalaris minor* (66.5%). However, *Ageratum conyzoides* L. (6.5%) and *Trifolium resupinatum* (7.3%) were poorly colonized²⁴.

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Weedy plants have the potential for the association of beneficial mycorrhizae for 'P' uptake. When mycorrhizal residing weeds are present in degraded land such lands it becomes fertile for the further cultivation of crop plants. Three dominant AM fungi genera were recovered in association of weedy plants from degraded land. Mycorrhizal infection when found more, it was positively intolerant for soil fertility. In present study, it isolated indigenous AMF spore and mass multiplied with restoring plant species and source of inoculums for further developed good experiments. Some weeds are helpful for reestablishing crop plants. Thus, weeds plant will benefit directly from the AM symbiosis through increased nutrient uptake and inevitably increased growth. Weeds are an important variable in crop productivity, economically, ecologically and also may serve to maintain diversity and agronomic beneficial species of AM fungi. It was find out the presence of weedy plants such as Dichanthium caricosum, Parthenium hysterophorus, Cynodon datylon, Dinebera retroflexa and Chrohrous capsularis in crop fields, it would be support for higher AM colonization and directly increased the biomass and yield of crop plants.

CONCLUSION

Study concluded that the weed species differed in the response to AM root colonization in both season in degraded land. While mycorrhizal symbiosis had no effects on the growth of non-competitive weeds, competitive weed growth was positively influenced by the present of the fungal symbiont. There were significant differences between the organic and conventional systems in the density and biomass of both non-competitive weeds and competitive weeds in both seasons.

SIGNIFICANCE STATEMENT

It was find out the presence of weedy plants such as *Dichanthium caricosum*, *Parthenium hysterophorus*, *Cynodon datylon*, *Dinebera retroflexa* and *Chrohrous capsularis* in crop fields, it would be support for higher AM colonization and directly increased the biomass and yield of crop plants.

When mycorrhizal residing weeds are present in degraded land such lands it becomes fertile for the further cultivation of crop plants. Three dominant AM fungi genera were recovered in association of weedy plants from degraded land. Some weeds when present in crop fields, it would be beneficial for enhancing productivity because it producing more AMF spore producing. Mycorrhizal infection when found more, it was positively intolerant for soil fertility. In present study, it isolated indigenous AMF spore and mass multiplied with restoring plant species and developed good source of inoculums for further experiments.

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