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INVESTIGATION OF ARBUSCULAR MYCORRHIZAL FUNGI FROM WEEDS PLANTS GROWN IN *TRITICUM AESTIVUM* FIELDS IN DISTRICT CHARSADDA, PAKISTAN

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A B S T R A C T

Arbuscular mycorrhizal (AM) symbiosis is a reciprocal association between fungi and more than 80% of terrestrial plants, and the association play an important role in plant growth and development. Arbuscular mycorrhizal fungi (AMF) colonization have been explored in various crops such as wheat and maize, however, AMF colonization in weeds has been poorly investigated. In order to explore the AMF colonization in weeds grown in wheat fields, a study has been conducted in which we identified first the weeds species and then AMF spores and colonization in wheat (*Triticum aestivum* L.) fields at four localities viz Ghazgai, Palosa, Sardeheri and Mangha dargi in District Charsadda, Khyber Pakhtunkhwa, Pakistan. Fifteen species belonging to 12 families were found distributed as wheat's weeds in the study areas. The most important families in terms of species representation were Asteraceae (3 genera and 3 species) followed by *Fabaceae* (1 genus and 2 species) *Euphorbiaceae, Cryophyllaceae, Leguminaceae, Papaveraceae, Poaceae, Brassicaceae, Cannabinaceae, Chenopodiaceae, Convolvulaceae* and *Polygonaceae*. The increase of AMF root colonization was high in Mangha dargi (25-119 %) followed by Ghazgai (40-90%), Sardeheri (34-80%) and Palosa (24-80%). Moreover, Great AMF (TSD) per hundred gm-1 of soil abundance were recorded in Ghazgai (65-131 gm-1) followed by Mangha dargi (40-119 gm-1), Palosa (46-115 gm-1) and lowest was in Sardheri (40-107 gm-1). The present investigation highlights the AMF colonization, identification, classification and documentation of weeds in different localities of district Charsadda, Pakistan.

Keywords: Arbuscular mycorrhizal fungi, *Titicum aestivum,* Weeds, District Charsadda

INTRODUCTION

Arbuscular mycorrhiza (AM) is symbiotic association between Arbuscular mycorrhizal fungi (AMF) and plant roots, which provides and shared advantages to both partners in terms of nutrients. Approximately, 85-90% of the land plants can be colonized by AM fungi as the remaining are weeds (Jordan *et al*., 2000) which are not relying on AMF for nutrient uptake presumably due to deep and extensive rooting system (Wang and Qiu, 2006; Brundrett, 2009). Although AMF has been used for weeds control (Cameron, 2010; Jordan *et al*., 2000), but the exact mechanism how they interact with weeds is not known. There are reports that AMF can change the structure and nature of weeds. It was further documented that these fungi are not always detrimental to weeds plants but minimize the crop yield loss arising

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from weeds plants and reduces the negative effects of weeds on crop plants (Jordan *et al*., 2000). AM Fungi have been reported to play a role in ecological relationship of weeds as they can affect the weeds growth without influencing the crop plants growth (Jordan *et al*., 2000). However, the colonization and spore density of AMF is still not well explored.

Weeds are plants grown out of place or unintentionally which compete with our major crops for nutrients, light and other requirements. The yield loss due to weeds has been estimated to be 34% every year (Oerke, 2006) and in some cases even 45- 95% (Ampong and De Datta, 1991) despite using control measures which is alarming. About 200 weeds have been reported to develop resistance against various herbicides due to excessive application of herbicides in our field crops. Herbicides are not supposed to be applied for weeds control in organic farming, which is a sort of farming without applying any chemical, and mostly employ cultural control methods for weeds control. The organic farming is aimed to avoid harming of natural environment by exploiting the traditional or cultural control methods (Basker, 2009).

The colonization of crop plants by AMF may be adversely affected in case of mechanical control of weeds possibly due to hyphal damages which has not been observed in case of herbicides application (Brito *et al*., 2013). In another control method termed as bio control living organism are being used for weeds control mostly as foliar pathogens. The idea of using living microbes for weeds control is fascinating but it should have been included soil microorganisms like rhizo-bacteria and AMF etc. having the potential for weeds control directly or indirectly (Boyetchko, 1996). Apart from this, tissue culture based approaches were used for observing bacteria, which is considered as one of the bio control method of weeds (Souissi *et al*., 1994).

Here, we aimed to identify the weeds species in *Titicum aestivum* field, and then we further explored the AMF spore density and colonization in weeds. The objects of this work were: (1) to explore the weeds species abundance in *Titicum aestivum* field; and (2) to uncover the AMF colonization in weeds in different localities of district charsadda, Pakistan.

MATERIALS AND METHODS

Study Site: Charsadda average temperature is about 22.5°C. The average rainfall in this part of Khyber Pakhtunkhwa has been reported to be 460 mm. The month June is normally the driest and warmest one with mean temperature of 33.3°C receiving approximately 11mm rainfall (Anonymous, 1998). The lowest mean temperature has been reported in January, which is about 10.4 °C. The land of this region is fertile and used for growing agriculturally important crops like Wheat, maize, tobacco, Sugarcane and Sugar beet etc.

Research sites: The study area was designed into four localities:

1. Ghazgai, 2. Palosa, 3. Sardeheri, s4. Mangha dargi

These localities were selected for field analysis and collection of weeds.

Collection of Weeds: Weeds samples were collected from four different area (Ghazgai, Palosa, Sardeheri and Mangha dargi) of study considering, habitat, life form, phonological status, and abundance. Collections of weed samples were based on random sampling during different time periods of wheat growth.

Preservation: After collection of all specimens were properly shady dried under room temperature in open space. The dried specimens were than mounted and preserved on herbarium sheet (Judd, 2002).

Identification of weeds: The total collected specimens were then subjected to identification. Weed samples were identified with the help of flora of Pakistan (Nasir *et al.,* 1972; Nasir *et al*., 1995).

Soil and Root Sampling of weeds species: The Survey was undertaken at 4 different sites located in Charsadda city. In each Location three cultivated fields was selected. In each field Root and soil samples of weeds plants were collected randomly. From each site, 3-4 healthy wheat plants were collected along with rhizospheric soil and roots at different stages (vegetative -fruiting stages). Roots and rhizosphere soil was dug out with a trowel at a depth of 0-15 cm after scrapping away the top 1 cm layer of soil. Samples were collected randomly from different site in each field, pooled and homogenized. Soil samples along with secondary and tertiary roots of three individual fields for fifteen weeds plants were collected during course of investigation from 2015. Rhizospheric soils (about 100 gm) were air dried for 2-3 weeks, and then stored in sealed plastic bags at room temperature.

Identification of AMF spores: The identification of these fungi was done by following the manual of (Schenck & Perez, 1990) and was also compared with reference species description demonstrated by INVAM (International culture collection of vesicular arbuscular

mycorrhizal fungi, 2014). For the characterization of AM fungi, various characteristics of spores such as spore morphology, shape, colour and size were studied.

Assessment of root colonization of weeds: For assessment of root colonization of weeds, the procedure of (Giovannetti and Mosse, 1980) was used. For Microscopic study, three fragments each about 1cm long was randomly chosen. Morphology of AMF entophyte was studied and expressed in percentage (%). The percent infection was calculated as follow:

Extraction and isolation of Spores from weeds: Rhizospheric soil samples were collected of wheat at vegetative and fruiting stages. 300 gm sample of rhizospheric soil was taken from different fields of the wheat plant at a depth 15-20cm. Wet sieving and decanting technique of (Gerdemann and Nicolson, 1963). Wet sieving techniques was used to extract the spores from collected soil samples, sieving techniques include different sizes; 30µm, 50µm, and 100µm. The spores were introduced onto slide in drop of glycerine. Spore density was calculated using known method demonstrated by (Stahl and Christensen 1982) which can be defined average number of spores per 100gram of soil.

$$\text{Percent colonization} = \frac{\text{Total number of colonized root segment}}{\text{Total number of colonized root segment examined}} \times 100$$

RESULTS AND DISCUSSION

Root colonization and Spore diversity in weeds: In the present study presence of AMF root colonization were investigated shown in Tables (2 & 4) and Figures (1-4 and 6). Our study also observed an increase in AMF root colonization over the wheat-growing season in all localities of Charsadda (Figure 1-4). The increase of AMF root colonization (TRC) percentage was highest in Mangha dargi (25-119 %) followed by the others localities of Charsadda Ghazgai (40-90 %), Sardeheri (34-80 %age) and Palosa (24-80%age). Our results are supported by (Bilalis et al., 2011) who demonstrated that colonization of weeds by AM fungi vary in response to different weeds. No effects were observed on the growth of non-competitive weeds by AMF symbiosis. Positive effects on the growth of competitive weeds was observed with the presence of AMF.

Table 1. General information about collected weeds from wheat crops of District Charsadda.

s.no	Name of weeds	Family	Group	Sub-group	Growth phase
1	*Euphorbia helioscopia*	Euphorbiaceae	Angiosperm	Dicotyledon	Flowering
2	*Stellaria media*	Cryophyllaceae	Angiosperm	Dicotyledon	Vegetative
3	*Medicago sativa*	Leguminaceae	Angiosperm	Dicotyledon	Fruiting
4	*Fumaria indica*	Papaveraceae	Angiosperm	Dicotyledon	Vegetative
5	*Cynodon dactylon*	Poaceae	Angiosperm	monocotyledon	Vegetative
6	*Conyza canadensis*	Asteraceae	Angiosperm	Dicotyledon	Vegetative
7	*Parthenium hysterophorus*		Angiosperm	Dicotyledon	Fruiting
8	*Taraxacum officinale*		Angiosperm	Dicotyledon	Vegetative
9	*Capsella bursa- pastoris*	Brassicaceae	Angiosperm	Dicotyledon	Flowering
10	*Canabis sativa*	Cannabinaceae	Angiosperm	Dicotyledon	Fruiting
11	*Chenopodium album*	Chenopodiaceae	Angiosperm	Dicotyledon	Fruiting
12	*Convolvulus arvensis*	Convolvulaceae	Angiosperm	Dicotyledon	Fruiting
13	*Vicia faba*	Fabaceae	Angiosperm	Dicotyledon	Flowering
14	*Vicia sativa*		Angiosperm	Dicotyledon	Fruiting
15	*Rumex nepalensis*	Polygonaceae	Angiosperm	Dicotyledon	Flowering

Much variation was observed in the external hyphae, internal hyphae, arbuscules and vesicles in all studied sites, which show diversity of AMF population in weeds species (Fig.5). The diversity of agronomical important AM fungal species can be retained with thse help of weeds (Vatovec *et a*l., 2005). Significant increase in number of AMF spores was documented with increasing number of weeds species (Chen *et al*., 2004). (Rillig, 2004) demonstrated reduced AMF hyphal length in those areas having dense population of invasive mycorrhizal weed as compared to area getting chemical or other management practices. (Veiga, 2012) observed that though maize presented a negative mycorrhizal growth response when grown alone. It was insensitive to AMF in the presence of weeds, while the coexisting weed species grew lesser when colonized by AMF. It was proposed that maize forms more beneficial symbiosis with AM fungi than other tested weed species. Our study also observed an increase in AMF spore density and over the wheat growing season in all localities of Charsadda shown in (Figure 1- 4 and 5) and AMF diversity Table (3). Great AMF (TSD) per hundred gm^{-1} of soil abundance were recorded in Ghazgai (65-131 gm^{-1}) followed by Mangha dargi (40-119 gm^{-1}) , Palosa (46-115

gm^{-1}) and lowest in Sardheri (40-107 gm^{-1}) results agree with (Vatovec et al 2005) stating that AMF not always adversely affect weeds growth but some weeds species may affect the diversity and abundance of AM fungi. Reduced crop losses have been reported in case of positive interaction of weeds and AMF, which arises from weeds. It has also positive effects on soil microbial community composition and soil property.

Table 2. AMF colonization percentage in different weeds of wheat crops of District Charsadda.

s.no	Name of weeds	Family	IH. (%)	EH. (%)	Arb. (%)	Ves. (%)
1	*Euphorbia helioscopia*	Euphorbiaceae	20	32	40	44
2	*Stellaria media*	Cryophyllaceae	10	16	20	50
3	*Medicago sativa*	Leguminaceae	45	30	33	40
4	*Fumaria indica*	Papaveraceae	40	54	50	60
5	*Cynodon dactylon*	Poaceae	44	50	55	74
6	*Conyza canadensis*	Asteraceae	44	45	65	70
7	*Parthenium hysterophorus*		40	40	62	79
8	*Taraxacum officinale*		40	50	54	50
9	*Capsella bursa- pastoris*	Brassicaceae	0	0	0	0
10	*Canabis sativa*	Cannabinaceae	115	10	10	22
11	*Chenopodium album*	Chenopodiaceae	10	13	15	20
12	*Convolvulus arvensis*	Convolvulaceae	30	40	44	58
13	*Vicia faba*	Fabaceae	40	40	54	69
14	*Vicia sativa*		45	40	50	70
15	*Rumex nepalensis*	Polygonaceae	34	17	25	30

Table 3. Spore density and diversity in different weeds of wheat crops of District Charsadda.

s.no	Name of weeds	Spore per 100gm of soil density	Abundance of Endogonace spores		
1	*Euphorbia helioscopia*	80	*Glomus mossae* *Glomus hoi* *Glomus tenebrosum*	*Acaulospora mella* *Acaulospora gadenskinesis*	*Sclerocystis microcarpa* *Sclerocystis spp.*
2	*Stellaria media*	110	*Glomus albidum* *Glomus hoi* *Glomus dimorphicum*	*Acaulospora mella* *Acaulospora delicata*	*Sclerocystis spp.*
3	*Medicago sativa*	65	*Glomus maculosum* *Glomus mosssae*	*Acaulospora mella* *Acaulospora gadenskinesis*	*Sclerocystis nigra*
4	*Fumaria indica*	50	*Glomus*	*Acaulospora mella*	*Sclerocystis spp*
5	*Cynodon dactylon*	66	*Glomus*	*Acaulospora gadenskinesis*	*Sclerocystis spp*
6	*Conyza canadensis*	70	*Glomus*	*Acaulospora mellaAcaulospora gadenskinesis*	*Sclerocystis spp*
7	*Parthenium hysterophorus*	74	*Glomus*		*Sclerocystis spp*
8	*Taraxacum officinale*	45	*Glomus ambisporum* *Glomus albidum* *Glomus maculosum*		*Sclerocystis spp*
9	*Capsella bursa-pastoris*	0	*Nill*	- *Acaulospora mella*	-
10	*Canabis sativa*	95	*Glomus aggregatum*		*Sclerocystis spp*
11	*Chenopodium album*	80	*Glomus geosporum*	*Acaulospora gadenskinesis*	*Sclerocystis spp*
12	*Convolvulus arvensis*	80	*Glomus mossae* *Glomus pustulatum*	*Sclerocystis microcarpa*	*Sclerocystis spp* *Sclerocystis spp*

13	*Vicia faba*	85	*Glomus*	*Acaulospora mella*	*Sclerocystis spp*
14	*Vicia sativa*	60	*Glomus*		*Sclerocystis spp*
15	*Rumex nepalensis*	75	*Glomus fasiculatum* *Glomus albidum* *Glomus hoi*	*Acaulospora gadenskinesis*	*Sclerocystis spp*

Table 4. Arbuscular mycorrhizal infection Dominancy in different four Localities of district Charsadda.

S. No	Species Name	Ghazgai		Palosa		Sardeheri		Mangha dargi	
		TSD	TRC%	TSD	TRC%	TSD	TRC %	TSD	TRC%
1	*Euphorbia helioscopia*	112	40	100	72	107	56	95	82
2	*Stellaria media*	125	35	110	24	90	80	100	80
3	*Medicago sativa*	100	55	69	44	85	73	119	78
4	*Fumaria indica*	123	60	115	34	49	70	80	90
5	*Cynodon dactylon*	110	70	87	62	59	65	70	40
6	*Conyza canadensis*	90	60	46	61	50	55	74	51
7	*Parthenium hysterophorus*	80	80	100	54	68	50	70	66
8	*Taraxacum officinale*	75	45	56	60	40	48	70	80
9	*Capsella bursa-pastoris*	65	74	50	80	45	34	65	44
10	*Canabis sativa*	110	70	80	40	60	40	80	34
11	*Chenopodium album*	123	90	85	60	75	45	83	62
12	*Convolvulus arvensis*	131	40	92	35	77	55	100	23
13	*Vicia faba*	100	60	76	55	60	80	25	26
14	*Vicia sativa*	85	74	65	35	54	78	40	66
15	*Rumex nepalensis*	90	82	55	55	40	70	90	75

TSD (total spore density per 100gm of soil) TRC (total root colonization percentage)

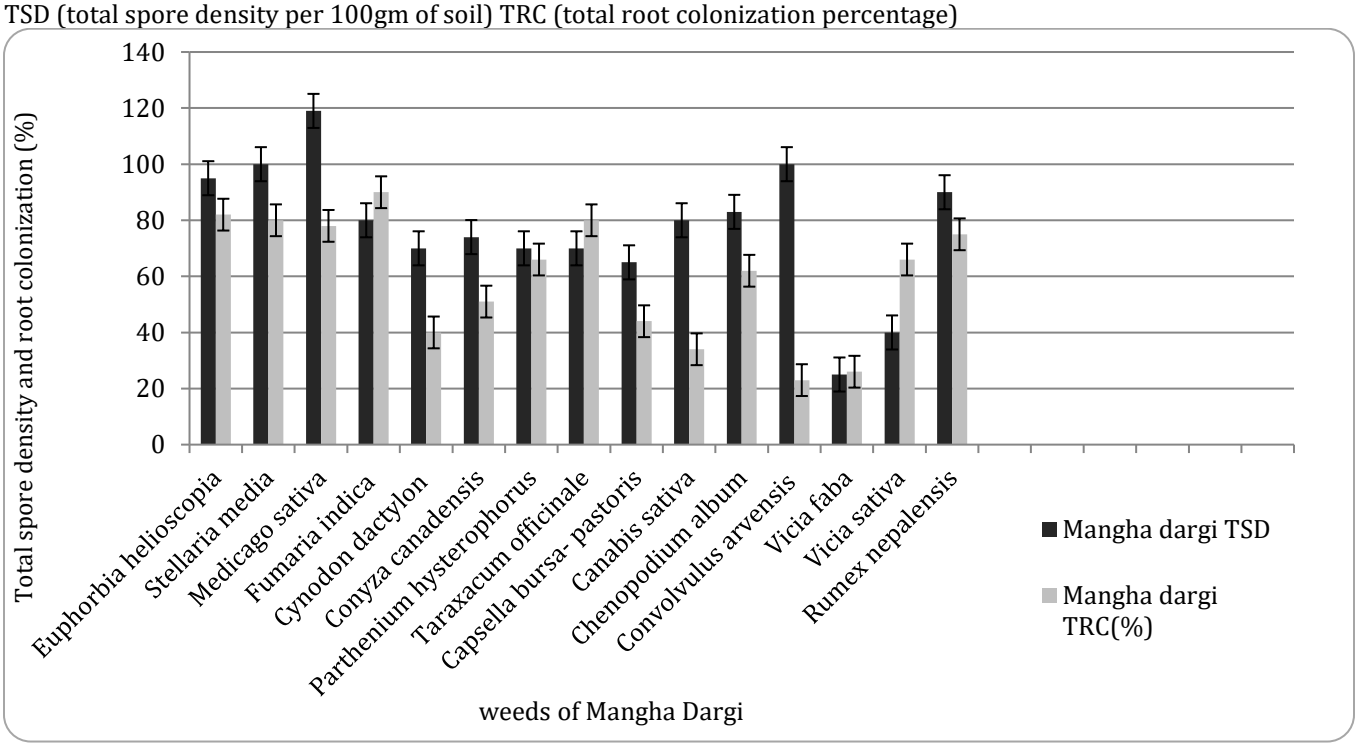

Figure 1. Total spore density (TSD) and Total root colonization (TRC) percentage in Mangha Dargi of district Charsadda.

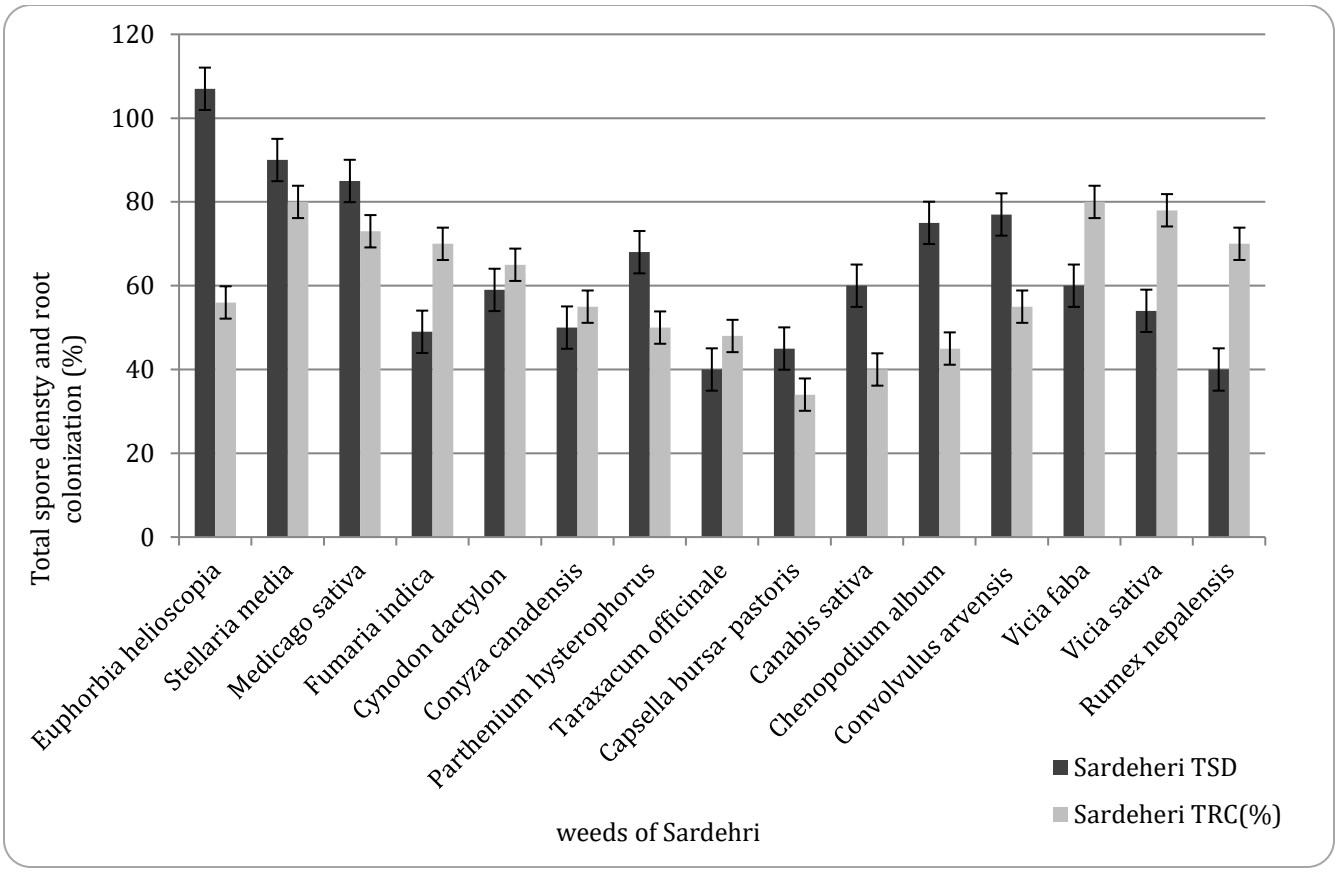

Figure 2. Total spore density (TSD) and total root colonization (TRC) percentage in Sardeheri of district Charsadda

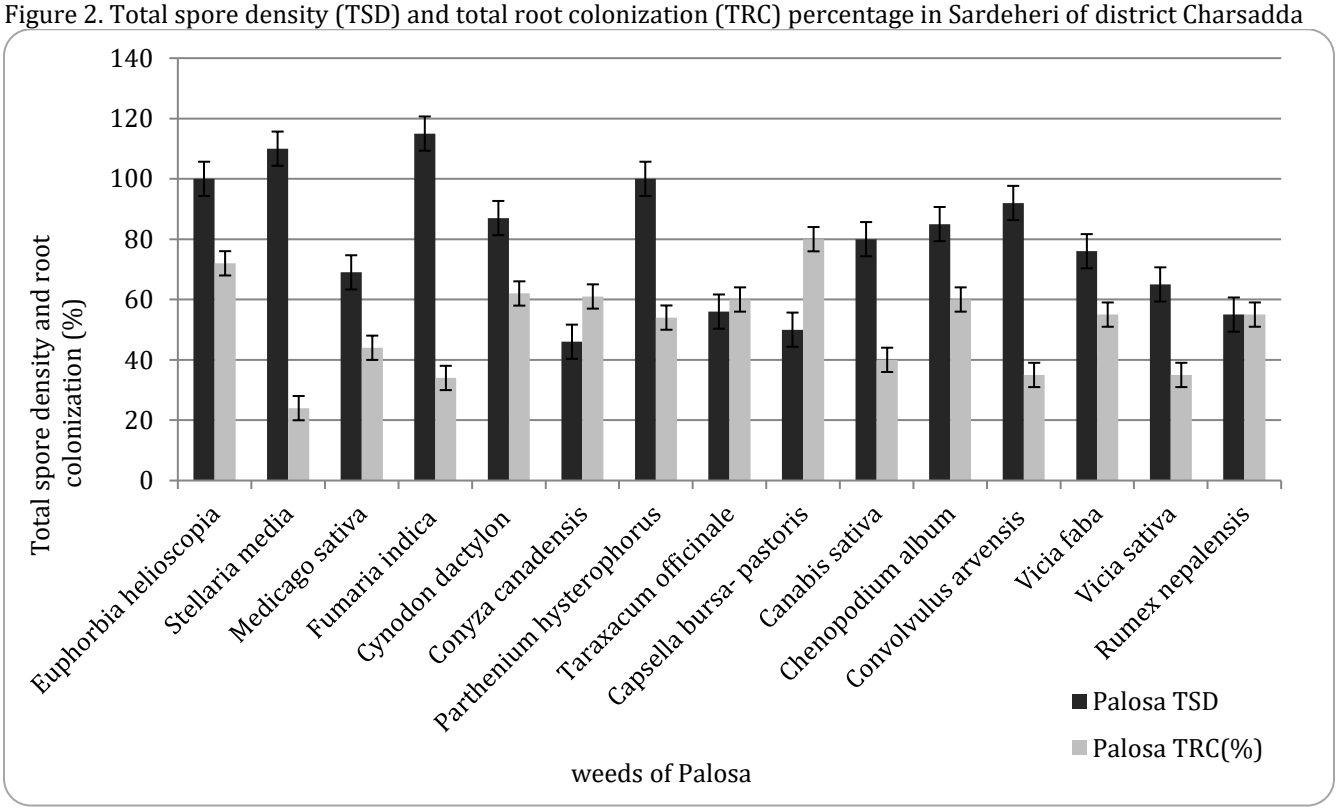

Figure 3. Total spore density (TSD) and total root colonization (TRC) percentage in Palosa of district Charsadda

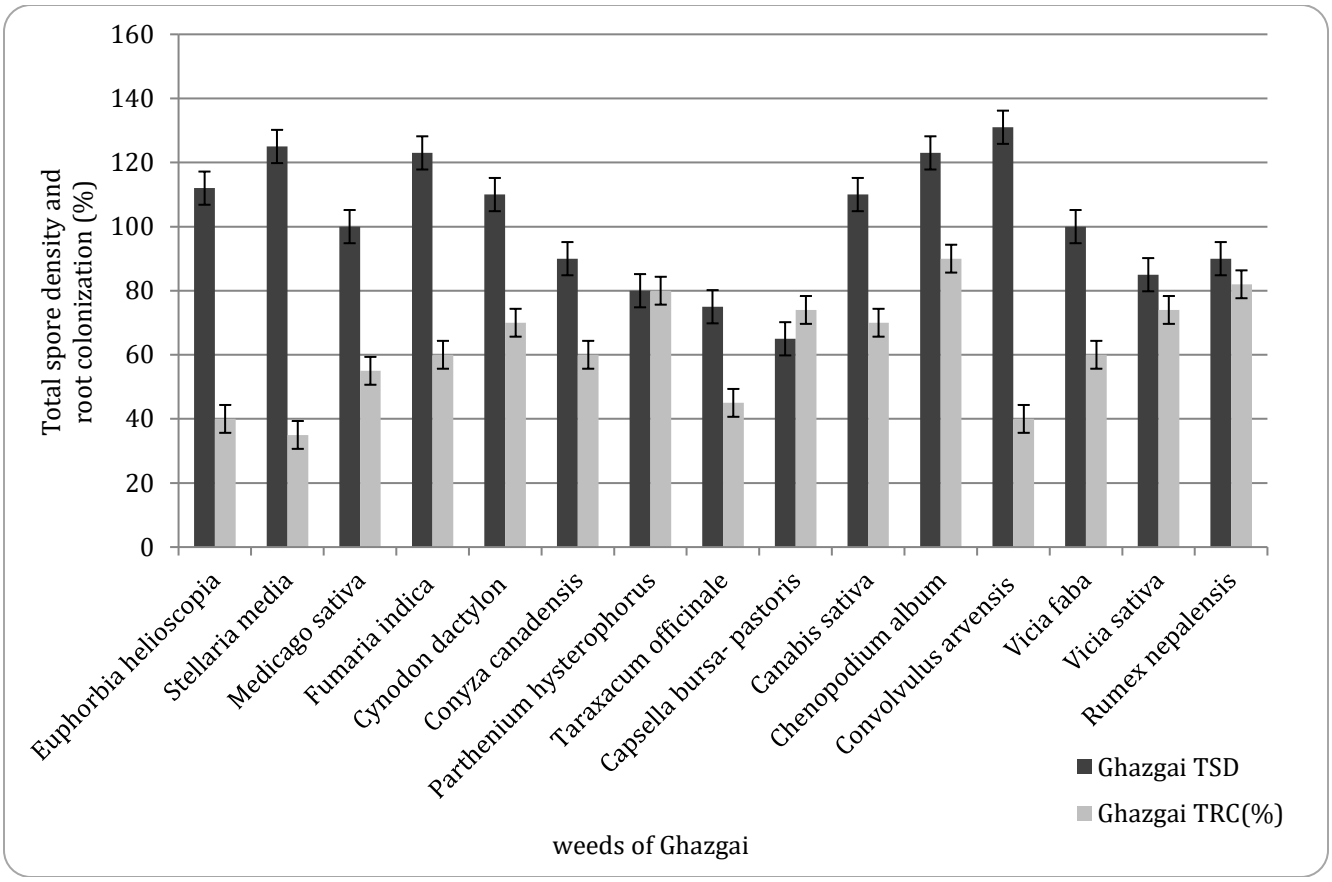

Figure 4. Total spore density (TSD) and Total root colonization (TRC) percentage in Ghazgai of district Charsadda

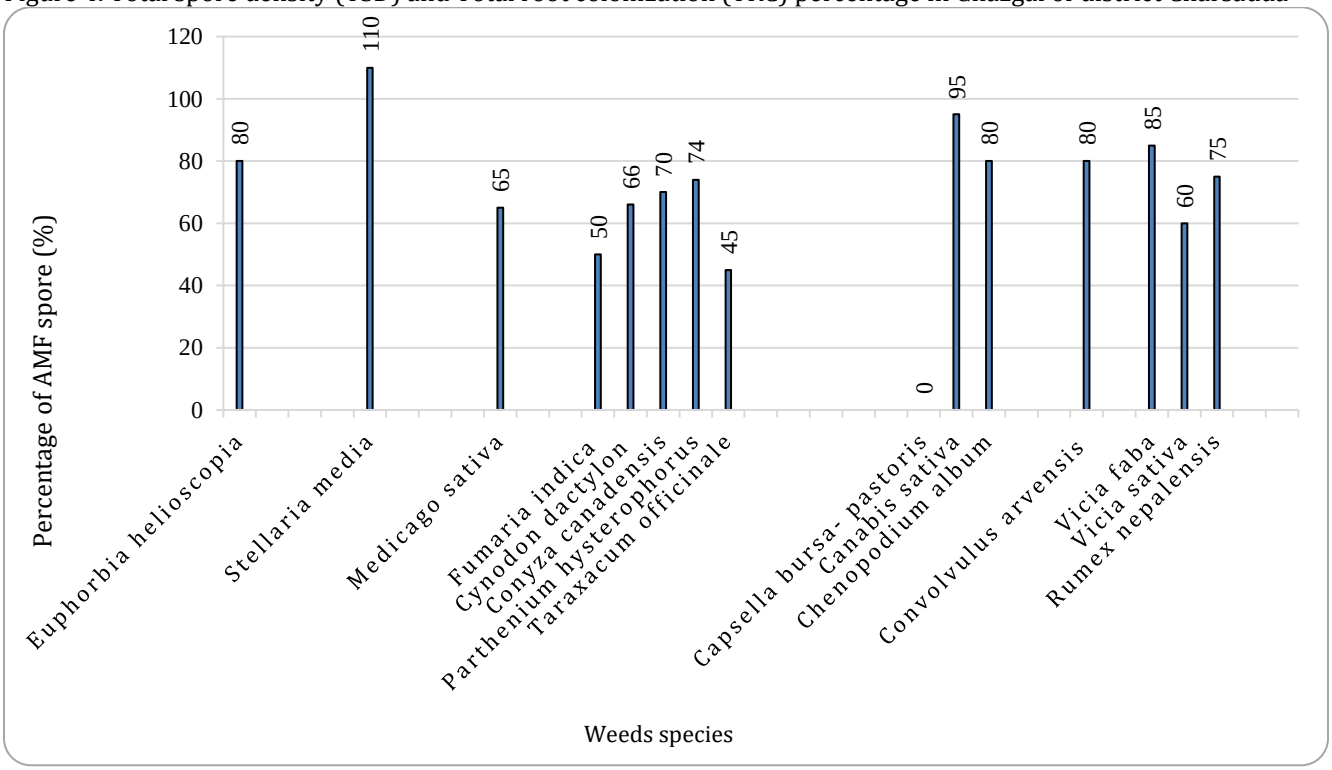

Figure 5. Spore diversity and density in different weeds species

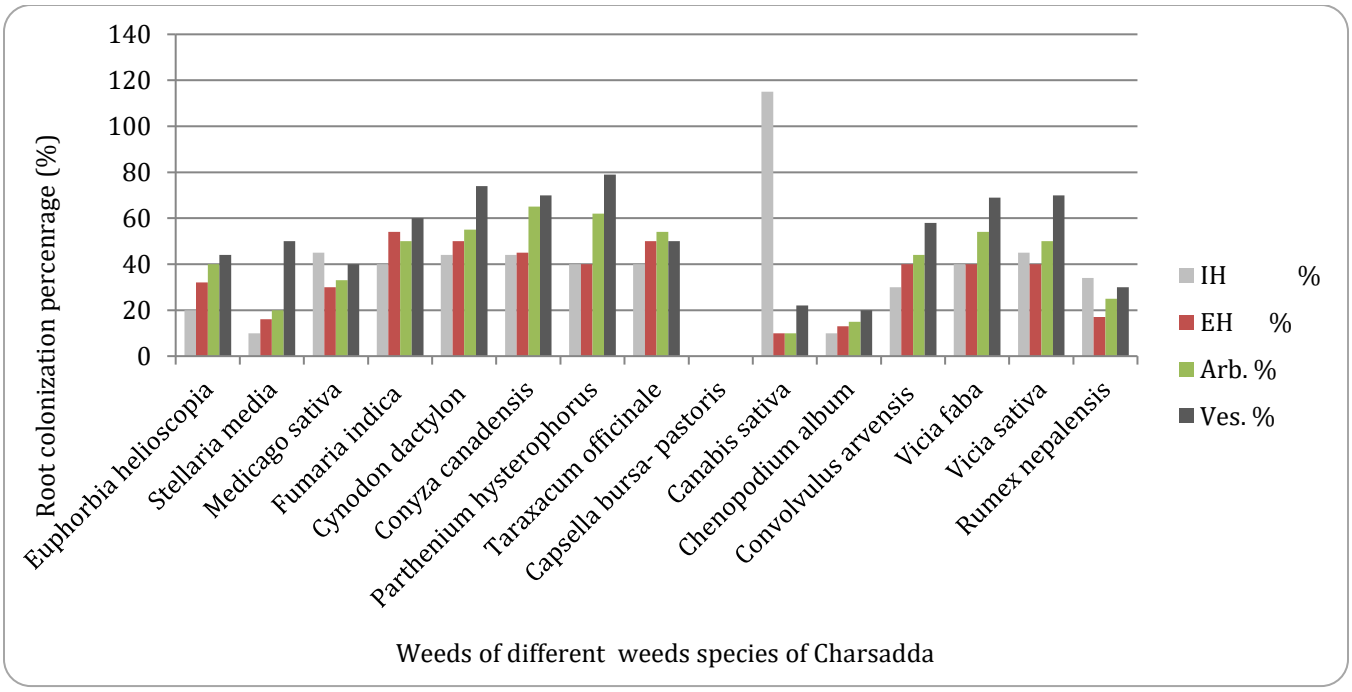

Figure 6. Root colonization by AMF structures IH (internal hyphae), EH. (External hyphae), Arb. (Arbuscules) and Ves. (Vesicles) in different weeds species.

CONCLUSION

It is concluded from our results that the most abundant weed family is Asteraceae followed by other families in wheat field. It is also cleared that the diversity of AMF spore density and root colonization varied from species to species, family to family and also affected by host plant growth stages (flowering/ vegetative stage). There is a specificity of AMF, natural diversity of AM fungi in the soil differ from species to species and differently influence root colonization and spore density. Spore density when studied in different location of district Charsadda, indicated that the dominant AMF area was Mangha dargi followed other localities which indicate that AMF root colonization varied by place to place. Furthermore, more research is required to explore the AMF colonization in weeds in other crops field.

Abbreviations: AMF (Arbuscular Mycorrhizal Fungi), TSD (Total Spore Density), TRC (Total Root Colonization), IH (Internal Hyphae), EH (External Hyphae)

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REFERENCES

Ampong-Nyarko, K. and S. K. De Datta. 1991. A handbook for weed control in rice. International Rice Research Institution, 1-49.

Anonymous. 1998. District Census Report of Charsadda, PCO, Govt. of Pakistan.

Basker, D. 1992. Comparison of taste quality between organically and conventionally grown fruits and vegetables. American Journal of Alternative Agriculture, 7: 129-136.

Bilalis, D., A. Karkanis, A. Konstantas, S. Patsiali and V. Triantafyllidis. 2011. Arbuscular mycorrhizal fungi: a blessing or a curse for weed management in organic olive crops. Australian Journal of Crop Science, 5: 858.

Boyetchko, S. M. 1996. Impact of soil microorganisms on weed biology and ecology. Phytoprotection, 77: 41-56.

Brito, I., M. Carvalho and M. J. Goss. 2013. Soil and weed management for enhancing arbuscular mycorrhiza colonization of wheat. Soil use and Management, 29: 540-546.

Brundrett, M. C. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant and Soil, 320: 37-77.

Cameron, D. D. 2010. Arbuscular mycorrhizal fungi as (agro) ecosystem engineers. Plant and Soil, 333: 1-5.

Chen, X., J. Tang, Z. Fang and K. Shimizu. 2004. Effects of weed communities with various species numbers on soil features in a subtropical orchard ecosystem. Agriculture, Ecosystems & Environment, 102: 377-388.

Gerdemann, J. and T. H. Nicolson. 1963. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Transactions of the British Mycological society, 46: 235-244.

Giovannetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist, 84: 489-500.

Jordan, N. R., J. Zhang and S. Huerd. 2000. Arbuscular-mycorrhizal fungi: potential roles in weed management. Weed Research-Oxford, 40: 397-410.

Khan, M. N., F. Hadi, A. Razaq and S. M. Shah. 2017. Utilitarian aspects of weeds and their ecological characteristics in Ochawala valley, District Charsadda, Pakistan journal of agricultural sciences**,** 5: 182-189.

Nasir, E., Ali, S. I., & Stewart, R. R. (1972). Flora of West Pakistan: an annotated catalogue of the vascular plants of West Pakistan and Kashmir. Fakhri, 102.

Nasir, Y. J., R. A. Rafiq and T. J. Roberts. 1995. Wildflowers of Pakistan. Oxford university press. Information System Division, National Agricultural Library, 264-266.

Oerke, E. 2006. Crop losses to pests. The Journal of Agricultural Science, 144: 31-43.

Phillips, J. M. and D. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society, 55: 158-161.

Rillig, M. C. 2004. Arbuscular mycorrhizae and terrestrial ecosystem processes. Ecology Letters, 7: 740-754.

Schenck, N. and Y. Perez. 1990. Manual for the Identification of VAM Fungi. 3rd. Synergistic Publication, Gainesville, Fl,USA, 3-17.

Souissi, T. and R. J. Kremer. 1994. Leafy spurge (*Euphorbia esula*) cell cultures for screening deleterious Rhizobacteria. Weed Science, 42: 310-315.

Stahl, P. D. and M. Christensen. 1982. Mycorrhizal fungi associated with Bouteloua and Agropyron in Wyoming sagebrush-grasslands. Mycologia, 74: 877-885.

Trappe, J. M. 1982. Synoptic keys to the genera and species of *Zygomycetous* mycorrhizal fungi. Phytopathology, 72: 1102-1108.

Vatovec, C., N. Jordan and S. Huerd. 2005. Responsiveness of certain agronomic weed species to arbuscular mycorrhizal fungi. Renewable Agriculture and Food Systems, 20: 181-189.

Veiga, R. 2012. Biological weed control with soil fungi. Antagonistic effects of arbuscular mycorrhizal fungi on the growth of weeds. Dissertation. Utrecht University Repository.

Wang, B. and Y.-L. Qiu. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza, 16: 299-363.

Contribution of Authors:

Yaseen Khan	:	Carried out experimental work
Tabassum Yaseen	:	Supervised the work and wrote the paper
Khushnood Ur Rehman	:	Provided the research materials
Muhammad Noor	:	Provided the research materials
Usman Jamshaid	:	Helped in execution of field trial
Rani Gul	:	Helped in execution of field trial
Gul Nawaz	:	Prepared graphs
Sulaiman Shah	:	Analyzed data statistically