J. Mycopathol, Res, ***49(1)*** *: 95—99 (2011)*

Influence of temperature, pH and moisture content of soil on dynamics of root colonization and spore density of VAM fungi in the rhizosphere of *Commiphora mukul* and *Moringa oleifera*

SUNITA KAUSHISH, AND ASHOK AGGARWAL
D.A.V. College for Girls, Yamuna Nagar, Haryana and Department of Botany, Kurukshetra University, Kurukshetra, Haryana 136 119

Received : 21.06.2010 Accepted : 07.02.2011 Published : 25.04.2011

The seasonal population dynamics of arbuscular mycorrhizal fungi was investigated in the rhizosphere of two medicinal plants. The effect and correlation of pH, temperature and moisture content of soil was studied. Sporulation was maximum during the winter season followed by summer season and minimum during rainy season in *Commiphora mukul*. Mycorrhizal root colonization was maximum during the summer season followed by rainy season and minimum during winter season. Negative correlation of spore count and root colonization with temperature was found to be significant at 0.05 level and correlation with pH and moisture content was found significant at 0.01 level. Spore count and root colonization were negatively correlated. In *Moringa oleifera*, though maximum mycorrhizal root colonization was observed during late summer season and minimum during winter season, the VAM spore density was maximum during the autumn or winter season ind minimum during summer or rainy season. Correlation of spore count and root colonization with temperature, pH and moisture content of soil was found to be significant at 0.01 level. In *M. oleifera* , all six VAM spore types (*Glomus, Acaulospora , Sclerocystis, Entrophospora* and *Gigaspora*) except *Scutellospora* were found whereas in *C. mukul*, only *Glomus, Acaulospora* and *Gigaspora* were found.

Key words: *Commiphora, Moringa,* VAM fungi, VAM spoes, VAM colonization, seasonal patterns

INTRODUCTION

The 'plant mycorrhizae pathogen environment complex' constitutes a standard condition to be maintained to ensure the sustainability of the environment. The spores of VAM fungi survive and perrenate for a long period in soil and germinate under suitable conditions when particular hosts are present. Germination, depends upon composition of native AMF communities, growth of congeneric plant species, change of season besides other biotic and abiotic factors and efficiency of the indigenous VAM fungi (Moore *et al.*, 2004, Muthukumar and Udaiyan, 2002). Host dependence of AM fungal population growth rates in soil may play an important role in the maintainance of AM fungal species diversity (Bever *et al.*, 1996) and suppression of mycorrhizal symbiosis may result in decreasing of the dominant plant species and an increase in species diversity (Hartnett and Wilson, 1999). In addition, plant diversity may increase or decrease if the dominant plant competitors are more weakly or strongly mycotrophic than their neighbours. Because the spore count and root colonization are not necessarily correlated with each other (Mendoza *et al.*, 2002), and the rate of extension of mycorrhiza formaton is not always related to spore density (Abbott and Robson, 1982), there is always a difficulty in studying the relationship among soil characteristics, plant species and climate with AM fungi and the magnitude of participation of AM fungi in plant nutrition. Seasonal fluctuations in moisture, temperature and soil nutrients status show high and dramatic effects on AM spore population, percentage of root colonization and their distribution as reported by Hasan and Khan (2006); Bouamri *et al.*(2006),. Soil moisture (Ruotsalainen *et al.*, 2002), temperature (Lugo *et al.*, 2003) growth rate and turnover of

plant roots are among the most frequently proposed drivers of AM seasonality. AMF community may vary with host plants even within the same ecosystem with different cropping patterns (Lskberg *et al.*, 2008; Lekberg and Koide, 2005). These differences are significant not only in terms of species composition but also in their seasonal dynamics. The knowledge of native AMF communities with *Commiphora mukul* and *Moringa oleifera* is not yet known. Such a study is very important in identifying and utilizing the most suitable AMF species for large scale inoculation programmes for conservation of medicinal plants in Haryana. The present study has thus been undertaken to study the seasonal changes in the rhizosphere of *Commiphora mukul* and *Moringa oleifera*.

MATERIALS AND METHODS

Roots and soil samples from the rhizosphere of the plants under study were collected at the end of each month. Five different plants of each type were randomly selected for study. About 10 g of the rhizospheric soil along with fine roots of 2 mm diameter were collected from 15-30 cm deep soil from each plant and taken in polythene bags. These samples were then mixed together to form a composite sample and stored at 5°C for further studies. Three replicates were taken for further analysis from each composite sample.

Estimation of VAM root colonization

Mycorrhizal root colonization in all the plants was studied by 'Rapid clearing and staining technique' (Phillips and Hayman, 1970).

Estimation of VAM spore population

VAM spores were isolated from soil samples using 'Wet sieving and decanting technique' (Gerdemann and Nicolson, 1963) and the quantification of VAM spores was done by 'Grid-line intersect method' (Adholeya and Gaur, 1994).

Identification of VAM fungal spores

intact VAM spores were examined and identified by using the manual of Schenck and Perez (1987), Morton and Benny (1990) and Mukerji (1996).

Soil moisture content was determined every month by taking 25 g of soil and weighed. It was dried at 105°C, cooled and weighed again to note down the loss of weight on drying. The moisture percentage was calculated by the following formula : Moisture % = Loss of weight on drying/Initial sample weight × 100

The pH variation was also recorded for the samples studied by mixing soil and distilled water (1:2) using digital pH meter.

Temperature of the soil was also recorded by soil-thermometer in the morning.

The data was analysed statistically using Pearson correlation method.

RESULTS AND DISCUSSION

The rhizosphere can be considered as a dynamic environment determined by reciprocal interaction between soil, plants and microflora associated with roots. The present study was undertaken to assess the rate of VAM colonization, arbuscule and vesicle formation in the root and endomycorrhizal spore population in the rhizosphere of *C. mukul* and *M. oleifera*.

The distribution of AM fungal species exhibited wide range of variation within different months of the year and it was related to soil pH, temperature and moisture content of the soil. In *C. mukul* (Table 1), sporulation increased from September to January (91.0 ± 2.44) and decreased from February onwards and finally decreased in August (11.3 ± 2.49). The highest amount of VAM colonization (100 ± 0) was observed during the months of July and September, whereas the lowest amount of colonization (42.3 ± 2.05) was observed during the month of January. Therefore, sporulation was maximum during the winter season followed by summer season and minimum during the rainy season. The mycorrhizal root colonization was maximum during summer season followed by rainy season and minimum in winter season. In *M. oleifera* (Table 2), maximum number of VAM spores were observed in the month of September (82.3 ± 2.05) and minimum in the month of July (16.0 ± 0.81). Mycorrhizal colonization was sparse during the month of December (31.3 ± 1.41) and increased during the month of June (91.9 ± 2.03). Though maximum mycorrhizal colonization was observed during late summer and minimum during winter season, the VAM spore density was maximum during autumn or winter season and was minimum during summer or rainy season.

Table 1 : Seasonal variation of mycorrhizal association in *Commiphora mukul*

Months	Mycorrhizal Spore-count/10 g. of soil	Percentage mycorrhizal root-colonization	pH of soil	Temperature of soil	Moisture content of soil
January	91.0 ± 2.44	42.3 ± 2.05	8.5 ± 0.41	21.8 ± 0.08	20.0 ± 4.08
February	32.7 ± 1.69	48.3 ± 6.23	7.5 ± 0.41	20.5 ± 00.12	12.3 ± 1.70
March	27.7 ± 1.24	80.4 ± 0.58	7.3 ± 0.25	29.1 ± 0.09	8.0 ± 1 63
April	25.7 ± 1.69	73.9 ± 0.94	7.0 ± 0.40	32.2 ± 0.08	8.7 ± 2.49
May	22.7 ± 5.55	52.3 ± 2.05	7.2 ± 0.21	32.5 ± 0.08	6.0 ± 1.63
June	17.7 ± 2.05	60.7 ± 4.52	6.5 ± 0.41	31.4 ± 0.12	4.2 ± 0.24
July	18.3 ± 1.24	100 ± 0	6.4 ± 0.43	30.3 ± 0.09	9.0 ± 2.94
August	11.3 ± 2.49	85.0 ± 4.08	6.1 ± 0.30	29.6 ± 0.12	5.0 ± 0.41
September	52.3 ± 2.05	100 ± 0	8.0 ± 0.41	29.6 ± 0.12	20.0 ± 4.08
October	62.0 ± 1.63	63.3 ± 1.24	8.1 ± 0.25	27.3 ± 0.25	17.7 ± 2.05
November	37.3 ± 1.69	51.3 ± 1.20	7.9 ± 0.33	23.1 ± 0.08	14.0 ± 4.32
December	80.3 ± 1.24	73.2 ± 1.28	8.2 ± 0.22	22.0 ± 0.12	22.0 ± 1.63
Correlation value	0.1	-0.288	**0.897	*-0.648	**0.920

** Correlation is significant at the 0.01 level Mean of three replicates
* Correlation is significant at the 0.05 level (±) Standard deviation

Table 2 : Seasonal variation of mycorrhizal association in *Moringa oleifera*

Months	Mycorrhizal Spore-count/10 g. of soil	Percentage mycorrhizal root-colonization	pH of soil	Temperature of soil	Moisture content of soil
January	45.7 ± 1.24	60.0 ± 14.1	7.6 ± 0.25	22.4 ± 0.17	11.3 ± 1.25
February	29.7 ± 1.24	63.6 ± 7.43	7.4 ± 0.29	21.4 ± 0.16	8.7 ± 2.62
March	24.7 ± 2.05	87.0 ± 4.93	7.4 ± 0.25	29.2 ± 0.16	6.5 ± 0.41
April	31.0 ± 2.94	87.5 ± 1.51	7.9 ± 0.25	32.0 ± 0.05	15.7 ± 3.30
May	30.0 ± 4.89	90.3 ± 4.92	7.1 ± 0.16	32.5 ± 0.08	22.0 ± 1.63
June	17.7 ± 2.05	91.9 ± 2.03	7.3 ± 0.30	31.2 ± 0.12	4.4 ± 0.26
July	16.0 ± 0.81	82.4 ± 6.54	6.3 ± 0.21	30.2 ± 0.08	4.9 ± 0.54
August	76.7 ± 2.49	77.3 ± 2.06	8.0 ± 0.21	29.9 ± 0.08	25 ± 4.08
September	82.3 ± 2.05	76.3 ± 6.54	8.2 ± 0.16	29.3 ± 0.05	22 ± 1.63
October	60.7 ± 2.86	72.4 ± 0.79	8.0 ± 0.41	28.1 ± 0.05	20.7 ± 2.49
November	54.3 ± 2.62	72.4 ± 0.79	8.0 ± 0.41	22.9 ± 0.08	17.3 ± 2.05
December	44.7 ± 2.49	31.3 ± 1.41	7.9 ± 0.29	22.0 ± 0.05	14.3 ± 3.29
Correlation value	0.1	-0.270	**0.787	**0.821	**0.807

** Correlation is significant at the 0.01 level
Mean of three replicates
(±) Standard deviation

From the results it was clear that AM fungal root colonization and spore density had seasonal dynamics. The growth of the host and spore density as well as root colonization are a function of plant/fungus combinations (Sanders and Fitter, 1992). The seasonal changes as well as the different range of spore-density and root colonization are due to a wide range of hosts. The sporulation rates of AM fungi have been found to be host dependent. This is probably one of the reasons that the plants under study have shown different results when compared. The relationship between AMF colonisation and soil moisture may be associated with the development of the root system due to increase in water content of soil, with the formation of new roots, simultaneous increase in nutrients absorption and liberation of root exudates, stimulating mycorrhizal spore germination and subsequent infection. Tender cortical tissue of new roots may be more susceptible to infection and colonization by AMF (Oliviera *et al.*, 2005). Lugo and Cabello (2003) studied seasonal variation in a South American grassland and mentioned maximum root colonization, number of arbuscules and vesicles in summer season. This is related to the condition that higher value of root colonization in summer-autumn period could be due to the capacity of the AM fungi

to obtain higher profits and growth at higher temperature and abundant rainfall. Similar was the observation of Gavito *et al.*, (2003). Mendoza (2004) studied seasonal variation of AMF in temperate grasslands of Argentina and related soil moisture with total spore density which was reported to be highest in summer and lowest in winter season. In *C. mukul* and *M. oliefera* the moisture content was low (4-25%) and accordingly the number of spores was lower. Similarly Pandey and Tarafdar (2002) while working on neem suggested 30-60% available water for maximum infection of VAM fungi and recorded increased VAM infection and highest biomass with increasing moisture content but to a certain optimum level. Similar study by Sankaranarayanan and Sundarababu (2001) on black gram explained that moisture levels of 60-70% were favourable for the establishment of *G. mosseae* and mycorrhizal colonization increased as moisture level increased up to 70% but declined above 70% moisture level. Temperature determines the VAM fungal activity. Maximum activity was found between 20-25°C in the plants under study. Posada *et al.*, (2008) studied seasonal dynamics of AMF with relation to physical, chemical and environmental factors in *Brachiaria decumbens* and found that AM fungi were influenced by all these factors. Heinemeyer and Fitter (2004) investigated the effect of temperature on AMF in *Plantago lanceolatus* and *Holcus lanatus* and found that percentage of colonized roots positively correlated with temperature in *P. lanceolatus* and no significant effect of temperature was seen in *H. lanatus*. Ingham and Wilson (1999) suggested that intense soil solarization killed mycorrhizal spores and reduced plant root colonization potential. Li *et al.*, (2005) studied the effect of 10°C, 15°C and 23°C temperature on AM colonization and reported reduced activity at sub-optimal temperature i.e. 10°C and maximum activity at 23°C. Shah and Zafar (2006) attributed rhizospheric pH to be responsible for differential AM colonization of pine seedlings and reported higher soil pH (6.39) to be showing higher levels of AM propagules and acidic soils with pH 5.34 to be fungistatic in nature. Mendoza (2004) also correlated spore density with nitrogen, phosphorus and pH of soil. Maximum root colonization and number of AM spores was reported at pH 7.2–7.4 by Selvaraj *et al.* (2001) whereas pH 6-7 was reported to be the best for mycorrhizal development by Sankaranarayanan and Sundarababu (2001). Minimum root colonization and maximum VAM spore number was observed in the month of September in *C. mukul* and *M. oleifera* which is supported by the observation of Fontenia *et al.* (1998).

The lesser number of spores in *C. mukul* and *M. oleifera* as well as their occurrence in dry season was related to low nutrient availability and plant phenology along with other soil features. It is in accordance with the study of Kumar (2002). Maximum root colonization in July in *C. mukul* is in confirmity with Mago and Mukerji (1994) where they observed 80.3% root colonization in the month of July. More arbuscular development was found to be due to the formation of more appressoria during moist conditions. The formation of more arbuscules and vesicles in *M. oleifera* might be due to an efficient association of the mycorrhizal fungi with the host. The significant increase in spore abundance in rainy season coincided with a sharp decline in the rate of germination of spores. It could be stated that the developing stage of the host plant and whether the plant is metabolically active could be important factor in the survival of AM fungi and spore production suggesting thereby that rainy season may be considered as the best season for the propagation of *Commiphora* and *Moringa* by the application of AM fungi as bioinoculants particularly for plants which are the red listed ones.

ACKNOWLEDGEMENTS

The author (SK) is thankful to University Grants Commission, New Delhi, India for providing teacher fellowship.

REFERENCES

Abbott, L. and Robson, A. 1982. Infectivity of vesicular arbuscular mycorrhizal fungi in agricultural soil. *Aust. J. Agric. Res.*, **33:**1049.

Adholeya, A. and Gaur, A. 1994. Estimation of VAMF spore in soil. *Myco. News*, **6**(1): 10-11.

Al-Karaki, G.M.; Michael, B. and Zak, J. 2004. Field response of wheat to arbuscular mycorrhizal fungi and drought stress. *Mycorrhiza*, **14(4)**: 263-269.

Bever, J.; Morton, J.; Antonovics, J. and Schultz, P. 1996. Host dependent sporulation and diversity of arbuscular mycorrhizal fungi in mown grassland. *J. Ecol*, **84:**71-82.

Bouamri, R.; Dalpe, Y.; Serahini, M. N. and Bennani, A. 2006. Arbuscular mycorrhizal fungal species associated with rhizosphere of *Phoenix dactylifera* L. in Morocco. *African J. Biotech.*, **5(6):** 510-516.

Fontenia, S.; Goday, R.; Rosso, R. and Havrylenko, M. 1998. Root association in *Austrocedrus* forests and seasonal dynamics of arbuscular mycorrhizas. *Mycorrhiza*, **8**: 29-33.

Gavito, M.E.; Schweiger, P. and Jakobsen, I. 2003. Phosphorus uptake by AMF hyphae: effect of soil temperature and atmospheric $C0_2$ enrichment. *Global Change Biology*, **9(1)**: 106-116.

Gerdemann, J.W. and Nicolson, T.H. 1963. Spores of mycor-

rhizal Endogone species extracted from soil by wet sieving and decanting. *Trans. Brit. Mycol. Soc*, **46:** 235-244.

Hartnett, D. and Wilson, G. 1999. Mycorrhizae influence plant community structure and diversity in tall grass prairie. *Ecology*, **80:**1187-1195.

Hasan, A. and Khan, M.N. 2006. Seasonal dynamics of vehicular arbuscular mycorrhiza and parasitic nematode *Holicotylenchus indicus* on phalsa. *Myco. News*. **18(3):** 9-12.

Heinmeyebr, A. and Fitter, A.H. 2004. Impact of temperature on the arbuscular mycorrhizal symbiosis: growth responses of the host plant and its AM fungal partner. *J. Expt. Bot.*, **5(396):**525-534.

Ingham, E.R. and Wilson, M.V. 1999. The mycorrhizal colonization of six wetland plant species at sites differing in land use history. *Mycoirhiza*, **9:** 233-235.

Kumar, G.S. 2002. Seasonal variations in the biodiversity of arbuscular mycorrhizal fungi in forest ecosystems. *J. Ecol.,* **14(1):** 35-38.

Lekberg, Y. and Koide, R.T. 2005. Arbuscular mycorrhizal fungi, rhizobia, available soil P and nodulation of groundnut in Zimbabw. *Agric. Environ.* **110:**143-118.

Lekberg, Y.; Koide, R. T. and Twomlow, S. J. 2008. Effect of agricultural management practices on arbuscular mycorrhizal fungal abundance in low input crooping systems of Southern Africa: A case study from Zimbabwe. *Biol Fertil. Soils*, **44:**917-923.

Li, L.F.; Yang, A. and Zhao, Z.W. 2005. Seasonality of arbuscular mycorrhizal symbiosis and dark septate endophytes in a grassland site in southwest China. *FEMS Microbiol. Eco.*, **54(3):** 367-373.

Lugo, M.A.; Gonzalez Maza, M.E. and Cabello, M.N. 2003. Arbuscular mycorrhizal fungi in a mountain grassland II. Seasonal variation of colonization studied along with its relation to grazing and metabolic host type. *Mycologia*, **95(3):**407-415.

Mago, P. and Mukerji, K.G. 1994. Vesicular arbuscular mycorrhizae in Lamiaceae.I. Seasonal variation in some members. *Phytomorphology*, **44(1&2):** 83-88.

Mendoza, R. 2004. Seasonal variation of arbuscular mycorrhizal fungi in temperate grasslands along a wide hydrologic gradient. *Mycorrhiza*, **10:** 8-15.

Mendoza, R.; Goldmann, V.; Rivas, J.; Escudero, V.; Pagani, E.; Collantes, M. and Marban, I. 2002. Poblaciones de hoi.gos microrrizicos arbusculares en relacion con propiedades del suelo y planta hospedante en pastizales de Tierra del Fuego. *Ecol. Austr.*, **12:**9-20.

Moore, M.; Opik, M.; Sen, R. and Moore, M. 2004. Native arbuscular mycorrhizal fungal communities differentially influence the seedling performance of rare and common Pulsatilla species. *Functional Ecol.*, **1(4):** 554-562.

Morton, J.B. and Benny, G.L, 1990. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes), A new order Glomales, two new suborders, Glomineae and Gigasporineae and two new families, Acaulosporaceae and Gigasporaceae. *Mycotaxon*, **37:** 471-491.

Mukerji, K.G. 1996. Taxonomy of endoniycorrhizal fungi. In: *Advances in Botany*, (Eds.) Mukerji, K.G., Mathur, B., Chamola, B.P. and Chitralekha, P., APN Pub. Corp. New Delhi, Pp: 211-221.

Muthukumar, T. and Udaiyan, K. 2002 Seasonality of vesicular arbuscular mycorrhizae in sedges in semi arid tropical grassland. *Acta Oecologica Int. J. Ecol.*, **23(5):** 337-347.

Oliveira, A.N., Luiz, A. and Oliveira, D. 2005. Seasonal dynamics ol arbuscular mycorrhizal fungi in plants of *Theobroma grandiflorum* Schum. and *Paullinia cupana* Marl, of an agroforestry system in Central Amazonia, Amazonas State, Brazil. *Brazilian J. Microbiol.*, **36(3):** 9-17.

Pandey, M. and Tarafdar, J.C. 2002. Effect of phosphorus, salinity and moisture on VAM fungal association in neem (*Azadirachta indica* Linn.). *Symbiosis*, **32(3):** 195-209.

Phillips, J.M. and Hayman, D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of colonization. *Trans. Brit. Mycol. Soc*, **55:** 158-160.

Posada, R.H.; Franko, LA.; Romas, C.; Plazas, L.S.; Suarez, J.C. and Alvarez, F. 2008. Effect of physical, chemical and environmental characteristics on arbuscular mycorrhizal fungi in *Brachiaria decumbens* (Stapf) pastures. *J.Appl. Microbiol.* **104(1):**132-140.

Ruotsalainen, A. L.; Vare, H. and Vestberg, M. 2002. Seasonality of root fungal colonization in low alpine herbs. *Mycorrhiza*;, **12:**29-36.

Sankaranarayanan, C. and Sundarababu, R. 2001. Influence of moisture and pH cn the efficiency of vesicular arbuscular mycorrhiza, *Glomus mosseae* against *Meloidogyne incognita* on black gram (*Vigna mungo* L). *J. Biol. Control*, **15(1):** 69-72.

Schenck, N.C. and Perez, Y. 1987. *Manual for the identification of vesicular arbuscular mycorrhizal fungi.* Publ. INVAM Florida University Gainesville, USA, Pp: 245.

Selvaraj, J.; Murugan, R. and Bhaskaran, C. 2001. Arbuscular mycorrhizal association of *Cichorium intybus* L. in relation to physico-chemical characters. *Myco. News*, **13(2):** 14-16.

Shah, A.M. and Zafar, R. 2006. Incidence of arbuscular mycorrhizal colonization of *Pinus wallichiana* in different forest stands of Kashmir Himalaya. *Myco. News*, **17(4):** 11-14