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Arbuscular Mycorrhizal Incidence and Infectivity of Crops in North West Frontier Province of Pakistan

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Abstract: Spores density and mycorrhizal colonization were studied in twenty-five rhizosphere samples of some selected soils and plant roots from fertile and marginal soils of North West Frontier Province of Pakistan. All tested plants are considered to be mycorrhizal plants. Highest number of mycorrhizal spores was found in Potatoes, Barley, rice and chickpea (>4000 spores kg^{-1} soil) in fertile soils. Fields of alfalfa, wheat, barley, oat and grasses gave the highest number of spores in marginal soil. The root infection levels varied in different crops from one site to another. Barley, Potatoes and oats showed the highest infection rates being 44, 40 and 33%, respectively in fertile well managed soil, whereas 52, 50 and 43% highest mycorrhizal infections rates were observed in barley, alfalfa and wheat, respectively in marginal soil. Generally, spores density in soil samples seemed to be dominated by the species of *Glomus fasciculatum*. However, spores of *Glomus intraradices* and *Glomus mosseae* were also identified in the samples. Soils under investigation have pH value ranged from 5.6 to 8.5 with low concentration of available phosphorus and high contents of total phosphorus. Results suggest that (V)A mycorrhizal fungal spores and root colonization varied in different crops from one site to another under different agro-ecological conditions.

Key words: Crops % AM fungi % spores density % spores identification % root infections % soil conditions

INTRODUCTION

The ability to exploit the natural resources constituting a major step towards economic prosperity for developing country like Pakistan as chemical fertilizers are expensive, short and may cause the problems of environmental pollution [1]. Scientists are currently interested in developing alternative technology to minimize the dependence on chemical fertilizers to encourage the use of bio-fertilizers on a large scale in farming communities [2-4].

The importance of soil microorganisms in affecting soil fertility has been known since Bousingault (1802-1889) the French agricultural Chemist and Beijerinck (1851-1903) the Dutch Scientist who discovered the relationship between legumes and nitrogen-fixing bacteria, but the influence of soil microorganisms on soil quality and sustainability has been emphasized more recently [5, 6]. Microorganisms cause a series of dynamic biological and biochemical reactions such as organic matter decomposition, new material synthesis, rock weathering, element transformation in the soil and thus affect the nutrients availability for plants [7, 8].

Root system is a unique micro-site for the association of symbiotic and non-symbiotic microorganisms. (Vesicular-) arbuscular mycorrhizal fungi [(V)AMF] are distributed worldwide [9], which are currently classified in the order Glomales [10]. The taxonomy is further divided into suborders based on the presence of (I) vesicles in the root for the suborder Glomineae or (ii) absence of vesicles in the root for the suborder Gigasporineae. The term vesicular-arbuscular mycorrhiza (VAM) was originally applied to symbiotic associations formed by all fungi in the Glomales, but because a major suborder lacks the ability to form vesicles in roots, (V)AM is now the preferred form of writing . The order Glomales is further divided into families and genera according to the method of spore formation.

On global basis, mycorrhiza occurs in 83% of dicots and 79% monocots, whereas all gymnosperms are mycorrhizal [11]. Dense (V)AM infections are common in most species of leguminosae and gramineae. Most of the

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economically important crops are infected by (V)AM fungi [12]. Non-mycorrhizal plants occur in habitats where the soils are very dry, saline, waterlogged, severely disturbed and where soil fertility is extremely high or extremely low [13]. Pesticides treatment tends to decrease their numbers [14]. Mycorrhiza is undoubtedly of extraordinary importance in plant production, plant and soil ecology and plays a key role in sustainable agriculture [15-17]. Infection of crop roots with (V)AM fungi can improve their uptake of nutrients, particularly of phosphorus and increase crop production [18-21]. Mamatha *et al*. [22], Atimanav and Adholeya [23] and Joner [24] reported improvement in yield and plant nutrient accumulation with mycorrhiza. Responses of some tropical and subtropical cultures to endomycorrhizal fungi have been reported by Jaizme and Azcon [25].

The endomycorrhizal fungi are obligate symbiotic fungi, the hyphae of which develop mycelium, arbuscules and in most fungal genera vesicles in roots. These hyphae can explore an area around the root far exceeds that available to root hairs. It is the ability of these hyphae to make immobile or fixed elements (P, Zn, Cu) in acid or alkaline soils more available to plants, especially in the plants with coarse root systems [12, 26-28]. In marginal soils fertilized with rock phosphate, combined application of (V)AM fungi with N_2-fixing microorganisms and other organic materials may substantially increase P availability and crops yield [29-32]. There are indications to show that (V)AM fungal associations with plant roots can help plants to overcome water stress by stomata regulation in plants [33-35].

The research on mycorrhiza suffers from a dilemma of high expectations, that are often not met by indisputable and consistent results [36]. It can not be claimed that there is good balance between promised progress, clear understandings and successful applications, such as the use of AMF inocula as a biofertilizer [37]. The modest outcome may be related to a widely spread research strategy of simplifying complex field situations by factorial pot experiments and the subsequent transfer back to field conditions [38].

There is a great lack of information on the ranges of specific soil variables under which specific AM fungal species occur and thus on conditions which may be tolerable or optimum for them. Very limited information is available about the incidence of AM in Pakistan [29, 39-42]. Hussain and Burni [43], Burni *et al*. [44] and Burni and Jabeen [45] reported mycorrhizal association in a few plant species. No detailed and systematic studies have been conducted on the precise status of mycorrhizal association of plant in different ecological zones of Pakistan and particularly in North West Frontier Province (NWFP).

The present research project was planned to evaluate AM fungal status, their identification and infection intensity in various field crops under different agro-ecological conditions of NWFP in both nutrients deficient marginal and fertile soils. The results obtain from the proposed studies will help in proper management of indigenous resources for the maintenance of fertilizers application for sustainable crops production, which will have an obvious social and economic impact on the farming community of the area.

MATERIALS AND METHODS

Collection of samples: Plant roots and rhizosphere soil samples were collected from the fields of marginal and fertile soils of various locations of different agro-ecological zones of North West Frontier Province of Pakistan during April and May, 2004 from different standing crops at maturity stage. Marginal soils are generally with low input agriculture consists of small or no fertilizers dressings, no weeding or weeding by hands and no pesticides application whereas fertile soils are with high input agriculture consists of high yielding crop varieties, high fertilization, weed control by means of herbicides and chemical control of diseases and pests. Soil and plant samples were collected randomly in 10 replicates for a composite sample from each site and were stored at 4°C. The samples were analyzed at the Institute of Crop and Animal Production in Tropics, University of Goettingen, Germany.

Isolation and identification of mycorrhizal fungal spores: Soil mass 15 g was suspended in tap water and was sieved using wet-sieving and decanting technique [46, 47]. Two sieves (500 and 45 µm-mesh size) were used throughout this experiment. Spores retained on 45 µm-mesh size sieve were recovered by sucrose gradient centrifugation [48]. The spores were then transferred to petri-dishes and the number was recorded using a (30-50x) binocular microscope [49]. Some spores were mounted in small glass capsules containing water and a drop of chloroform for identification.

Spores were cleaned from attached organic materials under a dissection microscope (WILD M5) at 50x magnification with fine tweezers. The morphological characteristics of these spores were determined as far as possible according to the key proposed by Trappe [50]. These characteristics included, shape, size, color, distinct

Table 1: (Vesicular-) arbuscular mycorrhizal spores concentration and their root colonization

Crop	Soil type	Spores concentration			Root
		Black	Brown	White	colonization (%)
1. Potato	Fertile	High	High	Medium	40
2. Oat	0	Low	Medium	Medium	33
3. Sunflower	0	Medium	High	Medium	29
4. Mungbean	0	Low	Medium	Medium	27
5. Wheat	0	Medium	Medium	Medium	25
6. Chickpea	0	High	High	Medium	32
7. Berseem	0	Low	Medium	Medium	23
8. Barley	0	High	High	Medium	44
9. Alfalfa	0	Medium	Medium	Low	26
10. Rice nursery	0	Medium	High	High	30
11. Tobacco	0	Low	Medium	Low	20
12. Alfalfa	Marginal	Medium	High	High	50
13. Chickpea	0	Medium	Medium	Medium	32
14. Grasses	0	Medium	High	High	45
15. Sunflower	0	Low	High	Medium	35
16. Wheat	0	Medium	Medium	High	43
17. Mungbean	0	Medium	High	Medium	35
18. Potatoes	0	Low	Medium	Medium	42
19. Berseem	0	Medium	Medium	Medium	31
20. Rice nursery	0	Medium	High	Medium	32
21. Tobacco	0	Medium	Medium	Low	35
22. Oat	0	High	High	Medium	36
23. Barlay	0	High	High	Medium	52
24. Pea	0	Medium	High	Medium	27
25. Tomato	0	Low	Medium	Medium	25

0-20 spores (Low), 20-60 spores (Medium) , >60 spores (High) in 15 g soil

wall layer, attached hyphae, sporocarps, bulbous attachment, clustering and surface ornamentation of spores.

Estimation of root colonization: Mycorrhizal chitin was stained with lactic-trypan-blue according to the procedure as described by Philips and Hayman [51] and Koske and Gemma [52]. This procedure include various steps i.e. dispigmentation of roots by 10% KOH, washing with tape water, acidification of root with 2N HCl, staining root with 0.1% trypan-blue and then storing in glycerin: lactic acid: water (1:1:1) solution. Segments of 0.5 cm of stained roots were placed without overlapping on a slide and mycorrhizal infection (mycelium, vesicles and arbuscules) were observed in each segment in order to estimate the infection levels [12]. The root infection percentages (colonization) were calculated from the number of infected segments out of total root segments during their examination microscopically on a glass slide.

Chemical analysis of soil: Soil pH values of samples were determined in 1 : 2.5 soil and water suspension with the help of pH meter by the method outlined by McClean [53]. Total phosphorus in soil samples was determined by using the yellow molybdovanadate reaction and the yellow colored phosphomolybdovanadate complex was measured by spectro photo meter as described by Page *et al*. [54], whereas available phosphorus by the method of Soltanpour and Schwab [55]. Total soil nitrogen and carbon were determined by Dumas method using carlo erba elemental analyser [56].

RESULTS AND DISCUSSION

Number of (V)AM fungal spores and root colonization: Results in Table 1 show the density and color of (V)AM spores isolated from soil and their root colonization in different crops under investigations. The number of mycorrhizal spores was markedly affected by the crop types and sites. Potatoes, Barley, rice and chickpea gave the highest number of spores (> 4000 spores kg^{-1} soil). Oat, sunflower, alfalfa, mungbean, wheat and berseem showed medium number of spores (1333-4000 spores kg^{-1} soil) whereas tobacco gave the minimum number of spores (<1300 spores kg^{-1} soil) in fertile soil. In marginal soil, alfalfa, wheat, barley, oat and grasses gave the highest number of spores (> 4000 spores kg^{-1} soil) while ckickpea, pea, sunflower, rice, mungbean, potatoes, berseem, tomatoes and tobacco showed the medium spores (1333-4000 spores kg^{-1} soil) number (Table 1 and Fig. 1).

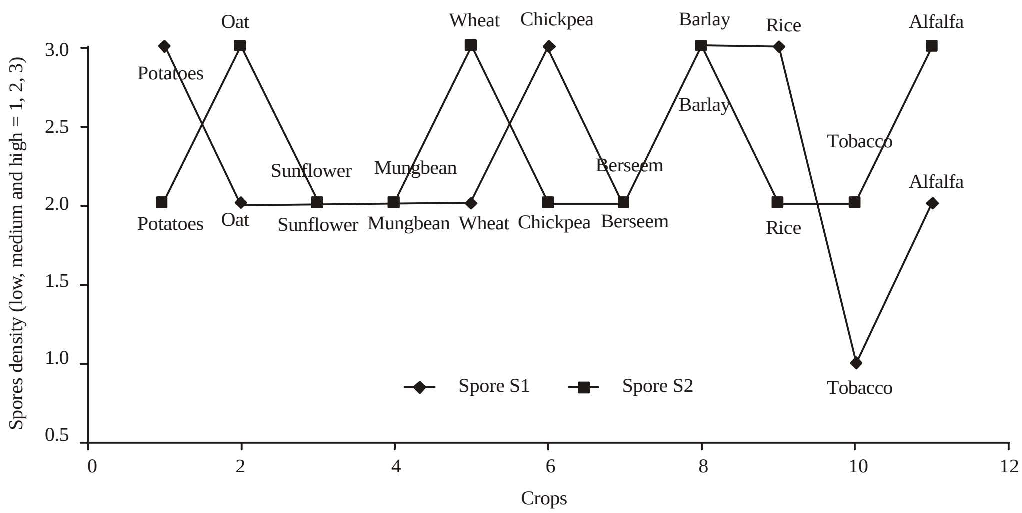


Fig. 1: (V)AM spores density in fertile and marginal soil

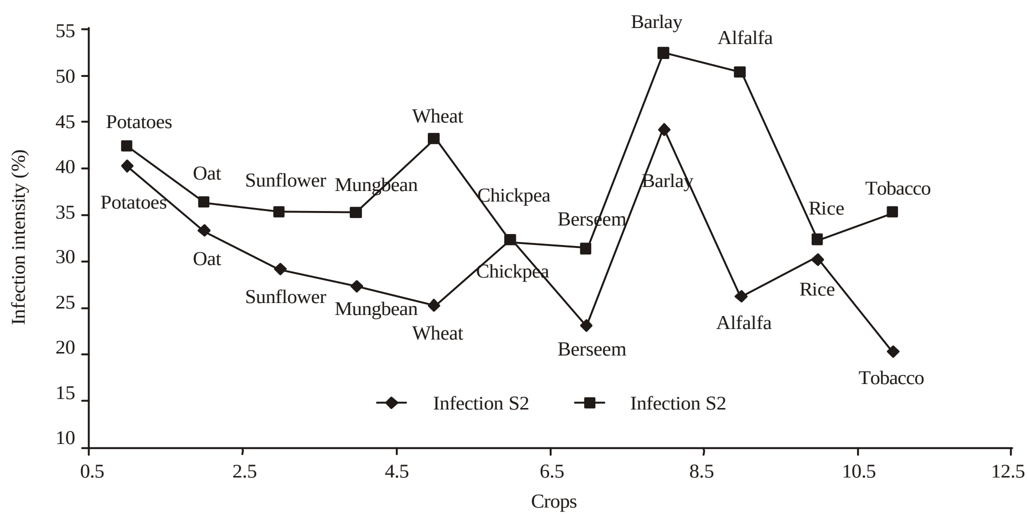


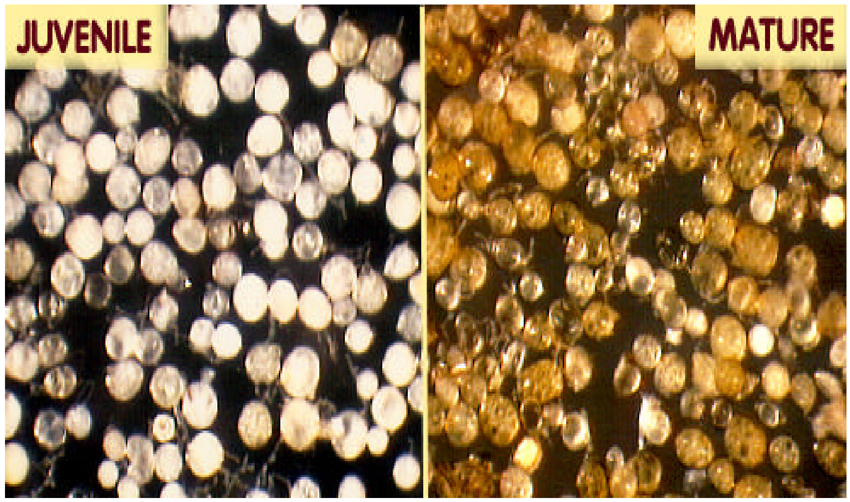
Fig. 2: (V)AM infection in fertile and marginal soil (S1 = Fertile soil, S2 = Marginal soil)

Data on root colonization of different crops by (V)AM fungi are presented in Table 1 and Fig. 2, which reveal that the root infection percentage varied in different crops from one site to another. Barley, Potatoes and oats showed the highest infection rates being 44, 40 and 33%, respectively, while the lowest infection rate was recorded in the case of tobacco and berseem in fertile well managed soil. In marginal soil, highest mycorrhizal infections rates of 52, 50 and 43% were observed in barley, alfalfa and wheat, respectively. The lowest mycorrhizal infection in this soil was observed in tomatoes and peas.

he highest number of mycorrhizal spores in the rhizosphere of barley, rice, potatoes and chickpea in fertile soil and that of barley, wheat, oat, grasses and alfalfa in marginal soil indicated that these plant species might be considered as good hosts for (vesicular-) arbuscular mycorrhizal fungi under the prevailing conditions. This may be attributed to the root exudates of these plants, which stimulate the germination of mycorrhizal spores and increase the infection percentage. The most plant species in Leguminosae and Gramineae families are normally mycorrhizal [12]. Morley and Mosse [57] reported that wheat, phaseolus beans and alfalfa vary in mycorrhizal infection according to cultivars and environmental conditions.

Soils, plants and environmental management factors mainly affect the mycorrhizal fungi and their development in an ecosystem. There are some indications that the diversity of (V)AM fungal species declines from natural ecosystem to high input agricultural systems. Relatively

a) b)



c)

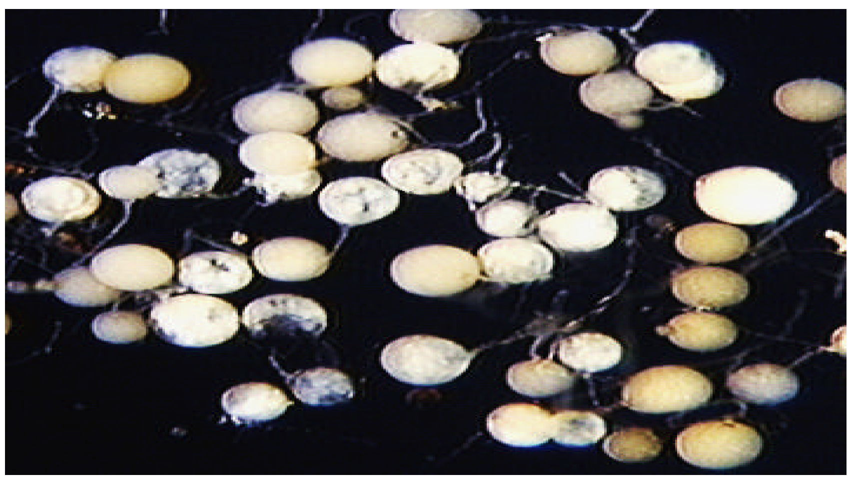


Fig. 3: Spores of a) *Glomus fasciculatum b) G. intraradices* and c) *G. mosseae* [59]

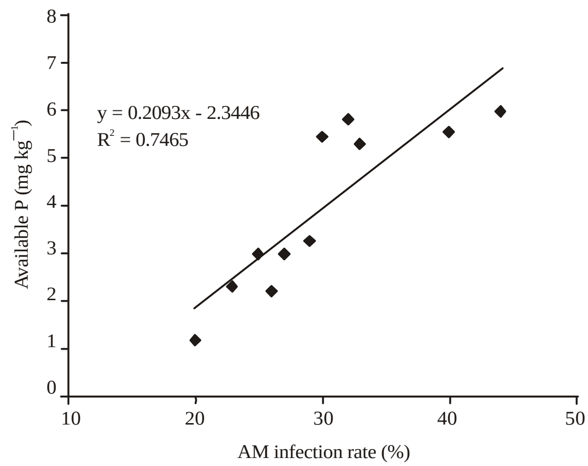


Fig. 4: Relationship between AM infection and P availability in fertile soil

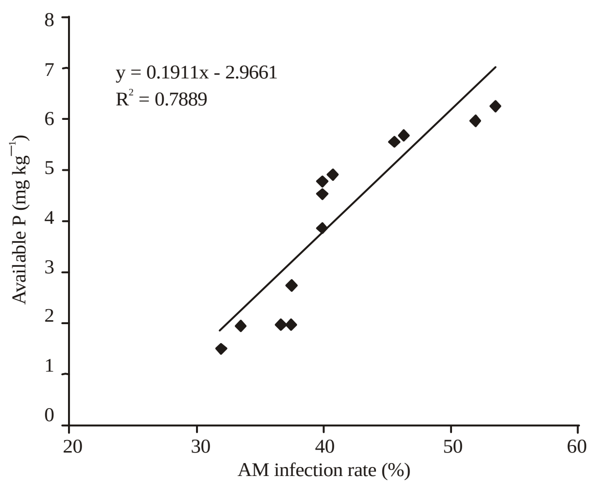


Fig. 5: Relationship between AM infection and P availability in marginal soil

high number of fungal species is found in marginal soil with low agronomic inputs. High fungal diversity in marginal soil may results in a more sustainable and less risky agricultural production provided that different fungal species could benefit the crop under the different potential stress conditions. Fertilizers and pesticides applications to the soils may counteract super optimum (V)AM fungal populations. The reactions of P fertilizers in soils are faster enough and the plants are not or only slightly dependent on (V)AM fungi and thus the reproduction of fungi will be low under this conditions, which will result low (V)AM populations and root colonization in such soils generally.

Identification of spores: Spores of *Glomus fasciculatum* (Fig. 3a) were found in abundance in all soil samples where as spores of *Glomus intraradices* (Fig. 3b) and *Glomus mosseae* (Fig. 3c) were also identified in the soil samples but in lower densities. Generally, spores density in soil samples seemed to be dominated by the species of *Glomus fasciculatum* but it still requires further confirmation by crop inoculation, re-isolation and re-identification. Indigenous (V)AM fungal communities generally contain several fungal species. Normally 5-15 species may be found in agro-ecosystems. The spatial distribution of (V)AM fungal species can vary and even when the number is the same at two different soil sites, the species composition of the fungal population can be completely different [58]. The spores of (V)AM fungi are very distinctive. They range in diameter from 10 µm for *Glomus tenue* to more than 1000 µm for some *Scutellospora* spp. The spores can vary in color from hyaline (clear) to black and in surface texture from smooth to highly ornamented. *Glomus* forms spores on the ends of hyphae, *Acaulospora* forms spores laterally from the neck of a swollen hyphal terminus and *Entrophospora* forms spores within the neck of the hyphal terminus.

Chemical analyses: Arbuscular Mycorrhizal fungi differ greatly in effectiveness and therefore it is of interest to have information on the ecological conditions and distribution of these fungi in an area for their specific management. Chemical analyses of the collected soil samples are presented in Table 2.

Data indicated that pH values of well managed fertile soil ranged from 8.3 to 8.5, total P 617.8 to 1084.6 mg kg^{-1}, available P from 1.20 to 5.98 mg kg^{-1} soils, total C 2.21 to 2.82% and total N ranged from 0.070 to 0.097%. In marginal soil, pH values ranged from 5.6 to 8.5, total P 484.5 to 1014 mg kg^{-1}, available P 1.46 to 6.2 mg kg^{-1} soils, total C 0.88 to 2.27% and total N ranged from 0.081 to 0.322%. Higher infection of root systems of barley, potato, oat and chickpea in fertile soil (Fig. 4) and that of barley, alfalfa, grasses and wheat in marginal soil (Fig. 5) with VA mycorrhiza is positively correlated with improved P availability in these soils having the property of phosphorus fixation due to high concentration of calcium and high pH values. (V)AM fungi produce organic acids, which may partially explain enhanced nutrient uptake by the roots of mycorrhizal plants. Another possible mechanism by which mycorrhizal fungi release inorganic phosphorus is through mineralization of organic matter. Soils under investigation contained low concentrations of available phosphorus and high contents of total

Table 2: Chemical analysis of soil

Crop	Soil type	pH (1 : 2.5)	Total P (mg kg^{-1})	Available P (mg kg^{-1})	Total C (%)	Total N (%)
1. Potato	Fertile	8.5	674.9	5.55	2.34	0.070
2. Oat	0	8.4	678.7	5.32	2.50	0.071
3. Sunflower	0	8.3	708.7	3.26	2.27	0.074
4. Mungbean	0	8.5	745.2	2.99	2.61	0.080
5. Wheat	0	8.4	1084.6	2.99	2.31	0.091
6. Chickpea	0	8.4	753.9	5.82	2.21	0.066
7. Berseem	0	8.4	617.8	2.32	2.43	0.075
8. Barley	0	8.4	621.8	5.98	2.32	0.097
9. Alfalfa	0	8.4	658.6	2.20	2.62	0.077
10. Rice nursery	0	8.4	702.2	5.44	2.71	0.093
11. Tobacco	0	8.5	792.9	1.20	2.82	0.093
12. Alfalfa	Marginal	8.3	834.7	5.94	2.27	0.082
13. Chickpea	0	8.5	695.6	1.95	1.81	0.103
14. Grasses	0	8.4	645.3	5.54	1.28	0.093
15. Sunflower	0	8.5	704.2	3.82	1.18	0.123
16. Wheat	0	5.6	529.1	5.64	0.88	0.091
17. Mungbean	0	6.2	488.6	4.50	1.29	0.128
18. Potatoes	0	5.9	484.5	5.52	1.41	0.161
19. Berseem	0	6.9	954.2	1.95	1.19	0.322
20. Rice nursery	0	6.7	682.7	2.72	1.69	0.268
21. Tobacco	0	8.2	954.4	4.76	1.11	0.104
22. Oat	0	7.9	678.7	4.88	0.91	0.084
23. Barlay	0	7.72	968.2	6.20	0.89	0.081
24. Pea	0	5.9	594.6	1.92	1.09	0.107
25. Tomato	0	6.2	1013.7	1.46	1.22	0.139

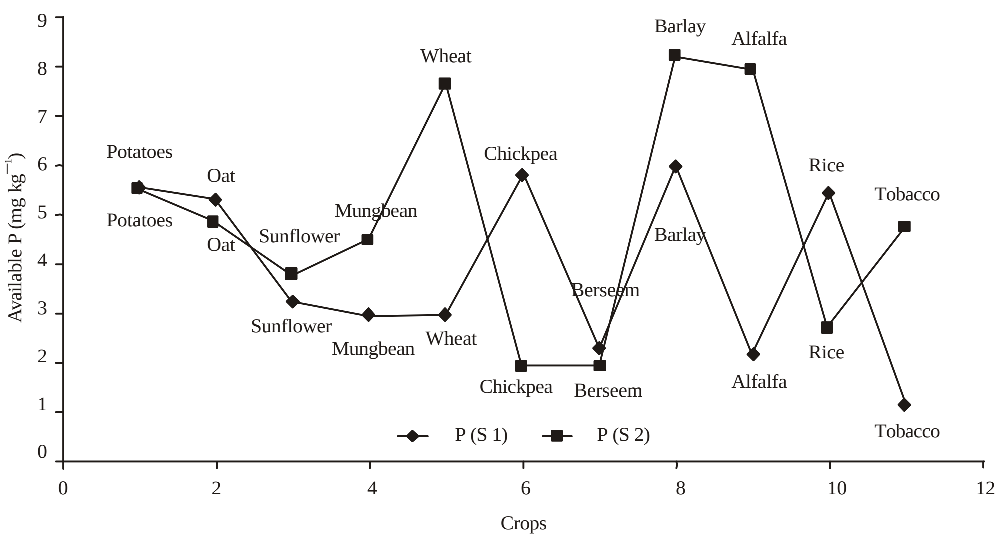


Fig. 6: Comparison of P availability in marginal and fertile soils (S 1 = Fertile soil, S 2 = Marginal soil)

phosphorus (Table 2). Comparatively higher P availability was recorded in marginal soil conditions (Fig. 6). Higher C percentage was recorded in fertile soil due to the addition of higher inputs. Addition of no organic matter and the possible utilization of soil C by the (V)AM as energy source for higher root colonization to solubilize more phosphorus might have caused the lower concentration of total C percentage in marginal soil.

CONCLUSIONS

It may be concluded from this study that (V)A mycorrhizal spores in soils and root colonization varied in different crops from one site to another under different agro-ecological conditions. Highest number of mycorrhizal spores was found in Potatoes, Barley, rice and chickpea in fertile soils and in alfalfa, wheat, barley, oat and grasses in marginal soil. The highest root infection rates were observed in barley, potato and oat in fertile soil and that in barley, alfalfa and wheat in marginal soil. Improved availability of soil P is correlated with the increased mycorrhizal root colonization of different crops in fertile as well as in marginal soil and comparatively more P availability was recorded in marginal soil conditions. Further comprehensive investigations are proposed for the occurrence, distribution and the identification of indigenous (V)A mycorrhiza in various field crops, their interactions with other soil microorganisms and management through agronomic practices for economically feasible crop production under different agro-ecological zones of North West Frontier Province of Pakistan. The problems of mass production of (V)AM fungus inoculum for field crops are needed to be concentrated as it is still not possible to propagate(V) AMF in axenic pure culture.

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