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Vesicular arbuscular mycorrhizal (VAM) studies in six pteridophytes in district Buner

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Abstract
Mycorrhizal study were conducted on the adventitious roots of six pteridophytes collected from different localities of District Buner. All plants were found heavily mycorrhizal. Apart from the marked dimorphism in external and internal hyphal elements, arbuscules and vesicles were noted which support the presence of multiple infections. Maximum Vesicular Arbuscular Mycorrhizal (VAM) infection were observed in the adventitious roots of *Pteridium aquilinum* (98%) and minimum VAM infection in *Asplenium dalhosia* (82%) and *Adiantum capillus* (82%). From the rhizosphere of plants, three VAM genera spores *Glomus spp, Sclerocystis spp* and *Gigaspora spp* were collected. All the fungal spores were studies for different micromophological features such as shape, color, wall, dimension, inner content, hyphae and presence or absence of septa. Plant species had a significant role in root tissue colonization by Mycorrhizal fungi.

Keywords: *Glomus spp, Sclerocystis spp, Gigaspora spp,* arbuscules, vesicles

1. Introduction
Pteridophytes has an ancient origin which occupied a very important position in the origin and evolution of vascular plants [1]. Mycorrhizal association between pteridophytes and fungi have been found in the fossil rhizomes of Rhynia and Asteroxylon, which were common in the Devonian to Carboniferous periods in the Paleozoic Era, and these mycorrhizas were considered to be the earliest arbuscular mycorrhizas [2]. Mycorrhizal association is a beneficial association between the roots of plants and fungi. Arbuscular mycorrhizas play a crucial role in the mineral nutrition of most plants. This symbiosis occurs across a wide range of environments, probably because mutualism enables plants to obtain nutrients more effectively [3]. The arbuscular mycorrhizal fungi diversity influences the composition and diversity of the plant community [4].
In the present study six pteridophytes (*Adiantum capillus, Adiantum caudatum, Asplenium dalhosia, Dryopteris blandfordii, Pteridium aquilinum and Pteris cretica)* has been investigated for vesicular arbuscular mycorrhizal (VAM) association in District Buner.

1.1. Introduction of the Area
District Buner has an area 1843 square kilometer lies between 34°-09´ and 34°-43´ North latitudes and 72°-10´ and 72°-47´ East longitudes in KPK province. The region is encircled by hills from all sides and separated from Swat valley by range of mountains reaching to an elevation of 10000 ft. The climate of the district is moderate and seldom rises above 37°C in summer. The annual rainfall is 81-88cm.

2. Material and methods
2.1. Collection of plants
Roots of the plants were collected from District Buner in 2017. The plants *Adiantum capillus* (in September) *Adiantum caudatum* (in November) *Asplenium dalhosia* (in December), *Dryopteris blandfordii* (in December) were collected in vegetative growth phase and *Pteridium aquilinum* (in December) *and Pteris cretica* (in December) were collected in reproductive growth phase.

2.2. Preparation of preservative (Fixative) solution
The classic plant fixative FAA Formaldehyde, Alcohol, Acetic Acid at 10%: 50%: 5% + 35% water) were prepared and use as fixative. The suitable quantity of the fixative (FAA) solution were poured in clean medium size glass bottles. The material of the individual plant was preserved in each bottle and labelled.

2.3. Preparation of KOH (10%) stain
KOH solution were prepared by mixing 10mg of KOH in 100ml water. The solution was stirred well and kept for staining procedure.

2.4. Preparation of acid Fuchsine stain
For the preparation of acid fuchsine stain 0.025gms fuchsine were dissolved in 220 ml lactic acid and then mixed with 10 ml distilled water and 16ml glycerin. The solution was stirred well and used for staining.

2.5. Staining procedures
The methodology of [5] were carried out for staining of roots.

2.6. Assessment of root colonization
Slide method referred by [6, 7] was followed for root colonization study. Simple binocular light microscope was used for the observation and measurement of different fungal hyphae using the following formula. Photographs were taken from the best representative slides.
% Mycorrhizal association =

$$\frac{\text{No.of mycorrhiza associated segments}}{\text{Total no. of segmented scored}} \times 100$$

2.7. VAM spores study
Soil samples were collected from the rhizosphere of selected pteridophytes. The spores were extracted following the methodology of [8].

2.8. Mounting of spores
Individual spores were picked up with the help of a needle and were mounted on slides along with a drop of Canada balsam. A coverslip was carefully placed on each slide. Selected spores were microphotographed at various magnifications. Spores were identified with the help of Keys [9, 10]

3. Results
In the present study, the survey of pteridophytes for VAM fungi showed variability in colonization and spore density. All the pteridophytes selected for study exhibited the presence of VAM association. Hyphal and vesicular stages of colonization were seen in all the pteridophytes.

3.1. VAM infection
General VAM infection in the roots of *Adiantum capillus, Adiantum caudatum, Asplenium dalhousia, Dryopteris blandfordii, Pteridium aquilinum* and *Pteris cretica* were 82%, 92%, 82%, 94%, 98% and 96% were recorded respectively. Root colonization in all species were characterized by the presence of external hyphae, internal hyphae, arbuscules and vesicles given in (Table-1, Fig-1).

Table 1: VAM infection in all pteridosphyte roots

Plant Name	% age of VAM infection	% age of External Hyphae	% age of Internal Hyphae	% age of arbuscules	% of vesicles
Adiantum capillus	82	38	10	82	4
Adiantum caudatum	92	66	56	82	54
Asplenium dalhousia	82	76	46	46	34
Dryopteris blandfordii	94	40	30	86	68
Pteridium aquilinum	98	70	34	98	82
Pteris cretica	96	80	56	62	26

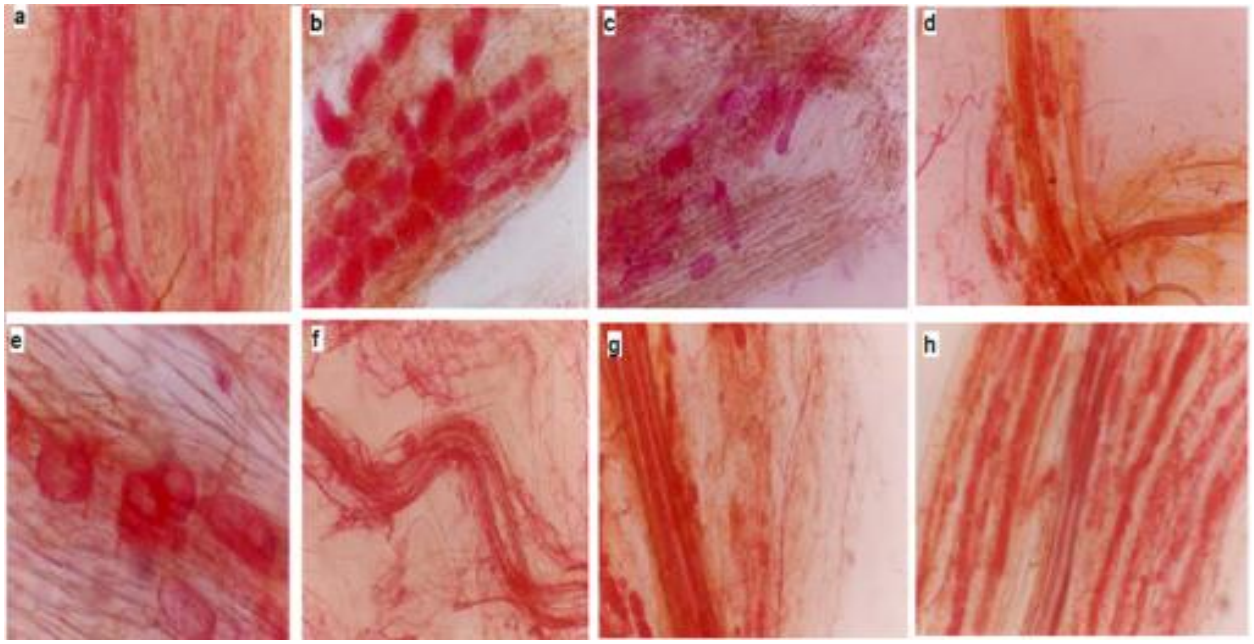

Fig 1: a: Arbuscules in the roots of *Adiantum capillus;* b-c: Arbuscules and vesicles in the roots of *Adiantum caudatum;* d: Vesicles in the roots of *Asplenium dalhousia;* e-f: Heavy hyphae and vesicles in the roots of *Asplenium dalhousia;* g-h: Hyphae and vesicles in the roots of *Pteridium aquilinum*

3.2. VAM Spores
Five *Glomus* species spores were collected from the rhizosphere of *Adiantum capillus* (Table-2, Fig-2). Three species of *Sclerocystis* and three species of *Glomus* were found in the rhizosphere of *Adiantum caudatum* (Table-2, Fig-2). Four species Of *Glomus* and 1 species of *Sclerocystis* were isolated from the rhizosphere of *Asplenium dalhousia* (Table-2, Fig-2). Three species of *Glomus* and 2 species of *Sclerocystis* were isolated from the rhizosphere of *Dryopteris blandfordii* (Table-2, Fig- 2). Two species of *Gigaspora*, 2 species of *Sclerocystis* and 1 species of *Glomus* were isolated from the rhizosphere of *Pteridium aquilinum* (Table-2, Fig-2). 1 species of *Gigaspora*, 1 species of *Sclerocystis* and 3 species of *Glomus* were recorded from the rhizosphere of *Pteris cretica* (Table-2, Fig-2). All the fungal spores has been studied for their shape, color, wall, dimension, inner content, hyphae and septa.

Table 2: Fungal spores recorded from the rhizosphere of all pteridophyte

Plant	Fungal species	Shape	Color	Wall	Dimension	Inner content	Hyphae	Septa
Adiantum capillus	*Glomus desecticola*	Globose	Brown	Double	91μm	Smooth	Present	-
	Glomus ambisporum	Globose	Dark brown	Single	91μm	Smooth	Present	Present
	Glomus multisubtensum	Globose	Brown	Double	104μm	Droplets	Present	Present
	Glomus morphicum	Globose	Brown	Double	273μm	Smooth	Present	-
	Glomus pustulatum	Globose	Dark brown	Single	143μm	Granular	Present	-
Adiantum caudatum	*Glomus ambisporum*	Globose	Dark brown	Single	104 μm	Smooth	Present	Present
	Glomus pustulatum	Globose	Black	Single	130μm	-	Present	-
	Sclerocystis spp	Irregular	Brown	-	78/364μm	Spiny	-	-
	Sclerocystis spp	Irregular	Dark brown	Single	130/676μm	Droplet	-	-
	Sclerocystis spp	Irregular	Brown	Single	117/650μm	-	-	-
Asplenium dalhousia	*Glomus botryoides*	Sub-Globose	Dark brown	Double	182 μm	Smooth	Present	-
	Glomus etunicatum	Globose	Translucent	Double	195μm	Smooth	Present	-
	Glomus convolutum	Globose	Translucent	Single	117μm	-	Present	Present
	Glomus australe	Globose	Dark brown	Double	143μm	-	Present	Present
	Sclerocystis spp	Irregular	Brown	Double	104/611μm	Smooth	Present	Present
Dryopteris blandfordii	*Glomus halon*	Globose	Yellowish brown	Double	260μm	-	Present	Present
	Glomus caledonium	Globose	Brown	Double	130μm	-	Present	-
	Glomus caledonium	Globose	Translucent	Double	221μm	Droplet	Present	-
	Sclerocystis spp	Irregular	Yellow	Single	78/325μm	-	-	-
	Sclerocystis spp	Irregular	Black	Single	91/663μm	Smooth	-	-
Pteridium aquilinum	*Glomus ambisporum*	Globose	Dark brown	Single	104μm	-	Present	Present
	Gigaspora spp	Globose	Black	Single	130μm	-	Present	-
	Gigaspora gigantea	Globose	Translucent	Double	195μm	Smooth	Present	Present
	Sclerocystis spp	Irregular	Brown	Single	104/351μm	Droplet	-	-
	Sclerocystis spp	Irregular	Brown	Single	130/650μm	Droplet	-	-
Pteris cretica	*Glomus dimorphicum*	Globose	Brown	Double	91μm	-	Present	-
	Glomus mosseae	Globose	Brown	Single	117μm	-	Present	-
	Glomus fistulosum	Globose	Brown	Double	143μm	Granulated	Present	Present
	Gigaspora spp	Globose	Dark Brown	Single	104μm	Smooth	Present	-
	Sclerocystis spp	Irregular	Dark Brown	Single	143/533μm	Granulated	-	-

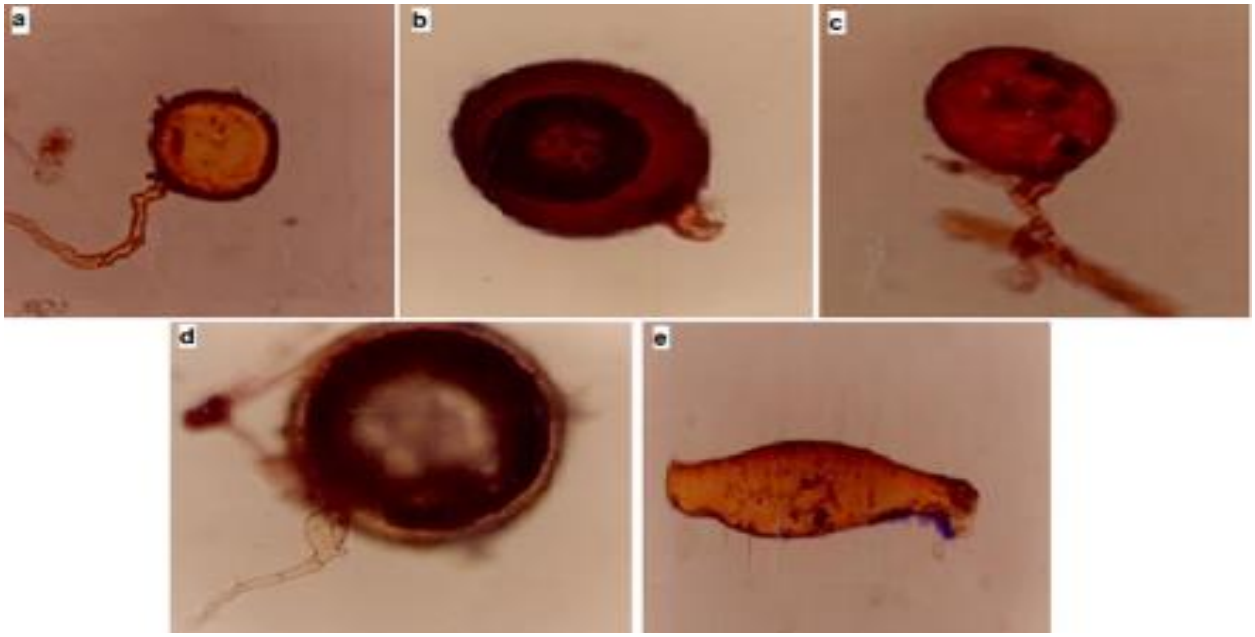

Fig 2: a: Spore of *Glomus desceticola;* b: Spore of *Glomus ambisporum;* c: Spore of *Glomus multisubtensum;* d: Spore of *Gigaspora giganta:* e: Spore of *Sclerocystis spp*

3.3. Discusssion

Mycorrhizal association in which fungus grows with the roots of a plant in a symbiotic relation in which both are benefited. It has been investigated that fungus helps in the absorption of nutrients from the soil by increasing the surface area of the roots. A lot of studies have indicated that VA mycorrhiza appear to be of common occurance in the pteridophytes [11, 12].

Hyphal adaptation for efficient absorption of substrates from the plant result in increased leakage of mineral nutrients to the host. Plant containing fungi acquire limiting mineral nutrients more effectively from hyphae within them than by other means [13]. Plants evolve recognition mechanisms to distinguish mycorrhizal fungi from pathogens. Specialized plant and fungi cells develop an interface zone where exchange occurs. Fungal hyphae increase their capacity to acquire the soil nutrients that limit plant growth. The plant becomes obligatory mycorrhizal, requiring the fungus for

growth at normal soil fertility levels [2].

In the present study *Adiantum capillus*, *Adiantum caudatum Asplenium dalhosia*, *Dryopteris blandfordii*, *Pteridium aquilinum* and *Pteris cretica* showed high VAM infection.

Our studies are well in lineage with [14] who reported highest VAM infection in *Angiopteris evecta* (81.36 %). *Sclerocystis* and *Gigaspora* spores were common in the rhizosphere of the species [15]. Reported significant VAM infection in *Asplenium cheilosorum* [16]. investigated the colonization of VAM in the roots of *Pteris multifida*, *Pteris vittata*, *Adiantum capillusveneris*, *Adiantum philippense* 31%, 24%, 23% arbuscular and 8%, 36%, 12% vesicular infection respectively. *Glomus* spores were highest in number in the rhizosphere of all species [11]. Reported highest arbuscular mycorrhizal root colonization (75%) in *Pityrogramma calomelanos*. *Glomus*, *Gigaspora* and *Sclerocystis* spores has been reported from the rhizosphere of the plant.

In the above studies *Glomus spp* has been reported from the rhizosphere of all the plant species which confirmed the findings of [17] who have reported the wide distribution range of this species.

3.4. Conclusion

From the present study it has been concluded that the mycorrhizal association helps in the root tissue colonization and diversification of the plant species. This may be helpful in the evolution of the plant species as both partners are get benefited.

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