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VAM infection and VAMF spores in *Withania somnifera* (L.) dunal and *Withania coagulans* Dun. (Stocks.) at fruiting stage

Tabassum Yaseen*, Muhammad Ibrar, Barkatullah

Department of Botany, University of Peshawar, Pakistan

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Abstract

The present paper deals with vesicular arbuscular mycorrhizal infection in roots and spores in the rhizospheric soils of *Withania somnifera* and *Withania coagulans* at fruiting stage. Soil and root samples were taken in May of 2010 from university of Peshawar and Bhadar baba at flowering and fruiting stage. Samples were used for direct spore counts, root colonization assessment to evaluate mycorrhizal colonization. Although *Withania somnifera and Withania coagulans* roots showed considerable arbuscular mycorrhizal (AM) colonization, ranging from 94% to 98%. Colonization rates were most influenced by available soil P, correlated positively with percentage of sand and soil pH, but correlated negatively with soil clay content. Field spore numbers varied from 200 to 263/50gm of soil. Fungal species identified *Glomus*, *Sclerocysts* and *Acaulospora* sp. were the most common species. To investigate the (V) AMF spore population in the rhizospheric soils of above mentioned plants.

Corresponding Author: Tabassum Yaseen ✉ sciences111@yahoo.com

Introduction

Arbuscular mycorrhiza (AM) is widespread symbiotic associations that are commonly described as the result of co-evolution events between fungi and plants where both partners benefit from the reciprocal nutrient exchange (Bonfante, 2008). Arbuscular mycorrhiza (AM) is the most common type of mycorrhizal association, occurring in 2/3 of land plants Hodge (2000). VAM fungi can improve their uptake of nutrients, particularly of phosphorus and increase crop production. (Tinker, 1975; Young *et al.,* 1988; Mamatha *et al.,* 2002; Atimanav & Adholeya, 2002) reported improvement in yield and plant nutrients accumulation with mycorrhiza.

Withania somnifera is an important medicinal and endangered plant growing as weed Ashwagandha, is used in Indian traditional system of medicine since long ago. The pharmacological activity of the root is attributed to the presence of several alkaloids; withanolides is obtained from leaves (Chatterjee & Pakrashi, 1995; Bone, 1996). It is necessary to study the growth and development of the plant like *Withania somnifera,* because general extinction of medicinal plant resources including *Withania somnifera*The drug is claimed to be effective in the treatment of rheumatic pain, inflammation of joints, nervous disorder and epilepsy. (Halder and Ray, 2006) *Withania somnifera*, a herb is commonly called as Ashwagandha. It belongs to the family *Solanaceae*. It is a very important herb in Ayurveda, the traditional Indian medicine (Haripriya *et al.,* 2009). To investigate the (V) AMF spore population in the rhizospheric soils of *Withania somnifera and Withania coagulans* and to identify the prevalent species of VAM entophytes in the rhizospheric soil of *Withania somnifera and Withania coagulans*.

Materials and methods

Withania somnifera and Withania coagulans roots were collected from the University of Peshawar and Bhadar baba. Roots were processed and stained after Philips and Hayman (1970). The stained root segments approximately 1cm long were mounted in lactic acid and measurements were taken and morphological characteristics were studied under microscope. By using the procedure of Bajwa and Javid (1997) intensity levels were also find out. Extraction of Endogonaceous spores. Endogonaceous spores were extracted by the wet sieving and decanting technique, Gerdemann and Nicolson (1963). In order to determine the genus and species, spores was done following the keys by Schenck and Perez (1987, 1990). Spores were micro-photographed with the help of Minolta x 700 with an adapter tube.

Results and discussion

In present research work the roots were studied for AM infection to find out the general AM infection. The results showed that the roots of *Withania somnifera* and *Withania coagulans* (94-98%). According to the results of the present study the plant showed heavy infection.

Table 1 a. Percentage and Intensity level of VAM infection in the roots of *Withania somnifera*.

VAM Structure	Total No. of root segments with infection	% of infection	Intensity level	Signs
Extend hyphae	35	70	Low	+
Internal hyphae	0	0	Low	+
Arbuscul	29	58	Low	+++
Vesicles	45	90	High	+
General infection		98	High	+++

Note: Total No. of root segments= 50; + =Very low infection; ++ =Moderate infection; ++ =High infection; ++++ =Dense

In the present investigations studied plants showed the percentage of vesicular infection was reasonably high at flowering stage reaching to maximum at fruiting

stage in both varieties. These results agree with the results of Iqbal and Bareen (1991), Nasim *et al.* (1996) and Burni *et al.* (1993). While presence of high vesicular infection can be related to the observations of Iqbal *et al.* (1988 III) who found high vesicular infection at the reproductive stage of the plant. In general the mycorrhizal infection increased with the age of the host plant. These results are in conformity with the finding of Nasim *et al.* (1996). Various workers such as Jalal-ud-Din and Anwar (1991), Ezawa *et al.* (1995), Fattah and Razak (1996) and Zhao *et al.* (1997) have also reported the relationship between the VAM development and the age of the host plant. Moreover, Al-Raddad (1995) observed that the type of crop and harvesting greatly affect the root colonization.

Table l b. Percentage and Intensity level of VAM infection in the roots of *Withania coagulans*.

VAM Structure	Total No. of root segments with infection	% of infection	Intensity level	Signs
Extend hyphae	2	4	Low	+
Internal hyphae	4	8	Low	+
Arbuscul	15	30	High	+++
Vesicles	14	24	Low	+
General infection		94	High	+++

Note: Total No. of root segments= 50; + =Very low infection; ++ =Moderate infection; ++ =High infection; ++++ =Dense

The heavy general AM infection in *Withania* species may be attributed to the deficiency of phosphorus in the soil. Manske (1990) showed that low availability of soil phosphorus increase VAM colonization. In addition, Gryngler and Vancura (1991) observed VAM colonization affected by soil phosphorus. A mycorrhizal fungus exists in soil as spores or as vegetative propagules in root segments Arora *et al.* (1991). Nasim *et al.* (1998) showed that spores are the means of identification of these fungi. The results also confirm the findings of Mosse and Bowen (1968) and Jalauddin (1993) who found that VAM are the regular component of soil microflora. Their number increased gradually from vegetative stage to fruiting stage. Our findings are in agreement with the work of Saif (1977) and Jalal-ud-din and Anwar (1991) who also found a general reduction of VAMF spores as the plants matured.

Table 2a. VAM Spores in the rhizospheric soil of *Withania* species.

Species	S.No.	Name of Spores	Spores density at fruiting
Withania somnifera	1	*Glomus*	97
	2	*Sclerocystis*	89
	3	*Acaulospora*	77
Total Number			263
Withania coagulans	1	*Glomus*	82
	2	*Sclerocystis*	58
	3	*Acaulospora*	60
Total Number			200

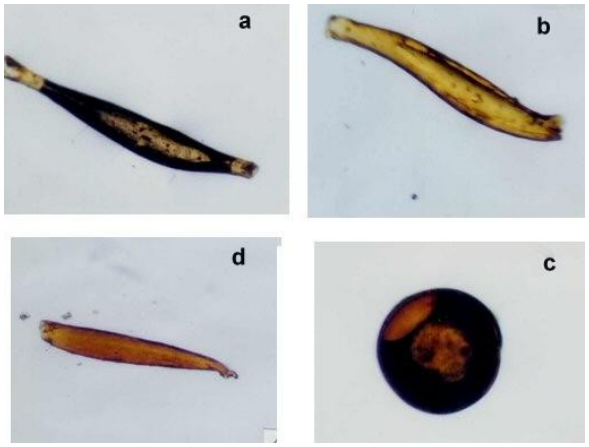

Fig. 1. VAMF spored. a, b and c represents *Sclerocystis* species; d represents *Acaulospora*.

In the present study different species of *Glomus* were most common and dominant at all growth stages. These results are in accordance with the findings of Morton (1998) and Land (1993) who found *Glomus* as a widely distributed and commonly occurring genus all over the world. The general VAM infection in

Withania somnifera and Withania coagulans was 98% and 94% respectively (Table No. a and 1b).

In the rhizospheric soil of both Withania species different VAM fungal species were recorded. *Glomus* species, *Acaulospora* and Sclerocystis spp were recovered from the rhizospheric soil (Figures, 1-4).

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