



Research article

Effect of edaphic factors on the diversity of VAM fungi

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Abstract: The present study deals with the diversity and distribution of VAMF at different sites with different selected plants. Maximum number of VAMF species were found at site IV (57 species) out of which *Glomus* species was most dominant (58%), followed by *Acaulospora* (19%), *Scutellospora* (8%), *Sclerocystis* (4.8%) and *Gigaspora* (1.6%) respectively. In site II 56 species of VAMF were observed with *Glomus* (55%), followed by *Acaulospora* (22.5%), *Scutellospora* (8%), *Gigaspora* (1.6%) and *Sclerocystis* (3.2%) respectively. In site III 55 species of VAMF occurred with *Glomus* (51.6%) followed by *Acaulospora* (22.5%), *Scutellospora* (9.7%), *Sclerocystis* (4.8%) and *Gigaspora* (0%) respectively. In site I 54 species of VAMF were found; out of these *Glomus* was highest 53% followed by *Acaulospora* (22.5%), *Scutellospora* (5%), *Sclerocystis* (1.6%) and *Gigaspora* (1.6%) respectively. These results suggest that selected study sites are rich in VAMF frequency and diversity. The Shannon-Wiener index confirms that diversity of VAMF fungal species varies with the test plant and maximum diversity was observed with *Ocimum sanctum* (3.948), and *Withania somnifera* (3.909) respectively. Maximum ANOVA value recorded in case of and *Withania somnifera* (0.20) and *Ocimum sanctum* (0.19) respectively. Maximum richness value was observed in case of *Ocimum sanctum* (0.3948) than *Withania somnifera* (0.0391).

Keywords: Arbuscular mycorrhizal fungi (AMF) - Vesicular-arbuscular mycorrhizal (VAM) - *Withania somnifera* - *Ocimum sanctum*.

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INTRODUCTION

Mycorrhizae are the mutualistic symbiosis (non-pathogenic association) between soil borne fungi and the roots of higher plants (Quilambe 2003). Mycorrhizal associations are found in wide range of habitats usually in the roots of angiosperms, gymnosperms and pteridophytes. They also occur in the gametophytes of some mosses, lycopods and psilotes, which are rootless (Mosse *et al.* 1981, Vyas *et al.* 2007, 2008). Arbuscular mycorrhizal fungi (AMF) have shown to be potentially able to take up both organic (Hodge *et al.* 2001, Campbell & Fitter 2001) and inorganic nitrogen from the soil (Govindarajulu *et al.* 2005). Vesicular-arbuscular mycorrhizal (VAM) fungi are essential components of ecosystem for both re-vegetation of the degraded lands and maintenance of soil structure (Caravaca *et al.* 2005), thereby reducing the risks of erosion and desertification.

Soil characteristics, plant species, and climate may all regulate the arbuscular mycorrhizal (AM) fungi community. The distribution of certain VAM fungal species has been related to soil pH, phosphorus level, salinity, soil disturbance (Abbott & Robson 1991), vegetation (Johnson *et al.* 1992), or hydrologic condition of the soil (Ingham & Wilson 1999, Miller & Bever 1999). In general terms, increase in soil pH, nutrient status and salinity in soil are related to a decrease in VAM root colonisation or spore density (Abbott & Robson 1991). Despite the importance of VAM fungi in the physiology and nutrition of plants, as well as in shaping plant communities, factors affecting the presence, diversity, spore density, and root colonisation by AM fungi in soil are poorly understood (Grime *et al.* 1987, Van der Heijden *et al.* 1998, Smith *et al.* 1999). One reason is the difficulty of establishing causation from correlation of soil and plant factors with VAM fungal populations. Another reason is that AM fungi can associate with a wide range of hosts present in community, but the sporulation rates of AM fungi have been found to be host dependent (Bever *et al.* 1996, Lugo & Cabello 2002). Host-dependence of VAM fungal population growth rates in soil may play an important role in the maintenance

of VAM fungal species diversity in grasslands (Bever *et al.* 1996), and suppression of mycorrhizal symbioses may result in a decrease in dominant plant population and an increase in species diversity (Hartnett & Wilson 1999). In addition, plant diversity may increase or decrease if the dominant plant competitors are more weakly or strongly mycotrophic than their neighbours (Hartnett & Wilson 1999).

An additional factor influencing populations of VAM fungi in soil, which may in turn affect the performance of plant species relative to each other, is the hydrologic condition of the soil, which may vary seasonally. The hydrologic condition of the soil plays an important role in determining plant community structure, and is even more important when soils are commonly subjected to periods of dryness and flooding (Chaneton *et al.* 1998). VAM fungi have been found in the roots of many plants in wetlands (Ingham & Wilson 1999, Miller & Bever 1999) or salt marshes (Brown & Bledsoe 1996). This is relevant because the fungi are believed to require well aerated soils, and are thought to have problems adapting to flooded conditions (Mosse *et al.* 1981). Nevertheless, little is known of VAM fungi patterns in wetlands or of the influence of the hydrologic condition of the soil on populations of AM fungus species.

Medicinal plants have been backbone of Indian traditional medicine system "Ayurveda". Among the mentioned plants in various Ayurveda texts two herbs Ashwagandha (*Withania somnifera*) and Tulsi/ Holy basil (*Ocimum sanctum*) are known for their extensive use in traditional Indian medicine. The major biochemical constituents of Ashwagandha are steroidal alkaloids and steroidal lactones in a class of constituents called withanolides. At present, 12 alkaloids, 35 withanolides, and several sitoindosides from this plant have been isolated and studied. A sitoindoside is a withanolide containing a glucose molecule at carbon 27. Much of Ashwaganda's pharmacological activity has been attributed to two main withanolides, withaferin A and withanolide D. These days many people cultivating medicinal plants to fulfil the increasing demands of pharmaceutical industries. Tulsi, the holy basil is one of the most cherished herbs for its many healing and health-giving properties in the Orient. Some of the main chemical constituents of tulsi are: oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, β -caryophyllene (about 8%) (Kuhn & Winston 2007) β -elemene (c.11.0%), and germacrene D (about 2%) (Puri 2002). Current research offers substantial evidence that Tulsi reduces stress, enhances stamina and endurance, increases the body's efficient use of oxygen, boosts the immune system, reduces inflammation, protects against radiation damage, lessens aging factors, supports the heart, lungs and liver; has antibiotic, antiviral and antifungal properties; enhances the efficacy of many other therapeutic treatments; and provides a rich supply of antioxidants and other nutrients

Thus prompted with above mentioned facts we undertook present study to understand how AM fungi play their role in association with the two above mentioned medicinal plants, in order to understand their bio-fertilizing potential which can be exploited accordingly.

MATERIALS AND METHODS

For the present investigation two test sites were selected, (I) Kariaya Village (II) Jaitpur Village in Shahdol district of central Indian state of Madhya Pradesh. The experiments were conducted for quantitative and qualitative estimation of AM fungi from rhizosphere and non-rhizosphere soil and roots of test plants.

The rhizosphere soil and root samples of selected test medicinal plants were collected from different soil depths (*i.e.* 0–10, 10–20, 20–30, 30–40 cm). The VAM spores were isolated from the collected soil samples by wet sieving and decanting method (Gerdemann & Nicolson 1963). Mycorrhizal spores were identified according to their spore morphology using conventional taxonomic key of Schenck & Perez (1990) and descriptions from <http://invam.wvu.edu/the-fungi/classification>. For the estimation of AM spores, a technique provided by Gour & Adholeya (1994) was followed. The soil pH was determined in 1:5 suspension of soil: deionized water ratio, electrometrically by glass electrode pH meter 335 (Jackson 1982). Statistical analysis of data for comparison of means, analysis of variance (ANOVA) was followed after Gupta & Kapoor (1997).

RESULT

Variance in relative abundance of VAMF spores was observed, with test plants *Withania somnifera* and *Ocimum sanctum*, growing in the Karaiya village and Jaitpur village, along soil depth gradient (Table 1). Maximum value was recorded up to 10 cm depth and minimum was recorded at 30–40 cm depth.

Table 1. Relative spore abundance* of VAMF species associated with test medicinal plants in different soil depths (cm) at different location.

VAMF Species	KARAIYA VILLAGE						JAITPUR VILLAGE					
	<i>Withania somnifera</i>		<i>Ocimum sanctum</i>		<i>Withania somnifera</i>		<i>Ocimum sanctum</i>		<i>Withania somnifera</i>		<i>Ocimum sanctum</i>	
	0-10	10-20	20-30	30-40	0-10	10-20	20-30	30-40	0-10	10-20	20-30	30-40
<i>Acaulospora bireticulata</i>	b											
<i>A. denticulate</i>	b	b							c	c		
<i>A. foveata</i>					c	b	a					
<i>A. mellea</i>	c	c	b	b	c	c	a		b	d	c	c
<i>A. nicolsonii</i>					c	b			b	b	b	b
<i>A. scrobiculata</i>					c	c	b				b	b
<i>A. spinosa</i>					c	c	b				b	b
<i>A. gerdemannii</i>	b	b	b	b					c			
<i>Entrophospora infrequens</i>												b
<i>Glomus ambisporum</i>	c	b			c	c		a		c	b	
<i>G. austral</i>	c							b				
<i>G. botryoides</i>					b					b		
<i>G. claroidium</i>									c	b		
<i>G. clarum</i>	d	c	b	b	d	d	c	b	e	d	e	c
<i>G. deserticola</i>	b								c	c		
<i>G. dimorphicum</i>	b											
<i>G. etunicatum</i>									c	b	b	b
<i>G. fasciculatum</i>	c	c			c	c	b		c	d	c	b
<i>G. heterosporum</i>					c	c	c			c	c	b
<i>G. hoi</i>	e	c	c	c	c	c	b		e	d	c	b
<i>G. intraradices</i>	c	c	b	b	d	c			d	d	b	b
<i>G. macrocarpum</i>									b	b		
<i>G. mosseae</i>	c	b	b	b					d	c	b	
<i>G. pustolatum</i>	c								b	b	b	
<i>G. versiforme</i>									b	b	a	
<i>Paraglomus occultum</i>									b	b	b	
<i>Scutellospora pellucida</i>									b	a		b

*Relative spore abundance: a, < 1%; b, 1-5%; c, 5-10%; d, 10-15%; e, > 15% of total VAMF spore population.

Table 2. Shannon-Weaver diversity index (H^I) and evenness (J^I) of VAM fungi associated with test medicinal plants at two different sites in different soil depths.

Site	Shannon Index with evenness	Soil depth (cm)				Total (Mean±SD)
		0-10	10-20	20-30	30-40	
Karaiya village Soil						
<i>Withania somnifera</i>	H^I	2.258	2.131	1.831	1.252	1.868±0.450
	J^I	0.88	0.89	0.88	0.90	0.88±0.009
<i>Ocimum sanctum</i>	H^I	2.20	2.048	1.818	0.899	1.741±0.580
	J^I	0.95	0.93	0.93	0.82	0.90±0.050
Jaitpur village Soil						
<i>Withania somnifera</i>	H^I	2.371	2.248	1.909	1.63	2.03±0.34
	J^I	0.84	0.83	0.87	0.91	0.86±0.03
<i>Ocimum sanctum</i>	H^I	2.04	2.143	1.947	1.767	1.974±0.15
	J^I	0.88	0.89	0.88	0.98	0.90±0.04

The Shannon-Weaver index value suggests that *W. somnifera* harbours more diverse morphotypes than *O. sanctum* (Table 2). Comparatively, soil of Jaitpur (H , 2.351) village harbour greater number of morphotypes in *W. somnifera* than of Karaiya (H , 2.250). However,

Shannon –Weaver index (H') value obtained from the different depth of rhizosphere of *O. sanctum* growing in Jaitpur village soil showed maximum value at the depth of 10–20 cm (2.143), and further deeper region showed linear decrease an H' value. *O. sanctum* growing Karaiya village showed maximum H' value up to 10 cm depth and below this H' value gradually decreased.

The evenness (J') of VAMF shows interesting trends, where there is little hike in J' value at 20–30 cm and 30–40 cm deep in soils from *W. somnifera* plants growing in Jaitpur village, at Karaiya village no such significant difference in J' value was observed (Table 2). Data of evenness (J') of VAMF in soils from *O. sanctum* in both the sites (*i.e.* Karaiya village soil and Jaitpur village) soil didn't showed definite trend. Where at Karaiya village soil J' value almost remains same up to 30 cm depth, with sudden significant reduction in J' further (Table 2). In contrast to this Jaitpur village soil J' value though remains same up to the depth of 30 cm but a significant increased at 40 cm depth (Table 2).

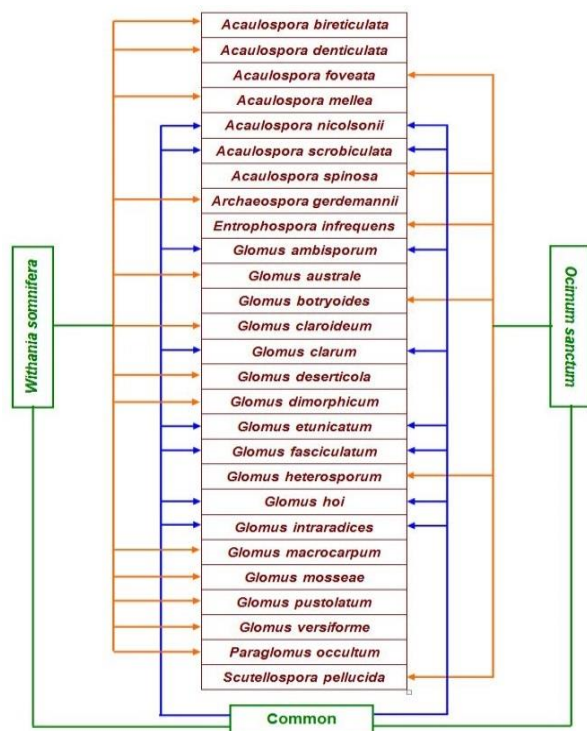


Figure 1. Distribution of VAMF species in the rhizosphere soil of *Withania somnifera* and *Ocimum sanctum*.

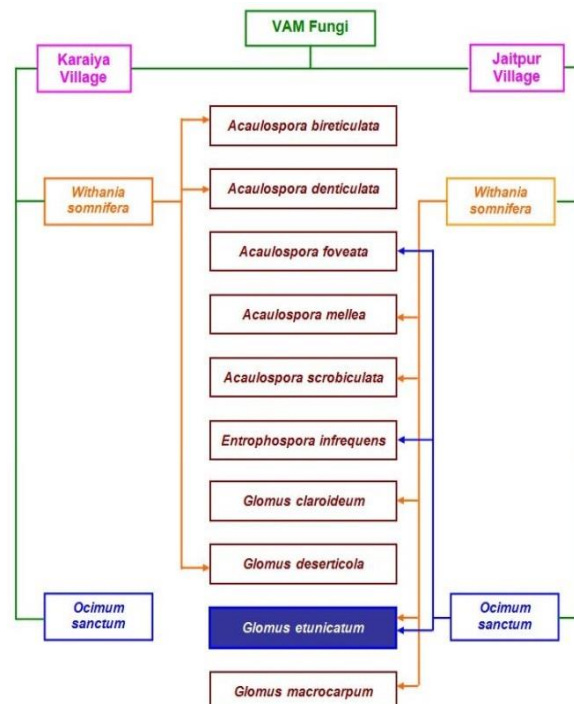


Figure 2. Occurrence of VAMF species associated with either *Withania somnifera* or *Ocimum sanctum* growing in Karaiya village and Jaitpur village.

The study revealed in total, 27 morphologically distinct VAM species isolated from the rhizosphere of *Withania somnifera* and *Ocimum sanctum* growing at the two study sites (Fig. 1). Out of 27 VAM fungal species, 13 different species were found associated only with *W. somnifera*, six species were found only with *O. sanctum* and eight species were found common in both the plants. Thus, a total of 21 species associated with *W. somnifera* and 14 species were found associated with *O. sanctum* (Fig. 1).

Among the 21 VAM species found associated with *W. somnifera*, five VAMF species viz. *Acaulospora mellea*, *A. scrobiculata*, *Glomus claroideum*, *G. etunicatum* and *G. macrocarpum* were not found in Jaitpur soil, whereas *A. bireticulata*, *A. denticulata*, *G. dimorphicum* were not found in Jaitpur village soil (Fig. 2). *Acaulospora* sp., *A. nicolsonii*, *G. clarum* and *G. hoi* were the prominent species of the VAM fungi which were isolated from surface to 40 cm. depths in the Karaiya village soil. *G. intraradices* and *G. mosseae* were isolated from the depth of 30 cm. *A. denticulata* and *Glomus* sp. were obtained from the depths of 10–20 and 20–30 cm. *G. ambisporum*, and *G. fasciculatum* were isolated from 0–10 and 10–20 cm depths. *A. bireticulata*, *G. australe*, *G. desrticola*, *G. dimorphicum*, and *G. pustolatum* were isolated from 0–10 cm depth in the Karaiya village soil (Table 1).

In the Jaitpur village soil, *A. nicolsonii*, *G. clarum*, *G. hoi* and *G. intraradices* were isolated from the topsoil to of 40 cm depth. *G. etunicatum*, *G. mosseae* and *G. versiforme* were collected from of 30 cm depth. *A. mellea* and *G. desrticola* were isolated from 0–10, 10–20, and 30–40 cm soil depth. *A. scrobiculata*, *G. australe*, *G. fasciculatum*, *G. macrocarpum*, and *G. pusotlatum* were isolated from 0–10 and 10–20 cm depth. *Acaulospora* sp. and *Glomus* sp. were isolated from 0–10 and 20–30 cm depth. *G. ambisporum* was isolated only 10–20 cm (Table 1).

Out of 27 VAMF species, 14 species were found associated with *O. sanctum* in both the sites (Fig. 1). Among the 14 VAMF species, three species viz. *A. foveata*, *Entrophospora infrequens* and *G. etunicatum* were not found in Karaiya village soil (Fig. 2). *A. nicolsonii* and *G. clarum* were the two VAMF species found very prominent in Karaiya village soil and isolated in all measured soil depth. *A. spinosa*, *G. fasciculatum*, *G. heterosporum* and *G. hoi* were isolated from the depth of 30 cm. Whereas, *A. scrobiculata*, *G. ambisporum* and *G. intraradices* were isolated from the depth of 20 cm. *G. botryoides* was isolated in topsoil (0–10 cm) and *Scutellospora pellucida* was isolated from 20–30 and 30–40 cm soil depth (Table 1).

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In Jaitpur village soil *Glomus clarum*, *G. fasciculatum* and *G. intraradices* were isolated from 40 cm depth. *A. nicolsonii*, *G. heterosporum* and *G. hoi* were collected from 30 cm depth while, *Acaulospora foveata*, *Glomus ambisporum* and *G. etunicatum* 20 cm depth. *A. spinosa* was isolated from 10–20, 20–30 and 30–40 cm soil depths, respectively. Here, also *Glomus botryoides* was isolated from the topsoil. *Entrophospora infrequens* was isolated from 20–30 and 30–40 cm depth and *Scutellospora pellucida* was isolated from 30–40 cm depth (Table 1).

The 14 VAMF species associated with *W. somnifera*, commonly occur in both the sites (*i.e.* Karaiya village soil as well as Jaitpur village soil) (Fig. 3). Among 14 VAMF species, 11 species associated with *O. sanctum*. It was also observed that 6 VAMF species viz. *Acaulospora nicolsonii*, *Glomus ambisporum*, *G. clarum*, *G. fasciculatum*, *G. hoi* and *G. intraradices* were found associated with both the test plants at in both the sites. However, three species *Acaulospora bireticulata*, *A. denticulata* and *Glomus desrticola* which are associated with *Withania somnifera* were found only in Karaiya village soil.

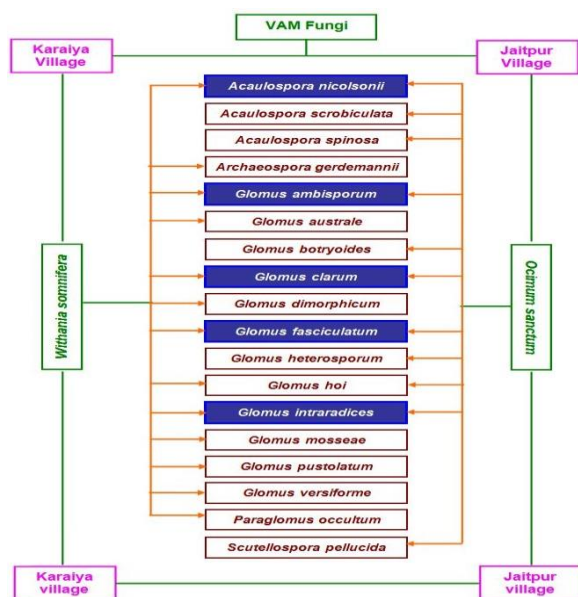


Figure 3. Common occurrence of VAMF species associated with *Withania somnifera* and *Ocimum sanctum* growing in Karaiya village or Jaitpur village.

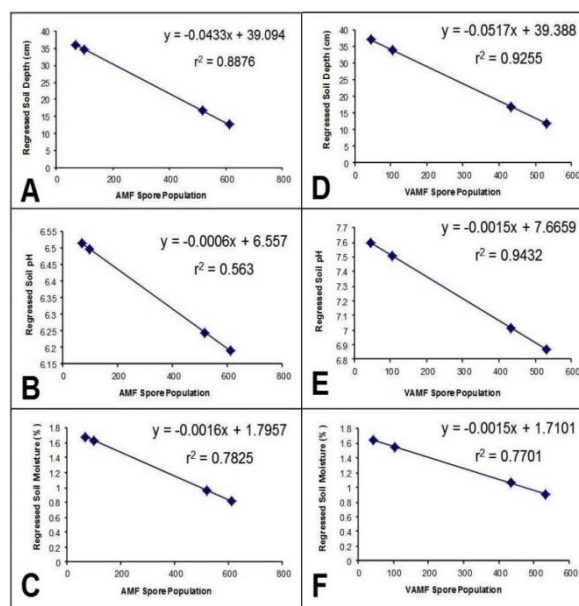


Figure 4. Regression of VA mycorrhizal fungal spore population with soil depth; soil pH; soil moisture percent in *Withania somnifera* (A–C) and *Ocimum sanctum* (D–F) at Karaiya village.

A linear regression analysis with coefficient of determination (= squared correlation coefficient or r²) of VAMF spore population with soil depth, soil pH, and soil moisture per cent in *Withania somnifera* and *Ocimum sanctum* at both the sites were presented in (Fig. 4 A–F) and (Fig. 5A–F). It is clearly evident from the result that the VAMF spore population showed a strong negative correlation with soil depth, pH and moisture of the soil. It is assumed that an increase in single variable (depth pH, or moisture) resulted in decrease in VAMF spore population in both the test plants at both the sites. In Karaiya village soil, depth and moisture of rhizosphere soil of both the test plants show highly significant correlation, while, variation found in correlation between soil pH and spore population of both the plants. In Karaiya village, VAMF spore population had weak correlation

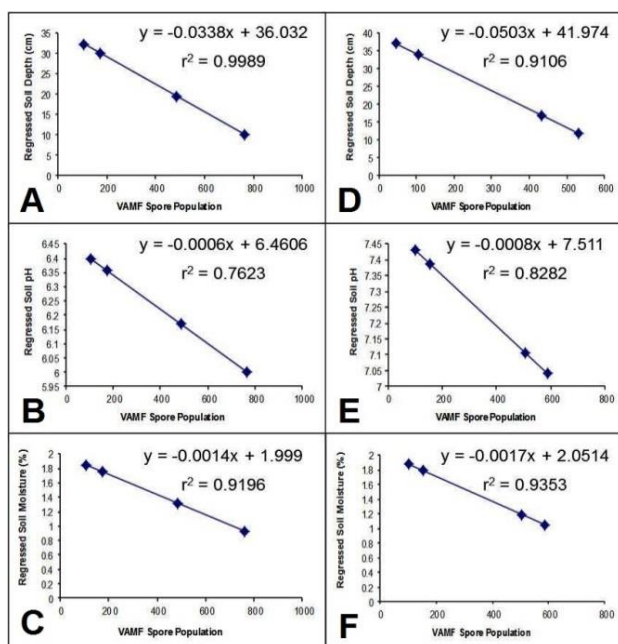


Figure 5. Regression of VA mycorrhizal fungal spore population with soil depth; soil pH; soil moisture percent in *Withania somnifera* (A–C) and *Ocimum sanctum* (D–F) at Jaitpur village.

Table 3. Comparative analysis of average values of soil pH, soil moisture, VAMF spore population and Shannon-Weaver diversity index with evenness at four soil depths from the Karaiya village and Jaitpur village

Soil Depth (cm) ^a	Soil pH (Mean±SD)	Soil moisture percentage (Mean±SD)	AMF spore population ^b (Mean±SD)	Shannon-Weaver diversity index (Mean±SD)	Evenness (Mean±SD)
0-10	6.55±0.46	0.97±0.10	623.88±91.64 ^A	2.217±0.13	0.88±0.04
10-20	6.62±0.56	1.09±0.07	485.75±40.89 ^{AB}	2.142±0.08	0.88±0.04
20-30	6.82±0.61	1.51±0.21	133.00±36.92 ^B	1.876±0.06	0.89±0.02
30-40	7.10±0.59	1.94±0.05	80.37±29.35 ^A	1.387±0.39	0.90±0.06

^a Treatments were compared using a one-way ANOVA and separated by Tukey's test.
^b Means in each column not sharing a common letter differ significantly (P ≤ 0.05).

($r^2=0.563$) with the pH of rhizosphere soil with *W. somnifera* in comparison to *O. sanctum* ($r^2=0.943$). In Jaitpur village soil, VAMF spore population showed similar trend as observed at Karaiya village soil with the depth and percent moisture of rhizosphere of both the plants. These two attributes significantly, correlated with the VAMF spore population (Fig. 5 A–F).

The data presented in table 3 show the comparative analysis of average values of soil pH, soil moisture, VAMF spore population and Shannon-Weaver diversity index at four soil depths from both the sites. The mycorrhizal population dropped significantly from the upper to lower soil depth level. Both the soils showed similar relationships for depths and mean total spore population (Fig. 6).

In the present study average soil moisture present initially increased two fold with the increasing depth (Fig. 7). Average soil pH found increased. Interestingly, soil pH values showed a general tendency to increase with increasing soil depth in both the site (Fig. 8).

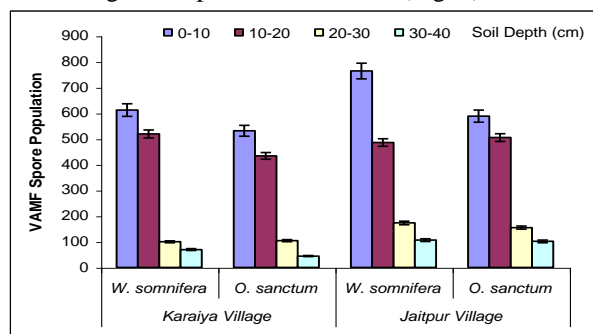


Figure 6. VA mycorrhizal spore population per 100 gm of rhizosphere soil of test medicinal plants in two different sites, at different soil depth.

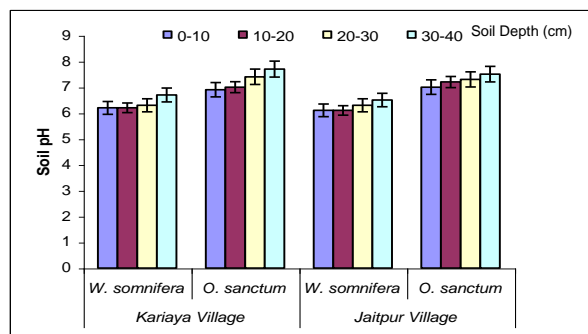


Figure 7. Soil moisture percent of rhizosphere soil of test medicinal plants in two different sites at different soil depth.

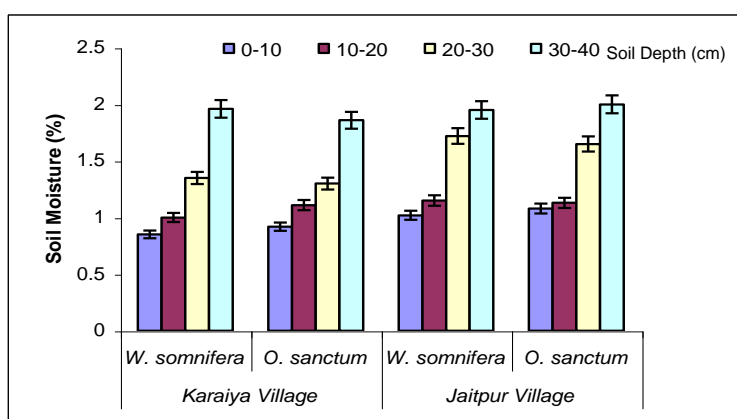


Figure 8. Soil pH of rhizosphere soil of test medicinal plants in two different sites at different soil depth.

DISCUSSION AND CONCLUSION

In the present study, the rhizosphere of two medicinal plants viz. *Withania somnifera* and *Ocimum sanctum* in different soil depth at two locations showed common as well as variant VAMF flora. Such variations in the VA mycorrhizal fungal community at different rhizosphere zone of plants have been reported earlier (Jakobsen & Nielsen 1983, 1986, Thompson 1991, Oehl *et al.* 2005). We investigated the rhizosphere soil over a depth range from surface to 40 cm depth. As expected from 0 to 20 cm depth the rhizosphere of both the plants contained the greater VA mycorrhizal fungal spore populations. Ecological studies on the community structure of arbuscular mycorrhizal fungi are generally restricted to the main rooting zone from 10 to 25 cm soil depth (Douds *et al.* 1995, Guadarrama & Alvarez-Sanchez 1999, Bever *et al.* 2001).

Data from both the site considered together, it was found that the fungal community composition changed with the soil depth, VA mycorrhizal fungal spore population were found decreasing with increasing soil depth. These data compliment the observations of Oehl *et al.* (2005) that VAM spore abundance and species richness decreased with increasing soil depth. Few studies also support, which done in the subsoil that increasing soil depth, a decrease was found in the percentage of roots colonized by AMF (Jakobsen & Nielsen 1983, Rillig &

Field 2003), in the number of infective propagules (An *et al.* 1990), and in the amount of extra radical AMF hyphae (Kabir *et al.* 1998).

In the present study maximum number of morphotypes as well as maximum percent population of spores was recorded under the genus *Glomus*. The genus *Glomus* is reported to be the dominant VAM fungi in some of the forest ecosystems (Sharma *et al.* 1986, Tamuli & Boruah 2002). Vyas & Soni (2004) and Vyas *et al.* (2006) have reported dominance of *Glomus* from Sagar. Dwivedi *et al.* (2004) suggested physico chemical properties of soil of Sagar are responsible for the occurrence of differential VAMF.

Here, many species were recorded in low numbers that too in one of the samplings only in the test sites. The rarity of some species may be an account of their narrow adaptability in contrast to *Glomus* species, which showed adaptability. Schenck & Kinloch (1980) attributed the abundance of *Glomus* species in the soils to their wide adaptability to different plants and environmental conditions.

Many species of VA mycorrhizal fungi were frequently found in the Jaitpur village. Interestingly, these species does not found in the Karaiya village soil such as *Aculospora foveata*, *A. mellea*, *A. scrobiculata*, *Entrophospora infrequens*, *Glomus claroideum*, *G. etunicatum*, and *G. macrocarpum*. However, their number decreases along with increasing soil depths. It is assumed that these VA mycorrhizal fungi, at least in central India preferentially inhabit undisturbed topsoil, rich in organic matter as occurring in Jaitpur village is as a good example. Another possibility is that they might need specific plant hosts.

Differences in VA mycorrhizal species in the rhizosphere region with two plants growing in two different soils may be attributed to the physico-chemical properties of both the soils. It is deduced from the results that soil of Jaitpur village is a natural soil, loamy in structure. Therefore, does not retain water, because pore size of soil particles is bigger which provide enough space for spores and mycelium to proliferate even in deeper zones. In contrast to the Karaiya village soil is a mixed soil having loam and clay 1:1 combination hence, it does not provides adequate space to VAMF spores to generate/ proliferate. Since, a clay soil particle has capacity to retain water, therefore moisture content in the soil remains for larger duration, which resulted in to poor occurrence of VAMF. Wet conditions are known for their deleterious effect on VAMF population (Dubey 2006).

Aculospora nicolsonii, *Archaeospora gerdemannii*, *Glomus clarum*, *G. fasciculatum*, *G. heterosporum*, *G. hoi*, *G. intraradices* and *G. mosseae* are frequently found in different rhizosphere zone with both the plants at both the sites. Oehl *et al.* (2003) called this type of VAMF species as AMF 'generalists' or even AMF 'weed' species (JPW Young Pers.com). We assume that even these AMF 'generalists' might fulfil different ecological functions.

Entrophospora infrequens and *Scutellospora pellucida* in particular associated with *O. sanctum* were found to occur more abundantly with increasing soil depth. Thus at least with respect to spore formation, these species appear to be specialized for deeper layers of the soils. This observation agrees with earliest findings of Mader *et al.* (2002), Jansa *et al.* (2003) and Oehl *et al.* (2004). The occurrence of *Scutellospora calospora* and *S. pellucida* spore were found to be negative correlated with soil contents of available phosphorous (Oehl *et al.* 2004). These findings suggest these possible reasons for the stimulation of development of *S. pellucida* in deeper soil layers, mainly the reduced mechanical soil disturbances and this effect to decreased supply of phosphorous.

In the present study there was highly negative significant correlation observed between soil parameters and fungal spore density in the samplings. The ability of the soil to support mycorrhizal population significantly decreases with increasing soil depth and is no doubt, greatly influenced by the total number of VA mycorrhizal propagules at a given depth. The average VA mycorrhizal spore population approaches zero at increased soil depths. Linear regression is a reasonably accurate statistical model for the data. However, mycorrhizae are absent at the soil surface, where there are no roots, yet linear models have a 'Y' intercept at zero depth. In reality, VA mycorrhizal spore population should be zero at the soil surface (zero depth), so linear models do not account for the absence of mycorrhizae at the soil surface. The use of narrow soil profiles (1–2 cm) for estimating fungal population could be a solution for developing a biological, nonlinear model that reflects the actual ability of the soil to support mycorrhizal formation.

Fibrous root systems such as those found in *W. somnifera* decrease with increasing soil depth. Data from cultivated soil (Sutton & Barron 1972, Smith 1978), from grassland soil (Sparling & Tinker 1975), and from semi-arid soil (Schwab & Reeves 1981) also support our results. These observations strongly support Redhead's (1977) conclusion that VAM decrease markedly below 15 cm and are consistent with similar observations of

Warcup (1951) for saprobic fungi. Mycorrhiza and fungal propagules of VAMF may occur at much greater depths in soil than those depths that we examined. It was found both colonization percent and intensity decreased with increasing depth in Tall grass or True prairie species, but *Glomus fasciculatum* was associated with forbs roots at depths to 220 cm.

These results suggest that spore viability may vary with soil moisture, and spore germination may occur at soil moisture levels that are not optimal for plant roots. Our data support previous observation of Trinick (1977) that the amount of moisture initially present in soil may affect mycorrhizal colonization of roots and thus the fungal spore density of soil. It was also observed that a significant linear relationship between moisture initially present in the soil and VA mycorrhizal spore population. Spore density of VA mycorrhizal fungi inversely propositional to moisture therefore losses the VAMF. Though relationship between soil moisture and spore population is highly significant relationship, get overriding factor is depth this can be justified simply by fewer roots, fewer mycorrhiza and fewer propagules in collected soil from lower depths.

Survival of VA mycorrhizal fungi and subsequent spore germination may depend on a species' adaptation and on the influence of physical parameters of the soil such as pH (Green *et al.* 1976). Friese & Koske (1991) found no significant correlation between VA mycorrhizal fungal spore clumping and soil pH. Bagyaraj (1991) points out that the interpretation of a pH effect on VAM fungal spore germination is difficult because many chemical properties of soil vary with changes in pH. Soil pH over a range of 4.8-8.0 significantly influenced germination of *Glomus epigaeum* Daniels & Trappe spores; optimum germination occurred at pH 7 (Daniels & Trappe 1980). The regression analysis of the VAMF spore population of the rhizosphere soil of test plants and soil pH shows a significant relationship. Spore density decrease as soil pH increases. Our results indirectly support Powell and Bagyaraj's (1984) conclusion that pH can influence spore germination in VAM fungal species, and that spore germination occurs within a range that is acceptable for plant growth. In spite of the significant relationship between soil pH and fungal population, the overriding factor seems to be the depth. The soil pH range covers less than one order of magnitude. As depth increases, there are fewer propagules to contribute to mycorrhizal population.

Direct cause and effect relationships between soil moisture or pH and mycorrhizal formation are equivocal. Peat & Fitter (1993) found no relationship between soil moisture and frequency of mycorrhizal colonization for British plants, and they reported that VAM occur at greater maximum soil pH values (*ca.* 6.0) than do ecto- or ericoid mycorrhizae. Soil from our study site ranged from pH 6.0 to 7.5. The occurrences of VAM at selected sites are consistent with the reports of Peat & Fitter (1993) and Read (1989). We conclude that soil pH has little direct effect on mycorrhizal population. Further Wang *et al.* 1993 had also reported field observations in Britain that percentage colonization and crop yield were little affected by soil pH ranging from 4.5 to 7.5.

This study shows that the frequency of genera and species of VA mycorrhizal fungi isolated from both the site varied with the above ground vegetation and with changes in soil moisture and soil pH. Currently, we have limited means for accurately determining the complex of genera and species that forming symbiosis with host plants in natural soil and that are responsible for variations in fungal density obtained from soil samples. Recent advancements in characterizing mycorrhizae with molecular markers will greatly improve our understanding of the ecology of these fungi.

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