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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(2): 1160-1173. OPEN ACCESS

Studying the infection of oil yielding plants by Arbuscular Mycorrhiza Fungi in Jahangira District Nowshera, Pakistan

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The present work was done with the aim of generating baseline information on studying the septicity of oil yielding plants with AMF in different locations of Jahangira District Nowshera, Pakistan. During the study, total of 10 oil yielding plants were evaluated at different growth stages (i.e. vegetative, fruiting and flowering) for investigating the infections of oil yielding plants by AMF root colonization. It was observed that *Tagetes erecta* (96±10.7) belonging to family Asteraceae shows highest percentage of AM infection followed by *Jasminum mesnyi* (90±13.9) which is an important member of Oleaceae. Root segments of *Cestrum diurnum* belonging to Solanaceae family showed (86±4.03) arbuscular mycorrhizal fungi infection while *Mentha longifolia* showed less infection (84±8.1). Similarly root segments of *Coriandrum sativum* had (80±9.4), *Jasminum grandiflorum* had (72±10.6), *Mentha arvensis* had (70±9.8), *Mentha X piperita* had (70±5.5) AM infection. Root segments of *Jasminum sambac* (62±2.5) and *Ocimum basilicum* (34±4.06) showed less AM infection. Only two types of structures (External hyphae and vesicles) were observed in *M. longifolia* and *M. arvensis* each. *Mentha X piperita* shows three types of fungal diversity Viz External hyphae, vesicles and arbuscule while *Tagetes erecta* represented by vesicles, external and internal hyphae. Similarly, *Cestrum diurnum* showed all types of diversity i.e hyphae (external and internal), arbuscules and vesicles. *Coriandrum sativum* was observed and investigated the (internal hyphae & external hyphae) and vesicles followed by *Ocimum basilicum*.

Keywords: Arbusclues; AMF; Hyphae, Jahangira; Oil yielding Plants; Vesicles

INTRODUCTION

AMF (Arbuscular mycorrhizal fungi) are ranked in the phylum Glomeromycota, which is a monophyletic group (Stürmer, 2012). Arbuscular mycorrhiza (AM) is a common symbiotic relationship generally defined as the co-association between mycobiont and plants in which both benefit from the mutual exchange of nutrients (Yaseen et al. 2011). About 70 to 90% land plants make symbiosis association for their survival on the globe (Smith & Read, 2008; Krüger, 2011). One of the key features of symbiosis association is arbuscles, which are established in plant roots with fungal body which provides bidirectional nutrient transfer between the plants and fungal partner. Phosphorus contents provides fungal partner in symbiosis (Harrison & van Buuren, 1995), nitrogen (Govindarajulu et al. 2005) and other nutrients (Liu et al. 2000) to the host plants. In a large range of habitats, such as pasture, forestland, as well as many frustrating conditions, arbuscular mycorrhizal fungi (AMF) occurs. Although the conditions of these ecosystems are stressful, AMF is mostly present in the sodic, saline and acidic soils (Wang et al. 2004). The common form of mycorrhizal association is present in agricultural systems is the arbuscular mycorrhizas association and main plant families may form mycorrhizae (Barea et al. 1992; Barea et al. 1993; Azcón-Aguilar & Barea 1997, Chen et al. 2018). Several researchers have studied the spreading of these fungi in various fertile land soil and their relationships native plants (Udaiyan et al. 1996; Hildebrandt et al. 2001; Wang et al. 2004). Mycorrhiza can be present in soil of almost any sort. Mycorrhizas are capable of forming all but a few vascular plant species including (Brassicaceae, Amaranthaceae and Cyperaceae). The presence of fungal symbionts is strongly influenced by the physiology of the plant (Gianinazzi-Pearson, 1984; Azcón-Aguilar & Barea 1997, Bidartondo, 2005). The universality of this symbiosis clearly shows that the eco-taxonomic characteristics of the fungal part with the native plants which are very different. About 3% of the higher plants create ectomycorrhizal associations, mainly forest trees from most of the families such as (Pinaceae, Fagaceae and Betulaceae) and many other leguminous woody plants, while Endomycorrhiza may be confined to a few species in the Ericaceae and Orchidaceae and the arbuscular mycorrhiza, is a common type (Azcón-Aguilar & Barea 1997). Many of the presented evidence suggest that mycorrhizal interactions is essential elements of habitat range and can in complex ways alter the structure and effectiveness of the composition of plant communities. In their growth responses to AM fungal symbiosis, co-occurring plant species may differ significantly (Eom et al. 2000, Bidartondo et al. 2002; Selosse et al. 2017). The adverse effects of plant stresses associated with soil, deficient from nutrients, organic matter, elevated pH and high salinity are stated to be reduced by ecto-endo mycorrhizal associations Oliveira et al. 2005. The diversity and identification of arbuscular mycorrhiza infection in oil yeilding plants (Medicinal) is well established (Bakhtiar et al. 2012; Yaseen et al. 2016; Khan et al. 2019; Naz et al. 2019; Yaseen et al. 2018). VAM infection in *W. somnifera* and *W. coagulans* at fruting stage is well defined by Yaseen & Muhammad, (2011).

Many studies have focused on associations of arbuscular mycorrhizal fungi with crops of District Charsadda, Mardan and surrounding areas is well studied by Yaseen et al. 2017-18, while fungal infection in ethno-medico plants of Shabqadar documented by (Yaseen et al. 2020), describing arbuscular mycorrhizal fungal spore numbers and root identification of disturbed and unwanted plants (Weeds) of Aster family (Asteraceae) by Yaseen et al. 2018. Similarly, AMF inacolation application productivity and growth of *V. unguiculata* (Yaseen et al. 2011) and *Cicer arietinum* (Yaseen et al. 2012), *Ipomoea aquatica* (Halder et al. 2015), AM fungi and rhizobium inoculation in *Pisum sativum* by Dhillon & Bhople, 2015, Mycorrhiza effect on phosphorous uptake and yield of wheat crop by Hussain et al. 2016, Biological efficacy and *in vitro* refinement of (VAM) vesicular- Arbuscular mycorrhizal fungi is well described by (Bholay et al. 2018).

Oilseeds are among the significant cash crops of Pakistan. For plants, they are imperative as reserves of energy to maintain the germination of the seed; for human these are considered as proficient food sources of energy. Seed is the fundamental and essential contribution for effective yield generation, which holds the way to the ranch efficiency and benefit. The oil obtained is either used for culinary purposes or used for medicinal and cooking purposes. Recently oil yielding plants attract more attention due to an increasing demand for their vegetable oils, livestock feeds, pharmaceutical biofuels and other chemical industries (Nial and Mahalik, 2020). The diversity and performance studies of native oil yielding plants were studied based on comprehensive and intensive explorations and observations in Jahangira District Nowshera, Khyber Pakhtunkhwa. The goal of our current study was to examine the dispersal of AMF infection in various oil producing plants in Jahangira, Nowshera district, Pakistan.

MATERIALS AND METHODS

Study area

The study was conducted at 33° 42 to 34° 09 N latitude and 71° 97 to 72° 15 E longitude in Jahangra District Nowshera, Khyber-Pakhtunkhwa, Pakistan. It is situated on the banks of River Kabul (Khan et al. 2016).

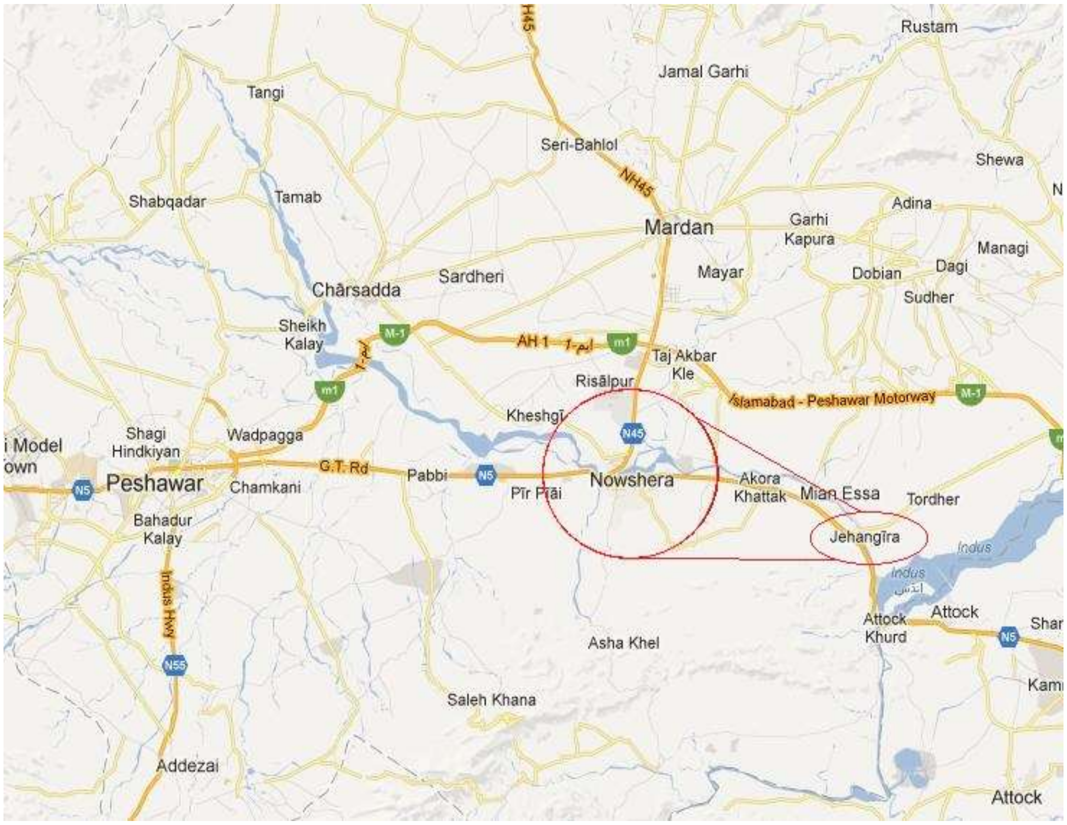


Figure 1: Map of the study site Jahangira, District Nowshera

Total population of the district is 1,518,540 and population of Jahangira is 353,490 (PBS, 2017). The area is bordered on the northern side by Mardan and Charsadda, on the east side by Peshawar District, on the northeast by Swabi District and on the south side by Kohat District while Attock is located to the South East (Figure 1). Climate of the district is warm and sub humid. The region receives maximum rainfall that is about 60% in the month of February followed by March and April. In summer the temperature goes up to 40°C while in winter the weather temperature dropped to 1°C (Ali, 2016; Zulfiqar et al. 2019).

Survey work

From the study area 10 samples of roots and soil each were collected from selected localities of Jahangira during 2019-20 in blooming seasons. Ten essential oil yielding plants Viz *Jasminum sambac*, *Jasminum mesnyi*, *Jasminum grandiflorum*, *Mentha longifolia*, *Mentha arvensis*, *Mentha X piperita*, *Coriandrum sativum*, *Ocimum basilicum*, *Tagetes erecta* and *Cestrum diurnum* belonging to different flowering families growing in Jehangira, district Nowshera, Pakistan. Plant samples distributed over five families categorized as Oleaceae (3 spp), Lamiaceae (4 spp), Apiaceae (1 spp), Asteraceae (1 spp) and Solanaceae (1 spp).The most important were Lamiaceae followed by Oleaceae, Apiaceae, Asteraceae and Solanaceae categorized by their respective species.

Soil collection and analysis

The soil sampling tubes have been used to collect soil samples up to a depth of 10-20 cm from each station. The samples were air dried, composite sample grinded, screened through 2 mm filter to remove the bits of stone and large root particles. Soil analysis and physiochemical characteristics were analyzed in the soil science laboratory of the Tarnab Agriculture Research Institute, Peshawar. Soil texture class, calcium carbonate content, OM (organic matter) content, N, P, K and pH were analyzed. Soil textures were analyzed through hydrometer (Koehler et al., 1984), pH meter were used for determination of pH by the method of Khan et al., 2017, for the assessment of soil organic matter, standardized $FeSO_4$ and $K_2Cr_2O_7$ solutions were used through the protocol of Nelson et al. 1996. AB-DTPA extractable N, P and K was analyzed in samples through method of Soltanpour, 1991 modified by Soltanpour, 2012.

Isolation and quantifications of AMF spores

The "Wet Sieving and Decanting Technique" described by Gerdemann & Nicolson, (1963) was used to remove the AM fungi spores.

Identifying AMF spores

The spores of AM fungi were identified, using the guidelines demonstrated by Morton and Benny, 1990 further modified by Kumar et al. 2009. The identification of these fungi was carried out following the Schenck and Perez Manual (1990) and was also contrasted with the reference species classification demonstrated by International culture collection of vesicular arbuscular mycorrhizal fungi (INVAM). Various characteristics of spores such as morphology, form, color and scale have been examined for the classification of AM fungi. Up to 50 spores were taken in a drop of lactic acid to analyze the size and form of the spore. The measurements of the spores have been calculated using micrometry.

Technique for cleaning and staining the root

A process recorded by Phillips & Hayman (1970) was used to clean and stain roots. Root samples were visualized using a compound microscope to track fungal structures such as vesicles, hyphae or arbuscules etc.

Analyzing the root colonization

Colonization and degree of AM fungi was demonstrated from the randomly selected root tissue of each plant analyzed using the frequency distribution method (Biermann and Lindermann, 1981). Percent root colonization for each plant was determined using the following equation.

$$\text{\% Root colonization} = \frac{\text{Total no. of colonized root segments}}{\text{Total no. of root segments investigated}} \times 100$$

RESULTS AND DISCUSSION

The current study was conducted in Jahangira, district Nowshera, KPK Pakistan. In assessment of the extensive use of crucial oils, fragrant chemical and other related materials, oil-producing plants have now been observed as a appreciated service and there has been a considerable rise in industrial turn over the last two decades (Burni et al. 2013). Owing to the rise in the values of chemical fertilizers and also with preventing soil contamination, mycologists are now trying to substitute for the use of organic fertilizers with bio-fertilizers such as AM fungi, which will be environmentally safe (Jalaluddin and Hamid, 2011). Therefore we are reporting ten essential oil yielding plants Viz *Jasminum sambac*, *Jasminum mesnyi*, *Jasminum grandiflorum*, *Mentha arvensis*, *Mentha X piperita*, *Mentha longifolia*, *Ocimum basilicum*, *Coriandrum sativum*, *Tagetes erecta* and *Cestrum diurnum* belonging to five flowering families categorized as Oleaceae (3 spp), Lamiaceae (4 spp), Apiaceae (1 spp), Asteraceae (1 spp) and Solanaceae (1 spp) in different flowering periods. Our results also agree with that of Yaseen et al. (2016) they also examined AMF spores types and its infection in some medicinal plants from 16 different families while Yaseen et al. (2018) also agree with our study and reported some weeds of family Asteraceae.The most important were Lamiaceae followed by Oleaceae, Apiaceae, Asteraceae and Solanaceae categorized by their respective species. Similarly our finding are agree with that of Burni et al. (2013) they also studied effect of AMF on essential oils of two pharmaceutically important Mentha species (*Mentha arvensis* and *Mentha longifolia*) belonging to family Lamiaceae in marginal soils. Burni & Hussain, (2011) also agree with our work and reported diversity of AMF morphology of some medicinal plants from family Lamiaceae. Root and soil sample were collected for illustration of arbuscular mycorrhiza fungi infection of oil yielding plants. The infection of AMF in medicinal plant roots has been widely studied by different workers (Huang et al. 2011; Yaseen et al. 2020) and agrees with our result. It was observed that *Tagetes erecta* (96±10.7) belonging to family Asteraceae shows highest percentage of AM infection followed by *Jasminum mesnyi* (90±13.9) which is an important member of Oleaceae as shown in Table 1, Figure 2. Similar, results were also made by Zaman et al. 2008 they also reported the same results for *Tagetes erecta* (80.1%). Our results are also agreed with Yaseen et al. (2018) they also reported highest AM infection of some members of Asteraceae. The results were presented on the bases on the occurrence of arbuscular vesicles. The quantity of establishment and concentration of roots colonization were compared among 40 different medicinal plants in which plants. 35 medicinal plants showed mycorrhizal infected roots by Zaman et al. (2008).

Table 1: List of plants showing their families and % roots colonization

S. No.	Plants Name	Family	Flowering period	%age of Root Colonization
1.	*Jasminum sambac* (Linn.) Ait.	Oleaceae	Warm season	62±2.5
2.	*Jasminum mesnyi* Hance.	Oleaceae	Feb-March	90±13.9
3.	*Jasminum grandiflorum* L.	Oleaceae	Warm season, rarely the whole year	72±10.6
4.	*Mentha arvensis* L.	Lamiaceae	July-Sep	70±9.8
5.	*Mentha longifolia* (L.) L.	Lamiaceae	May-Nov	84±8.1
6.	*Mentha X piperita* L.	Lamiaceae	Aug-Oct	70±5.5
7.	*Ocimum basilicum* L.	Lamiaceae	Throughout the year	34±4.06
8.	*Coriandrum sativum* L.	Apiaceae	From spring to Summer	80±9.4
9.	*Tagetes erecta* L.	Asteraceae	From spring to Summer	96±10.7
10.	*Cestrum diurnum* L.	Solanaceae	Jan-April, Aug-Oct	86±4.03

Table 2: List of plants showing different attributes of AMF

S. No.	Plants Name	Morphological diversity of AMF			
		Internal Hyphae	External Hyphae	Vesicles	Arbuscules
1.	*Jasminum sambac* (Linn.) Ait.	✓	✓	✓	x
2.	*Jasminum mesnyi* Hance.	✓	x	✓	✓
3.	*Jasminum grandiflorum* L.	✓	x	✓	✓
4.	*Mentha arvensis* L.	x	✓	✓	x
5.	*Mentha longifolia* (L.) L.	x	✓	✓	x
6.	*Mentha X piperita* L.	x	✓	✓	✓
7.	*Ocimum basilicum* L.	✓	✓	✓	x
8.	*Coriandrum sativum* L.	✓	✓	✓	x
9.	*Tagetes erecta* L.	✓	✓	✓	x
10.	*Cestrum diurnum* L.	✓	✓	✓	✓

Table 3: Summary of different spores

S. No	Diversity	No of Species	%
1.	Internal Hyphae	7	70%
2.	External Hyphae	8	80%
3.	Vesicles	10	100%
4.	Arbuscules	4	40%

Table 4: Physico-Chemical Analysis of Soil of Jahangira District Nowshera, KPK

S No.	Physicochemical analysis	
	Parameters	Values (%)
1.	pH	8.0
2.	Textural class	Loamy
3.	$CaCO_3$ (%)	8.0
4.	Organic matter (%)	0.69
5.	Total P (%)	7.4
6.	Total K (%)	2.00
7.	Total N (%)	0.034

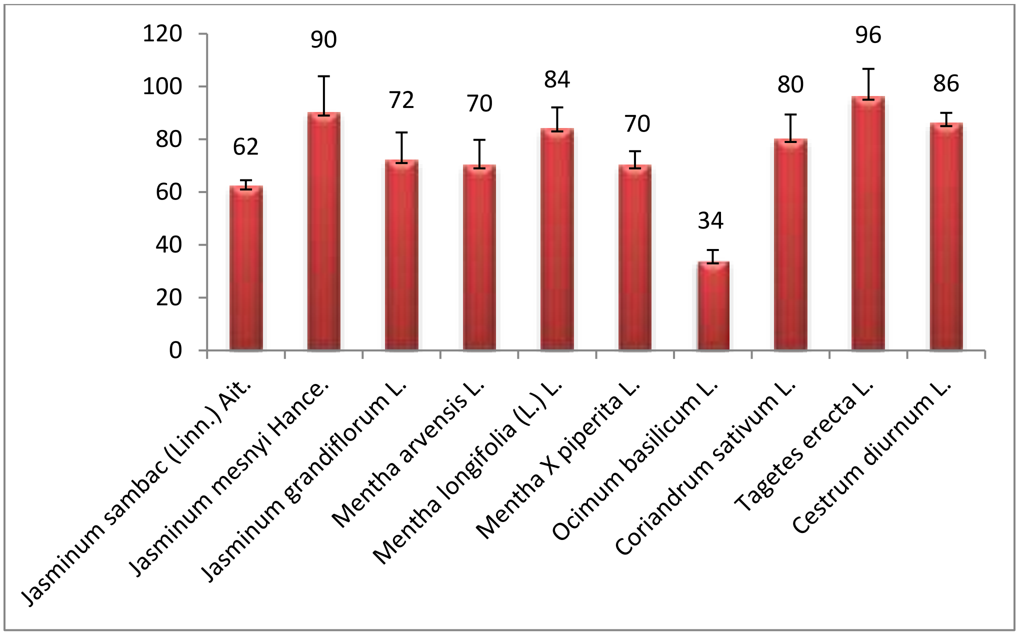
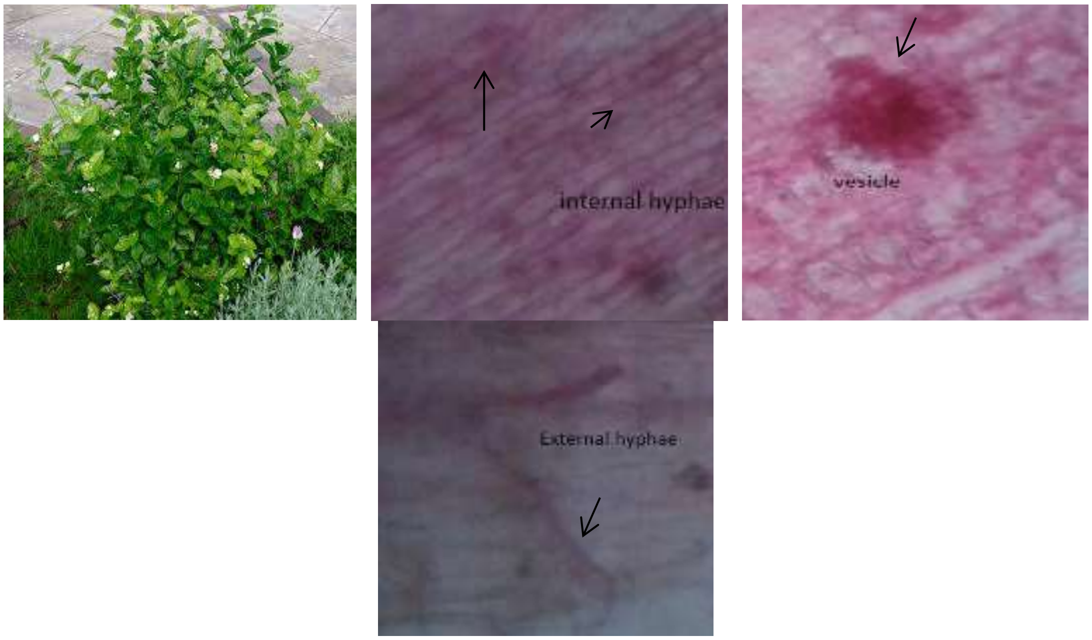
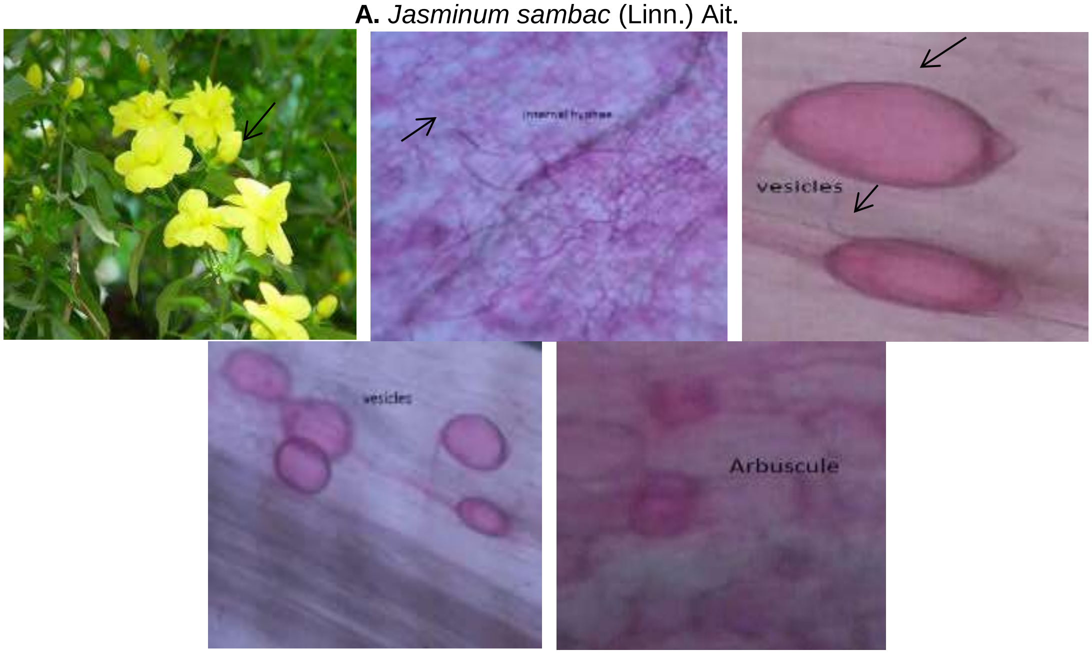


Figure 2: AM fungi detected in important oil yielding plants of Jahangira, district Mardan

Root segments of *Cestrum diurnum* belonging to Solanaceae family showed (86±4.03) arbuscular mycorrhizal fungi infection while *Mentha longifolia* showed less infection (84±8.1). Different workers are agree with our results but some of prominent are; Similarly root segments of *Coriandrum sativum* had (80±9.4), *Jasminum grandiflorum* had (72±10.6), *Mentha arvensis* had (70±9.8), *Mentha X piperita* had (70±5.5) AM infection. Root segments of *Jasminum sambac* (62±2.5) and *Ocimum basilicum* (34±4.06) showed less AM infection. Mycorrhizal association and arbuscules richness were well established by Copetta et al. 2006 in *Ocimum basilicum*. AMF colonization of *Coriandrum sativum* are well represented by Zeng et al. (2013) and Farahani et al. (2008). Spore density (223) and root colonization (98%) in 100 gm soil for *Mentha piperita* were also studied by Rajkumar et al. (2012) while % root colonization for *Mentha arvensis* (85%) reported by Zaman et al. 2008 and very less for *Mentha* sp. (30%) by Radhika & Rodrigues, (2010) which support our results. AM with arbuscules, which are the structural and practical criteria for symbiosis (Zubek et al. 2011) and are present in all plant species under investigation including Apiaceae, Asteraceae, Hypericaceae, Boraginaceae, Malvaceae, Linaceae, Lamiaceae, Oenotheraceae, Saxifragaceae, Solanaceae, Scrophulariaceae, Rutaceae, Ranunculaceae, Valerianaceae and Violaceae (Zubek & Błaszkowski, 2009). Internal hyphae and External hyphae, vesicles were detected in *Jasminum sambac*. (Figure 3-a) followed by Internal hyphae, vesicles and arbuscule in *Jasminum mesnyi* (Figure 3-b) & *Jasminum grandiflorum* as shown in (Figure 3-c). Only two types of structures (External hyphae and vesicles) were detected in *Mentha longifolia* (Figure 3-d) and *Mentha arvensis* (Figure 3-e) each. *Mentha X piperita* shows three types of fungal diversity Viz External hyphae, vesicles and arbuscules (Figure 3-f) while *Tagetes erecta* represented by internal hyphae, external hyphae and vesicles (Figure 3-g). Similarly *Cestrum diurnum* show all types of diversity Viz internal hyphae, external hyphae, vesicles and arbuscules (Figure 3-h). *Coriandrum sativum* observed in the occurrence of only internal hyphae and external hyphae, vesicles (Figure 3-i) followed by *Ocimum basilicum* (Figure 3-j). Arbuscular mycorhizal colonization was considered with arbuscules and/or vesicles and intra-radical hyphae. Vesicular and hyphal colonization was detected in sixteen species of plants, while four species of plants displayed arbuscular mycorrhizal colonization (Table 2 & 3). Vesicular, hyphal and arbuscular colonization was also investigated in 10 plant species by Radhika & Rodrigues, (2010) they also support our results. It was concluded that soil were mostly loamy with pH (0.8) and $CaCO_3$ (8.0%), OM (0.69%), total P (7.4%), K (2.00%) and N (0.034%) as shown in Table 4.



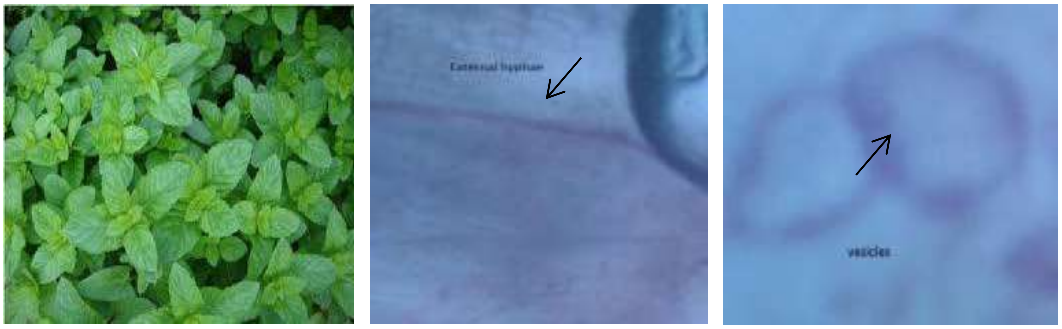
A. *Jasminum sambac* (Linn.) Ait.



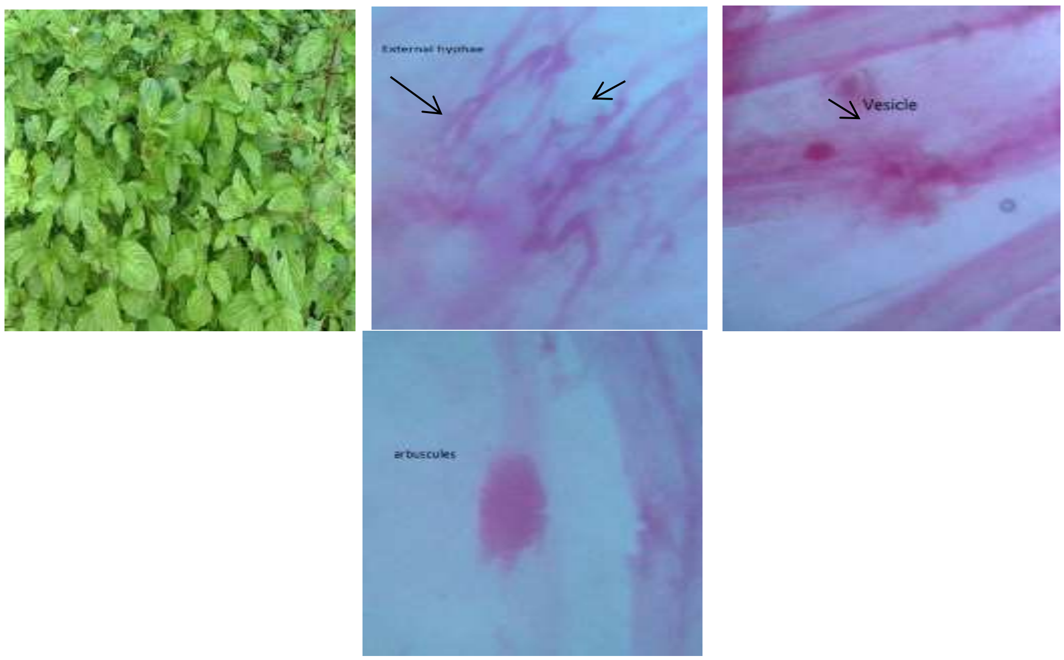
B. *Jasminum mesnyi* Hance.

C. *Jasminum grandiflorum* L.

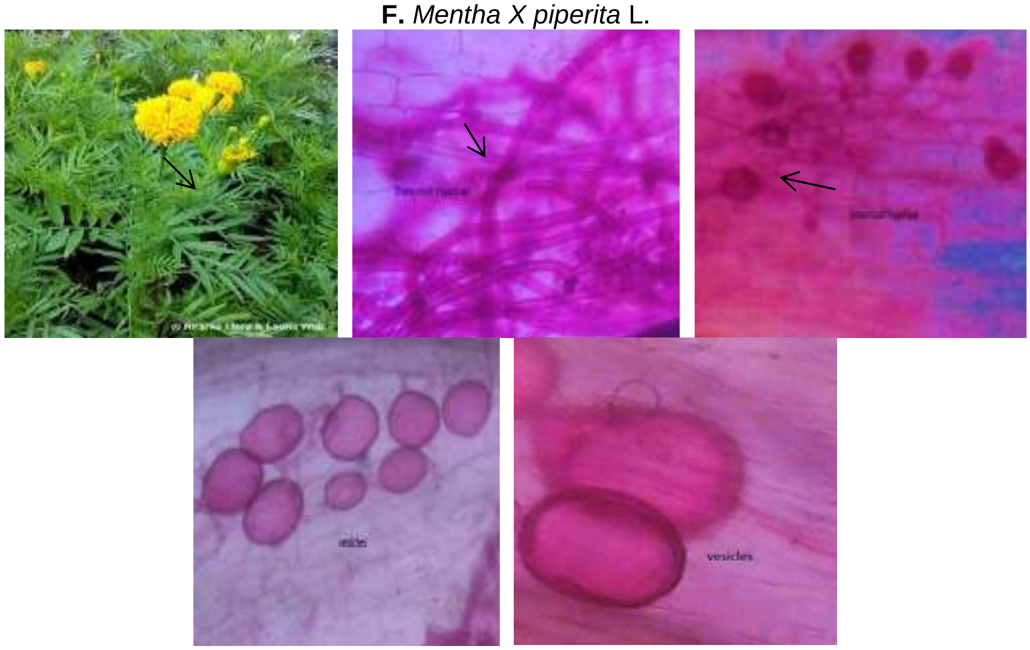
D. *Mentha longifolia* (L.) L.



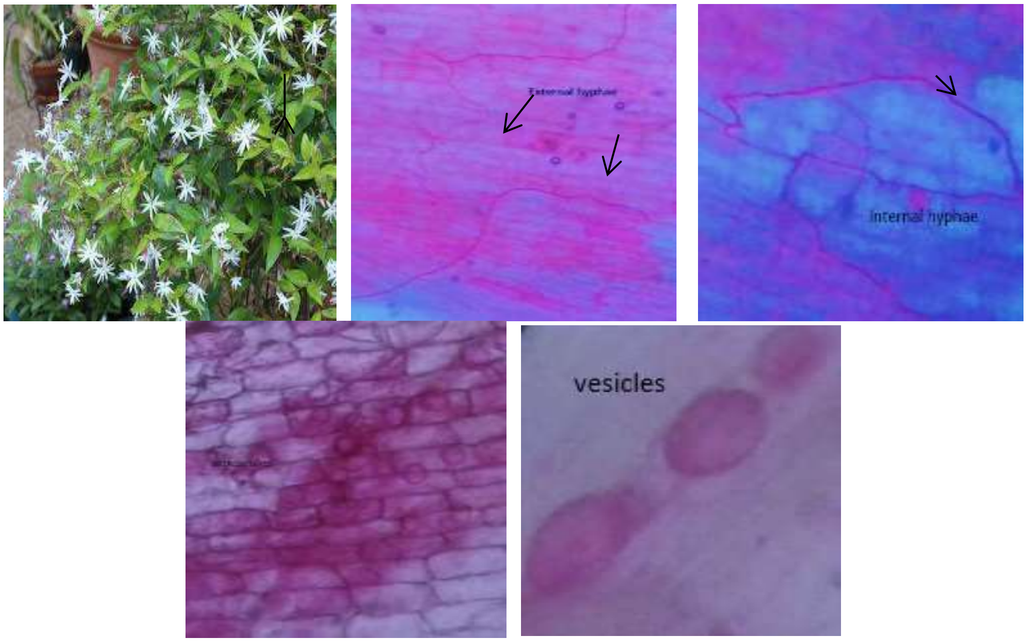
E. *Mentha arvensis* L.



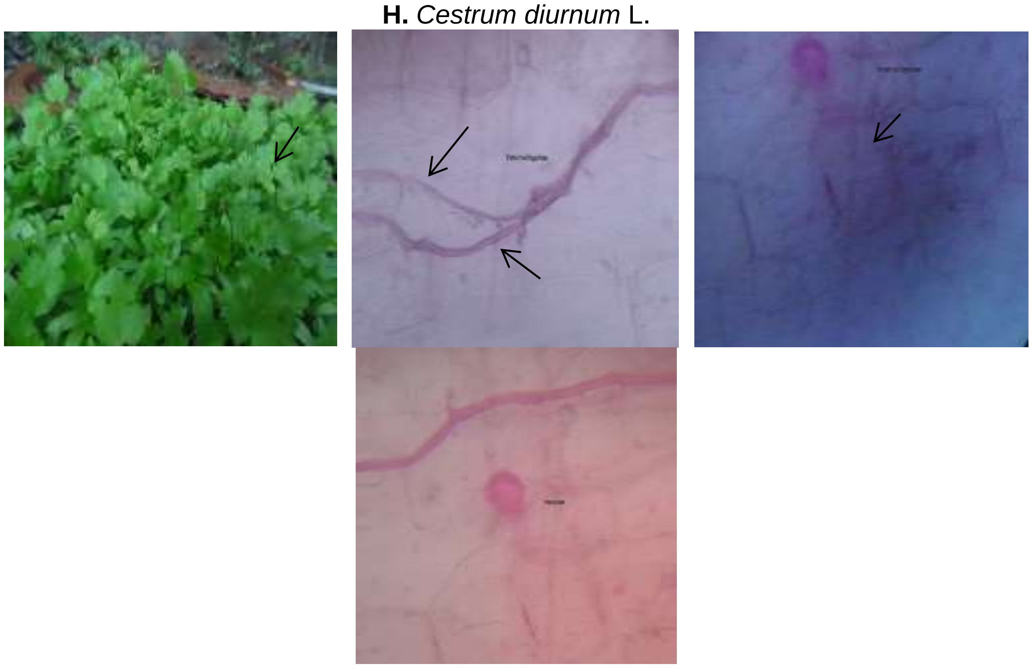
F. *Mentha X piperita* L.



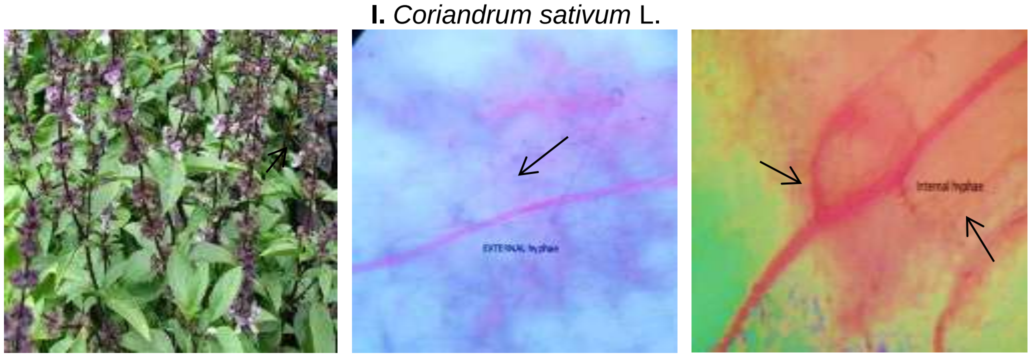
G. *Tagetes erecta* L.

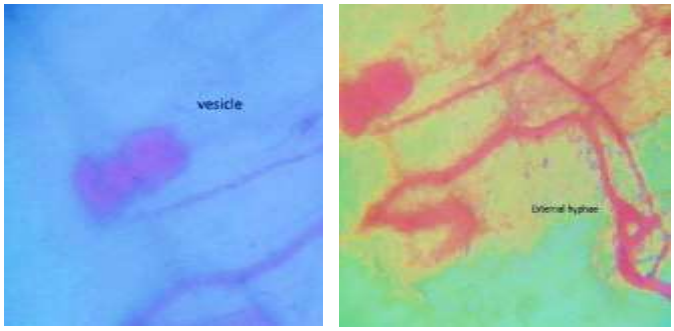


H. *Cestrum diurnum* L.



I. *Coriandrum sativum* L.





J. *Ocimum basilicum* L.

Figure 3: (A-J) Arbuscular Mycorrhizal Fungi (AMF) root colonization study of 10 oil yielding plants collected from Jahangira District Nowshera, KPK, Pakistan.

CONCLUSION

AMF effectively increased the growth of oil-producing plants, according to this report. Oil plant growth is influenced by soil mycorrhizal infectivity, and soil microorganisms' functional abilities are influenced by AM fungi inoculation. Inoculation of field crops with AM fungi has been shown to increase oil plants productivity while also improving soil bio-functioning. For better yields and sustainability, it's also worth considering having a comprehensive understanding of AMF ecology, a better fungal strain, and adaptability to environmental conditions.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The authors are thankful to Department of Botany, University of Peshawar for providing research facilities. Special thanks to Mr. Kamran Khan (Assistant Chemist, Tobacco Research Station Mardan) for soil analysis.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: AS and TB Performed the experiments: SS, HA and SMH .Analyzed the data: AZ and AA Contributed materials/ analysis: SW wrote the paper: MNK Proof Reading/Revision: TY and FAO

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