ARBUSCULAR MYCORRHIZAL FUNGAL INVESTIGATION IN THE WEEDS OF THE WHEAT FIELD OF TEHSIL TANGI, DISTRICT CHARSADDA, KHYBER PAKHTUNKHWA

Tabassum yaseen[1*], Muzamil shah[1], Khushnood Ur Rehman[2] Ali Mujtaba Shah[3], Gul Nawaz[1],Rani Gul[4]

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ABSTRACT

A study was conducted regarding arbuscular mycorrhizal fungi (AMF) spore density in weeds plants of soil selected from the Tehsil Tangi. The spore density was observed in all weeds plants in the host growth stage. The maximum arbuscular Mycorrhizal Fungi (AMF) spore density was found in the Chenopodium album (93.00±74.02) and Scandix iberica (89.00±92.08) at the vegetative stage. The Glomus species is dominant in the rhizospheric soil of weeds plants followed by Aculospora, and Sclerocystis. The lowest Glomus spore density is present in Fumaria parviflora (10.67±15.89). The spore density of AMF had a strong positive correlation with soil pH and a negative correlation with the Phosphors content of the soil. The highest vesicles are investigated Euphorbia heliscopia (14.33±9.5) and the lowest in Cirsium arvense *(3.33±1.53). Arbuscules were high in Euphorbia heliscopia (18.67±18.72) and lowest in* Cirsium arvense *(2.00±1.73). The highest External hyphae were found in Silene conidia (4.00±1.73) and lowest in Anagallis arvensis. Internal hyphae were in* Ranunculus muricatus *(6.33±5.13) and lowest in Melilotus indica (0.67±0.58). While absent* in *Brassica nigra* (Brassicaceae).

Key Words: Arbuscular mycorrhizal fungi, Charsadda, Spore density, and weeds.

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[1]Department. Of Botany Bacha Khan University Charsadda
[2]Department of Botany Islamia College Peshawar
[3]Department of Livestock Production Faculty of Animal Production & Technology Shaheed Benazir Bhutto University of Veterinary & Animal Sciences Sakrand-67210, Pakistan
[4]Department. Of Agricultural, Agronomy Bacha Khan University Charsadda

Corresponding author email: tabassumyaseen@bkuc.edu.pk

INTRODUCTION

Cultivated crops are inhabited by self-growing unwanted plants called weeds. When ancient men started growing selected plants for food and medicinal purposes, unwanted plants also started growing on themselves which were termed as weeds (Dangwall *et al.,* 2010). Normally all plant families have such unwanted plant species which grows on itself but a few selected families which mainly comprising *Fabaceae, Poaceae, Brassicaceae,* and *Asteraceae* contribute to the major weeds discovered till yet (Marwat *et al.,* 2009). The total number of weeds occurring throughout the world is 30000, but the most effective and main damage-causing species are about 50 to 200, which produce a catastrophic effect on major cultivated crops (Mahmood *et al.,* 1992). Similarly, 250 known weeds species were recognized to be usually present cosmopolitan in agricultural fields. The failure to engage the target crop production is mainly influenced by weeds, which imposes huge financial loss and main limiting factor to crops in premature growth stages, post-harvesting, and threshing problems (Noorka*, et al*., 2013). Some weed plants were utilized by men in ancient times for fiber and drug production has been termed as weeds now due to inter-conversion with plant species that have much better quality and quantity. The loss occurring in cultivated crops is mainly causing by weeds to acquire better environmental factors i.e. minerals, water, nutrients from soil, and light energy. Due to the above reasons, weeds are termed more costly than to agriculture crops then pathogens, pests, and insects. For eradication of weeds off the crops, the identity of the occurring weeds is of utmost importance before the application of any weedicide. Weeds were controlled by different methods from time to time i.e. it includes physical, chemical, and biological methods. As technology advances and hybrid crops took place, the removal of weeds got extra-ordinary importance (Marwat and Hashim, 2002). Invasive species affect the indigenous genera on a global level which is immense and irreversible. The quality and quantity of crops are lowered and production costs are increased by weeds as they directly compete with the cultivated crop for obtaining environmental facilities and production of allelochemicals which negatively affect the growth of said crop (Gupta, 2004). Forty-eight percent loss in yield of wheat (*Triticum aestivum* L.) is caused by broad-leaved and grass-like mix weeds (Khan and Haq 2002). The problem of weeds can be controlled by the application of chemical substances which proved to be effective in handling it (Kahramanoglu and Uygur, 2010). Due to its efficiency, almost 66% by volume pesticides used in the production of cultivated crops are herbicides. At the national level, the annual losses in wheat production exceed 28 billion rupees (Khan *et al.,*2001). The low yield of weeds in the country and throughout the world is characterized by the damages of weeds which decrease its yield by 37 up to 50 percent. The harvesting cost is increased and the quality of crops is decreased in size and nutrients, the waterways are clogged and escalate the fire hazards. For that reason, weed control is of most importance for getting optimum yield and quality grain of wheat. Regarding many crop problems in Pakistan, weeds are the no one limiting factor. Weeds compete for nutrients shelter sunlight and hence reduce crop quality (Hussain *et al.,* 2007). The weeds problem in Pakistan reduces the crops yield by18-30% (Ashiq and Cheema, 2005). While considering the loss in the world it reaches about 37-50% (Waheed *et al.,* 2009). *Avena, Chenopodium album, Coronopus didymus, Phalaris minor,* and *Rumex dentatus* L. are among the top weeds of Pakistan, which even cause the crop to cost more than pests and diseases (Siddiqui *et al.,* 2010). The symbiotic association of fungal hypha and roots of higher plants is known as mycorrhizae (Sadhana, 2014). AM fungi can enable harvests via increased uptake of nutrients making their vigorous against the Weeds species, as weeds species are non-mycorrhizal and are not benefitted by fungal association. So the

AM fungal association is a prospective alternative to herbicides which are not only costly but also damages the environment (Cameron, 2010). It is estimated that 25% of quality herbal and pharmaceutical products are dependent upon the medicinal plants as a raw material (Van and Wink, 2008). Though the economic loss is related to weeds, some studies show their positive impact, as the above-ground species sustain mycorrhizal communities in the soil, when used conservative practices (Kabir and Koide, 2000). The relationship between weeds and AMF still needs more considerations under organic supervision of fields. According to (Khan *et al.*, 2001) weeds the major competitors of crop plants regarding light, moisture, nutrients, and space.

MATERIAL AND METHOD

Site of survey work

Fifteen roots and soil samples were collected from a different area of Tehsil Tangi during the session (2017-2018). Plant samples belong to different families, In which *Brassicaceae, Poaceae, Papaveraceae, Apocynaceae, Fabaceae Euphorbiaece Ranunculaceae, Polygonacecs*, *Primulaceae, Chenopodiaceae, Asteraceae, Apiaceae* has shown in Table 1.

Table 1: Weeds species in the wheat field of Tehsil Tangi District Charsadda.

S.N	Weed collected	Families	Local name
1	*Fumaria parviflora*	Papaveraceae	Shahtara
2	*Melilotus indicus*	Fabacece	Lewane
3	*Ranunculus muricatus*	Ranunculaceae	Ohhpa
4	*Coronopus didymus*	Brassicaceae	Sqabote
5	**Camelina sativa**	Brassicaceae	Swat sag
6	*Euphorbia helioscopia*	Euphorbiaceae	Mandaro
7	*Rumax identatus*	Polygonaceae	Shallhe
8	**Anagallis arvensis**	Primulaceae	Mongote
9	*Chenopodium album*	Chenopodiaceae	Sarme
10	*Cirsium arvensis*	Asteraceae	Azghake
11	*Medicago denticulate*	Fabaeceae	Peshtara
12	*Scandix iberica*	Appiaceae	Gazare
13	*Silene conoidea*	Apocynaceae	Kargamewa
14	*Poa annua*	Poaceae	Jodare
15	*Brassica nigra*	Brassicaceae	Sharsham

Collection of plant and soil sample

The plants were carefully dug up along with there rhizospheric soil in triplicate and transported to the laboratory in polythene bags. The plant roots were gently washed under tap water to remove soil particles, and the rhizospheric soil was dry by shade condition the roots were fixed in formalin acetic acid (F.A.A) by 10:90 by volume.

Assessment of root colonization

The + slide method of (Giovannetti and Mosse,1980) was followed for the current study. Fifty root segments of the individual plant have randomly selected all samples of root were cut into pieces about 1 cm long for microscopic study. Morphology of Vesicular Arbuscular mycorrhizal entophyte was contemplated and count in percentage (%). The given formula was used for the calculation of infection in percentage:

Percentage (%) mycorrhizal infection = $\frac{\text{No of the infected segment}}{\text{Total No. Segments}} \times 100$

Extraction of Spores

Rhizospheric soil samples of tomato plants were collected at the vegetative stage. 50gm of rhizospheric soil sample was taken from each field. Wet sieving and decanting method' described by (Gerdemann and Nicolson, 1963) were followed for the isolation of spores for which 50 gm soil was homogenized with 100 ml of water mixing in a beaker. The beaker was left for 24 hours and when the soil particles settled down. Soil mixture solution passed through a 2-mm sieve to screen out large debris and wash the residue several times. The small amount of the residue was put into Petri dishes for studying the spores. The suspension of soil was enraged and passed it through the different number of sieves 140, 170, 400-µm size successively. For the determination of soil texture, the number of sieves can be decreased or increased for the purpose of extraction (Dalpe, 1993). The remaining residue was examined under a microscopic study which was collected from each sieve on the filter papers in Petri dishes.

Identification of spores

The spore's photo was captured through different magnification (4x and 10x) and was followed by the procedure of (Schenck and Perez, 1990) for the identification of spores.

Physiochemical Analysis of soil

The method of was followed for the collection of Soil samples from a different area of District Charsadda. Different soil samples of 50gm of rhizospheric soil were taken from the field and dissolved into 100ml water and cover the beaker for 24 hours. When dry then Soil samples before processing for analysis (Jalaluddin and Anwar,1991).

In the current study, the hydrometer method of (Ghee and Bauder,1986) was followed for the determination of soil texture. The mean value of all samples, Clay were recorded (21.59±15.14), Silt (36.05±5.31), and sand (42.50±15.79) respectively. The organic matter of the study area (1.27±0.20) was recorded and followed by Walkley- Black method (Nelson and Sommers, 1982). Electrical conductivity was observed during the present study of Tangi (0.41±0.12) of the saturated extract was determined by Solubridge conductivity meter as given by (Black 1965). Soil pH of Tangi was (6.14±0.65) of saturated paste determined by pH meter as recommended by (Jackson, 1967).

Results and Discussion

During the present study, about fifteen weeds species belonging to eleven families and location Tehsil Tangi showed a mycorrhizal association.

Spore Density

The plants showed from least possible to maximum colonization. Three types of AMF spores (*Glomus, Sclerocystis,* and *Acaulospora*) were isolated from the rhizospheric soil of both the plants studied. *Glomus* species investigated in different families of weeds Tehsil Tangi District Charsadda shown in Fig1.In which the highest glomus was investigated in *Chenopodium album*

(*Chenpodacece*), *Scandixiberica* (*Apiaceae*) *Euphorbia heliscopia* (*Euphorbiacea*e), and the lowest range was recorded in *Poaannua* (*Poaceae*) and *Fumaria pureflora* (*Papareracec*e). The predominance of *glomus* species under varying soil conditions might be because they are widely adapted to the varied soil condition and survive in acidic as well as in alkaline soils. Maximum diversity of AM species was reported in *Chenopodium album.* The *Glomus* was found more abundant followed by *Sclerocystis* and *Acaulospora* species our result similar to (Yaseen *et al*., 2018) who investigated that Glomus the most frequent specie among all types of spores studied. Glomus showed the highest adaptive value with both the plants. The maximum collective percentage of all types' spores was recorded as 36.18% and 35.43% respectively from the rhizospheric soil of both C. *Canadensis* and *P. hysterophorus* at location 2, which was Umarzai. About 38.98% and 48.24% of Glomus spores were isolated from the rhizosphere of *C. canadensis* and P. *hysterophorus* respectively, which evidenced that the Glomus was the most frequent among all the collected spores. The high spore density is present in *Chenopodium album* (93.00±74.02) while this plant-soil is a minimum amount of Phosphorus and nitrogen and the lowest spore density and the high level of Phosphorus are present Different soil nutrients like P and N has negative effects of the AMF. Our result agrees (Muthukumar *et al*. 2006) who reported that the 107 Medicinal and aromatic plants belonging to 98 genera in 52 families examined, 79 were AM, and 38 harbored a dark septate endophytes (DSE) association. Typical Arum- and Paris-type mycorrhizas are first reported in the presumed non mycorrhizal family Amaranthaceous Similarly, DSE associations were recorded for the first time in 9 plant families and 37 plant species. The results showed that inoculation with mycorrhizal fungi increases nitrogen and phosphorus content by the plant. The highest amounts of N and P content were the *Glomus* species are less number. This showed that some AMF species had better efficiency than others did in nutrient content (Lisette *et al*., 2003). *Acalospora* species investigated in a different family of weed Tehsil Tangi District Charsadda shown in Fig 2. In which the highest *Acalospora* was investigated in *Silene conidia* (Apocynaece), *Circium arevensis* (Asteraceae), and the lowest was recorded in *Poa annua* (Poaceae) and (*Fumaria pureflora* (*Papareraecec*) who described that the morphology of AM type is the result of interaction between both the plant and fungal species. It is, therefore, essential to examine a wider range of plants growing in different habitats to understand that variation in the morphology of AMF. Our results agree with (Kubato *et al*., 2005). The high *Acluspora* are investigated in *Silene conidia* (64±81.36) and this plant phosphate and N the inoculation significantly increased the total phosphorus content in plant tissue, acid phosphatase activity, and percentage of root colonization our result with agreeing (Moreira *et al*. 2006). *Sclerocystis* species investigated in a different family of weeds Tehsil Tangi District Charsadda shown in Fig 3. In which the highest *Sclerocystis* was investigated in *Medicago denticulate* (*Legumanaece*), *Melilotus indicus* (Fabaceae), and the lowest was recorded in *Silene conidia* (*Apocynaece*), *Brassica nigra* (*Brassicacec*), while absent in *Scandixiberica* (Apiaceae), and *Rumax identatus* (Polygonaceae). Our result agrees with (Guo *et al.,* 2006). The highest number is *sclerosis* is recorded in *Medicago denticulate* (5.00±8.66), Phosphate, and nitrogen. Total Nitrogen increment is due to an increase in Nitrogen fixation and high P application, these can be incremented with the help of AMF instead of soil Nitrogen addition (Mortimer *et al.,* 2008). The mycorrhizae have the beneficial effect on the phosphate nutrition of crop plants in soil low in phosphorous has been quoted (Chen et al., 2005) The mycorrhizal abundance in the soil can be attributed to the availability or otherwise of its nutrient content which is attributed to the amount of Nitrogen, and Phosphorus and Potassium among others. The reported that AMF colonization is influenced by the mineral salt level in the soil (Hopper,1983).

AMF root Infection

Fifteen samples of roots and soil were collected weeds from wheat field of Tehsil Tangi. These belong to 11 different families. Vesicle species investigated in a different family of weeds Tehsil Tangi District Charsadda shown (Fig 1) in which highest vesicles species investigated in *Euphorbia heliscopia* (Euphorbacece) and the lowest is recorded *Circium arevensis*(*Asteraceae*) and *Chenopodium album* (Chenpodacece) and While totally absent in *Brassica nigra* (*Brasicaece*) presence of high vesicular infection can be related to the observations of (Iqbal *et al.,*1988) who found high vesicular infection weeds plants. In general, the mycorrhizal infection increased with the age of the host plant. Vesicles were in-group forms. They were of different shapes and are thin-walled. *Brassica nigra* (*Brassicaceae*) which have no any mycorrhizal infection. These results conform to the finding of (Yaseen et al., 2017) who investigated that external and internal hyphae having the highest value as compare to vesicles and arbuscules. In the present investigation studied plants showed, the percentage of vesicular infection was reasonably high at the flowering stage reaching to a maximum at the fruiting stage in both varieties. These results agree with (Iqbal and Bareen ,1991). The high vesicles are present in *Euphorbia heliscopia*(14.33±9.50). In the field, we observed a higher incidence of hyphae in the moisture and a higher incidence of vesicles in the dry condition in the roots. Agrees with the general pattern of root colonization reported in other vegetation types. In general, arbuscules production tends to increase during the flowering period when the demand for soil nutrients increases, whereas the incidence vesicles tend to be more frequent during the post-flowering period (Allen *et al*., 1998). Arbuscules investigated in different species of a different family of weeds Tehsil Tangi District Charsadda shown (Fig 2) in which the highest abuscules was present in *Euphorbia heliscopia* (Euphorbacec), *Scandix iberica* (Apiaceae), and *Coronopus didymus* (Brassicaceae) and the lowest is recorded in *Chenopodium album* (Chenpodacece) and *Cirsium arvensis* (Asteraceae)While absent in *Camelina sativa* (Brassicaceae). The highest arbuscular mycorrhizae (AMF) colonization rate was recorded among the root samples collected from topsoil. The lowest colonization was found in the roots of (*Astreacece*) while the arbuscules were intracellular and formed from the hype. Similar observations were also recorded by (Yaseen *et al*., 2011). External hype species investigated a different family of weeds Tehsil Tangi District Charsadda shown in Fig 3. In which the highest External hype was present in *Silene conidia* (Apocynaece) and the lowest is recorded in *Chenopodium album* (*Chenpodacec*e) and *Anagallis arvensis* (Primulaceae) while absent in *Euphorbia heliscopia* (*Euphorbacec*e) and *Melilotus indicus* (Fabaceae). This may be due to the fungal toxic present in root tissue that reduces the mycorrhizal association. Our result is agreed by Fuchs and (Hasel wandter, 2004). Internal hype species investigated in a different family of weeds Tehsil Tangi District Charsadda shown Fig (4) in which the highest internal hype was present in *Ranunculus muricatus* (Ranunculacec) and *Fumaria parviflora* (Papareracece) and the lowest range is recorded in and *Chenopodium album* (*Chenpodacece*) while absent in *Cirsium arvensis* (Asteraceae)*, Poa annua* (Poaceae) and *Medicago denticulate* (Legumanaece) That is generally accepted as conventionally AMF less host *Camelina sativa* (Brassicaceae) which have no any mycorrhizal infection. Our results agree with the result of (Yaseen *et al*., 2017) Who reported that external hyphae having dominant value followed by internal hyphae and arbuscules and vesicles having the least value in wheat at both the vegetative and fruiting stage.

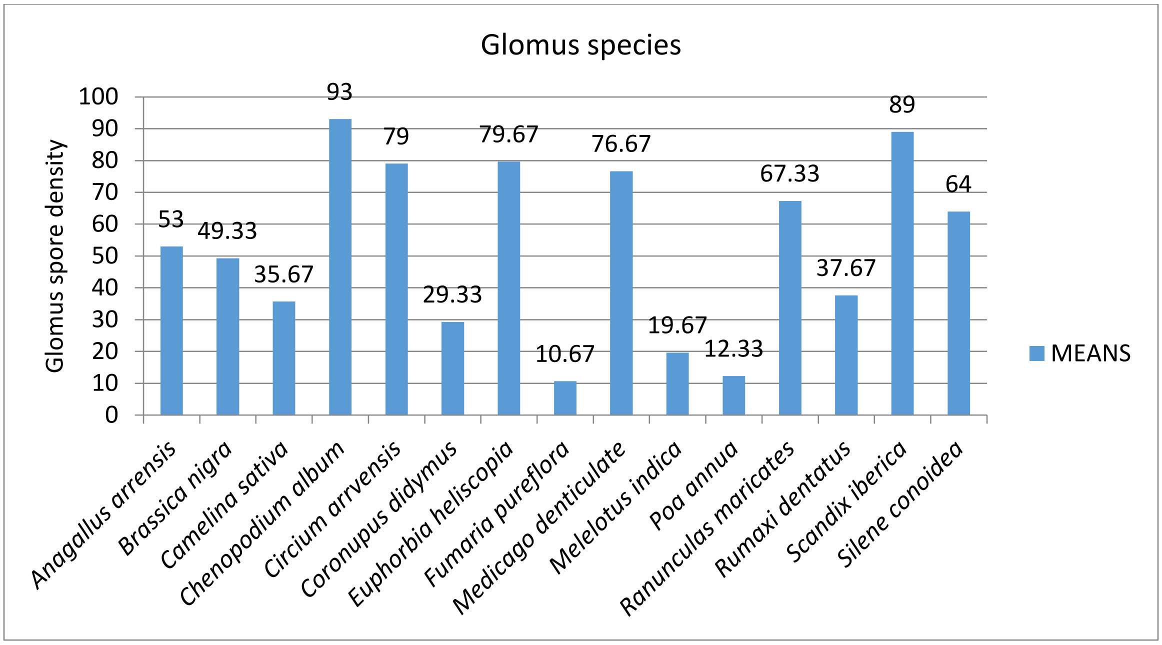

Fig 1: Investigation of *Glomus* species in the field of wheat of Tehsil Tangi District Charsadda.

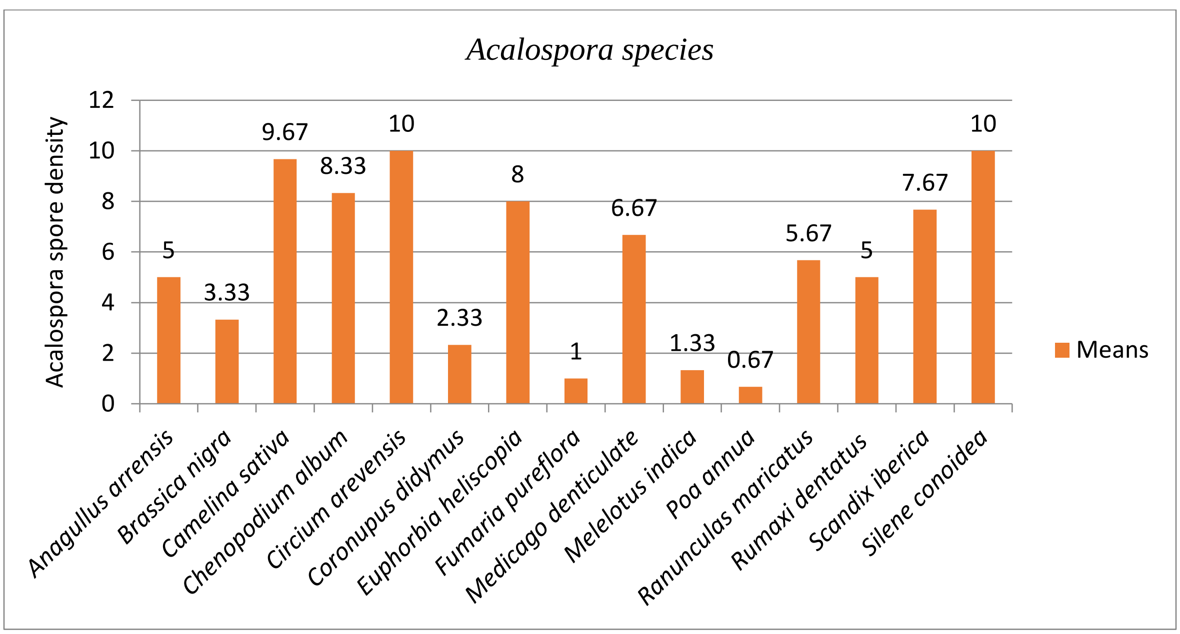

Fig 2: Investigation of Acalospora species in the field of wheat of Tehsil Tangi District Charsadda.

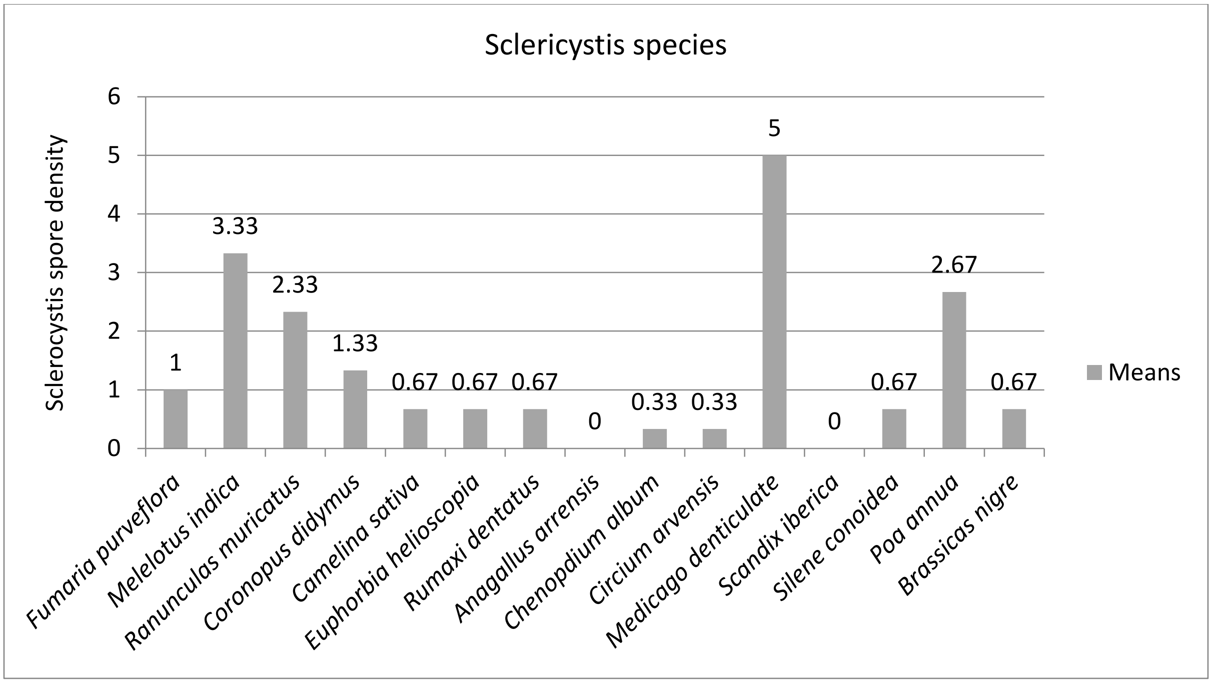

Fig 3: Investigation of *Sclerocystis* species in the field of wheat of Tehsil Tangi District Charsadda.

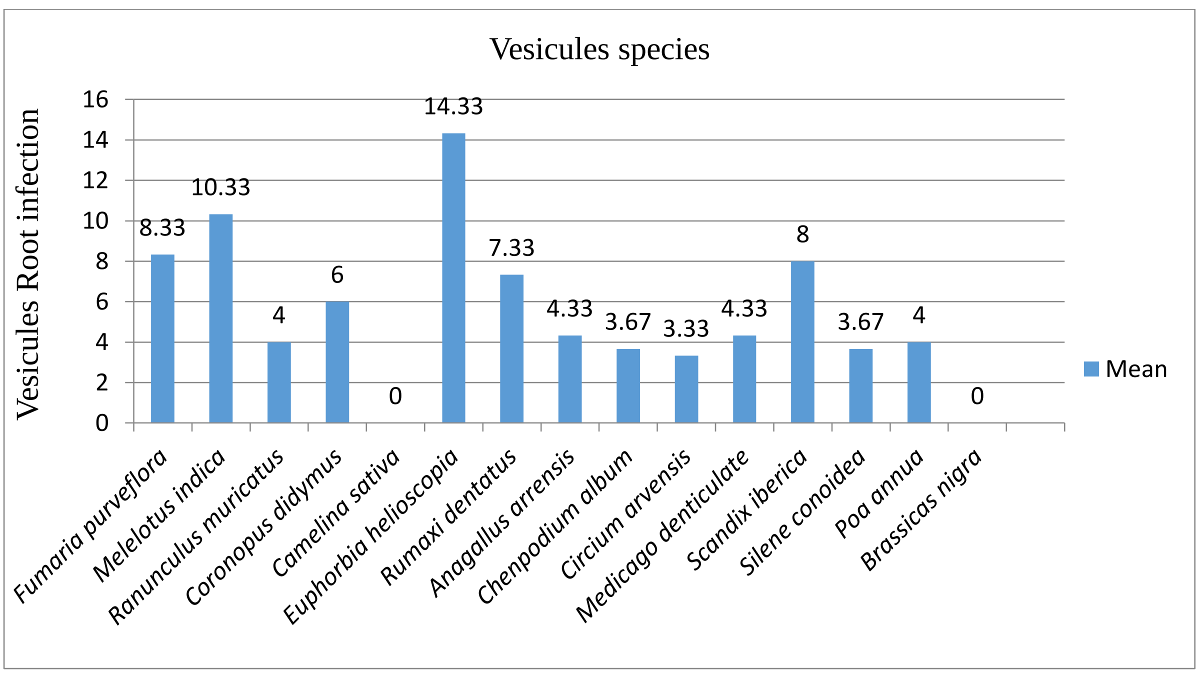

Fig 1: Investigation of Vesicle species in the field of wheat of Tehsil Tangi District Charsadda.

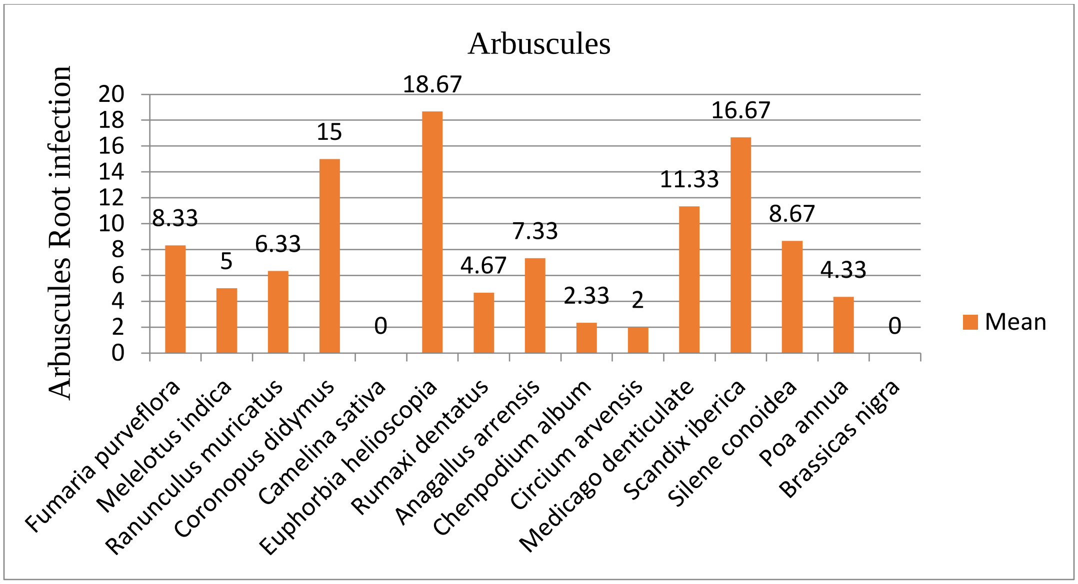

Fig 2: Investigation of Arbuscules species in the field of wheat of Tehsil Tangi District Charsadda.

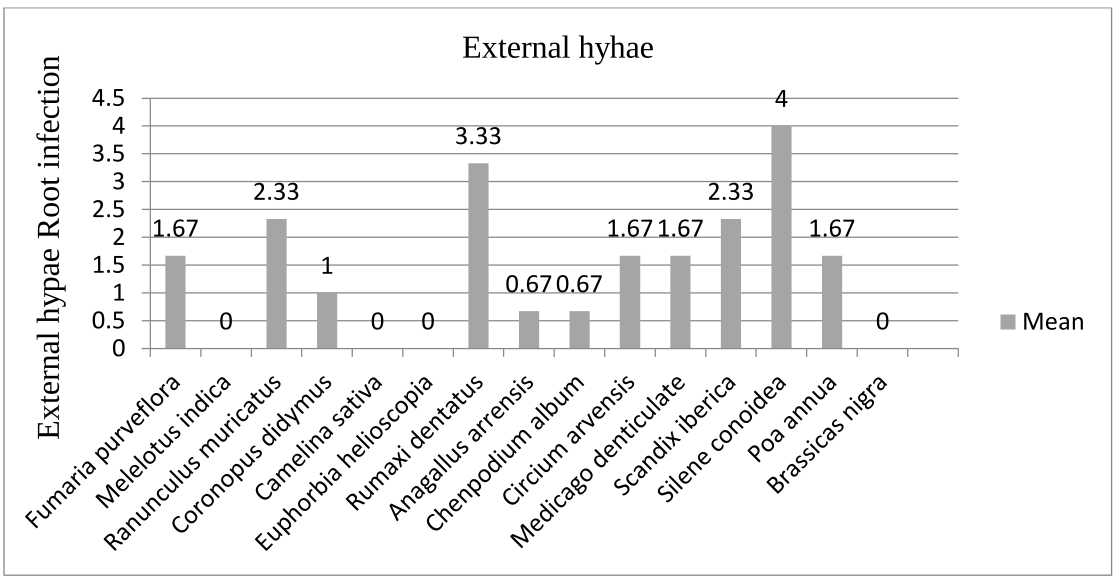

Fig. 3: Investigation of External hype species in the field of wheat of Tehsil Tangi District Charsadda.

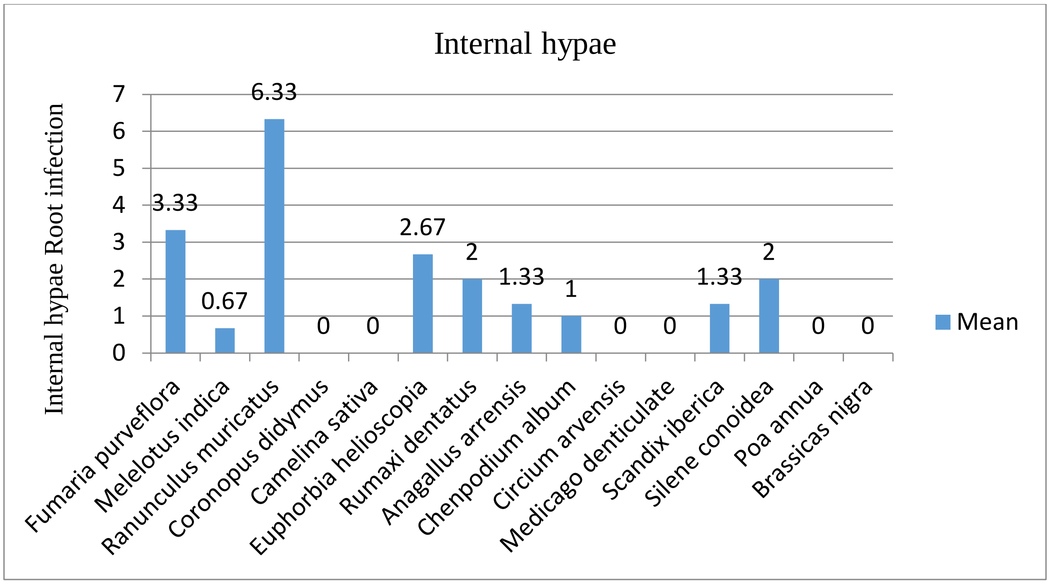

Fig. 4: Investigation of Internal hype species in the field of wheat of Tehsil Tangi District Charsadda.

Fig.5. Spores sp.

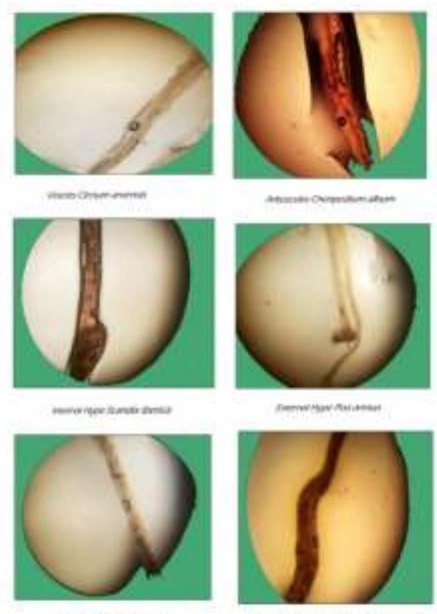

Fig.6. Root colonization

CONCLUSION

Result of the present study showed that the occurrence of arbuscular mycorrhizal fungi varies with host ranges as well physiochemical of soil though they are ubiquitous, they showed specificity in association with host plants. All the AMF species occur in the studied areas and not all the species had the same effect on their symbiont. Different species and different isolates within species can have different effects on one growth. During the present investigation, a total number of 15 weeds plants from the wheat field of Tehsil Tangi, District Charsadda were studied. From the result of the present study, it can be concluded that there is a high incidence of arbuscular mycorrhizal fungi (AMF) associations in weeds plants in the study area. All the weeds plants of selected locations studied were colonized by different AM fungi species. Since a large number of weeds plants are present in different locations of Tehil Tangi, extensive research work is required to create a database of mycorrhizal species colonizing these weeds plants and to determine their efficiency in promoting. It is concluded that there was a high spore density in the wheat field. This also showed that AMF sporulation is seasonal and dependent on host plant species, pluvial precipitation, soil moisture content, and soil chemistry. The study also demonstrated the influence of different plant densities on the diversity of arbuscular mycorrhizal fungi. As to the dependency of AMF on soil parameter. Glomus was dominant in this study. Further long time studies are necessary to elucidate the ecological role of AM fungi in Tehsil Tangi.

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