

## VIABILITY OF VAM SPORES FROM WHEAT FIELDS IN SOIL BASE CULTURE UNDER STORAGE

Q.M.K. ANWAR\* AND M. JALALUDDIN

Department of Botany,  
University of Karachi, Karachi-75270, Pakistan.

### Abstract

Axenic soil base VAM cultures of 6 different *Glomus* spp., viz., *Glomus fasciculatum*, *G. macrocarpum*, *G. monosporum*, *G. mosseae*, *G. radiatum* and *G. warcupii* extracted from the soil of wheat field and stored separately in pots at 5 and 10 ( $\pm 1$ )°C or kept under natural conditions showed a gradual loss in viability in 2 years. Loss in viability of spores was significantly higher ( $p < 0.001$ ) when the axenic culture of *Glomus* spp., were stored under natural condition with temperature ranging from 10-25°C during winter and 22-38°C during summer season. *G. macrocarpum* retained highest viability ( $p < 0.001$ ) after 2 years.

### Introduction

Vesicular arbuscular mycorrhizae (VAM) are ubiquitous soil borne symbiotic fungi which provide an intimate link between the soil and nutrient absorbing organs of plants (Harley & Smith, 1983). VAM fungi optimize the uptake of phosphorus in plants which results in increase in yield (Khan, 1972; Harley, 1989). Production of VAM inocula and their storage in a viable condition for a longer period of time is still a serious constraint (Ferguson & Woodhead, 1982). The present report describes the survival capability of spores of 6 different *Glomus* species under three different storage conditions.

### Materials and Methods

Spores of *Glomus* spp., viz., *G. fasciculatum* (Thaxter) Gerdemann & Trappe emend. Walker & Koske, *G. macrocarpum* Tulasne & Tulasne, *G. mosseae* (Nicholson & Gerdemann) Gerdemann & Trappe, *G. warcupii* McGee, *G. radiatum* Trappe & Gerdemann and *G. monosporum* Gerdemann & Trappe were extracted from soil of wheat field of Sindh using centrifugal floatation technique (Jenkins, 1964). Axenic soil base VAM culture containing soil, vermiculite, spores and hyphae were separately air dried at 5% moisture level as per recommendation of Ferguson & Woodhead (1982). In each axenic soil base VAM culture, spores were considered as main infective unit. The cultures were then put into steam sterilized 8 cm diam., plastic pots @ 300g/pot. There was 3 replicates of each treatment and the pots were randomized in a growth chamber at 5 and 10°C. A comparable set of pots were kept under natural condition in a screen house. The viability of VAM spores was determined by Vital Stain method (Menge & Timmer, 1982) after extraction of spores from culture (Jenkins, 1964) at 0, 180, 360,

\*Department of Biology, DHA Degree College (Men), Khayaban-e-Rahat, Phase VI, DHA, Karachi, Pakistan.

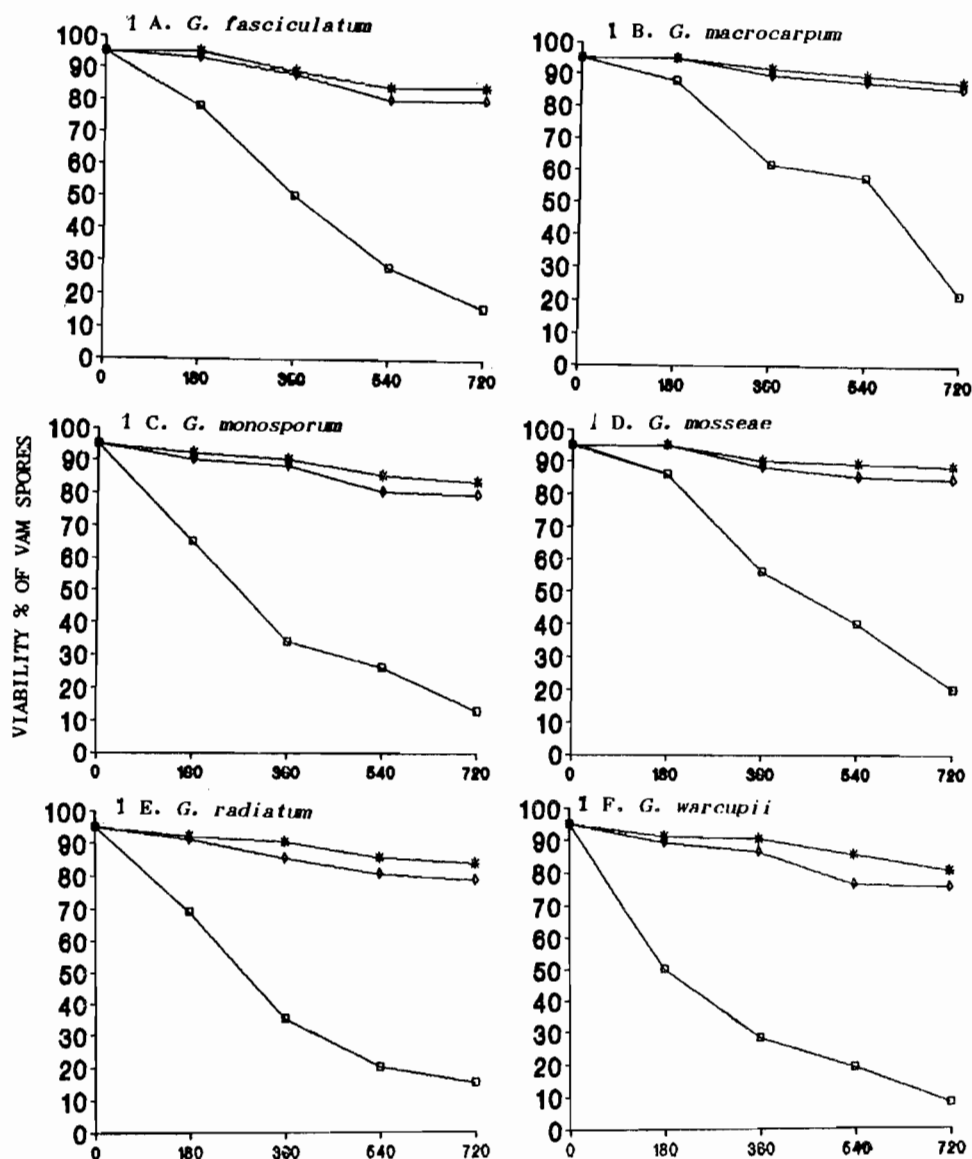


Fig. 1. Reduction in viability of spores of *Glomus* spp. under storage.

\* = 5°C, ◇ = 10°C □ = Natural Conditions

LSD<sub>0.05</sub> (Time) = 3.05, LSD<sub>0.05</sub> (Temperature) = 2.36, LSD<sub>0.05</sub> (*Glomus* species) = 3.36.

540 and 720 days interval. Viability percentage of VAM spores was determined by the formula given below:

$$\text{Viability of VAM Spores} = \frac{\text{No of viable spores}}{\text{Total No. of spores studied}} \times 100$$

## Results and Discussion

Spores of all the six *Glomus* spp., showed 95% viability at 0-days. During storage for upto 720 days the viability reduced to 80-88% and 75-86%, respectively, at 5 and 10°C. Storage of VAM spores under natural conditions in a screen house showed greater decline in the viability of spores with the passage of time since 8-22% viability was recorded after 720 days (Fig.1). Temperature in the screen house showed considerable fluctuation during the storage period (Table 1).

The data on the viability percentage of VAM spores at 720 days was subjected to means of FANOVA (Table 2). Result of the analysis showed that all the 6 *Glomus* species differed significantly in the viability with the passage of time ( $p < 0.001$ ). The data on viability collected under 3 different temperature conditions also differed significantly ( $p < 0.001$ ) in respect of the viability percentage. Interactions between *Glomus* species and temperature and time and temperatures were found significant ( $p < 0.001$ ) whereas the interactions between *Glomus* species and time; time-temperature-species was non-significant.

There are various reports which indicate that with the passage of time the viability of VAM spores is generally reduced. Mosse (1981) found VAM spores viability to be optimum during the first year, declining in the second year with complete loss in viability in the third year which is in accordance with our result. Haris *et al.*, (1987) also found that the infectivity of older VAM spores was less than that of the freshly stored spores, whereas, Daniels & Skipper (1982) reported that if the inoculum is dried and stored for two weeks the ability of infection (viability) is reduced. Similarly Mohankumar & Mahadevan (1988) observed that the number of spores and quantum of infection decline sharply after 6 months of storage and no VAM infection was formed after 18 months. Our results corroborate well with all these findings. Louis & Lim (1988) found a better germination percentage of VAM inocula stored upto 6 months at 25 to 30°C

**Table 1. Fluctuation in Temperature under national canditions.**

Time	Minimum temperature °C	Maximum temperature °C
<b>Winter season</b>		
Day	15 (± 2.5)	25 (± 1.4)
Night	10 (± 1.6)	18 (± 2.8)
<b>Summer season</b>		
Day	25 (± 2.5)	38 (± 4.4)
Night	22 (± 2.2)	31 (± 3.4)

**Table 2. Factorial analysis of variance (Fanova) on the viability of van spores in pot culture.**

Source of variation	Sum of squares	DF	Mean square	F	Probability
Time (Months)	37803.68	04	9450.92	145.70	<0.001
Temperature (Conditions)	85788.35	02	42894.17	661.30	<0.001
VAM species	4201.85	05	840.37	12.95	<0.001
<b>Interaction</b>					
Time x Temperature	29753.31	08	3719.16	57.33	<0.001
Time x Species	1461.42	20	73.07	1.12	Ns
Temperature x Species	2550.62	10	255.06	39.33	<0.001
Time x Temperature x VAM species	1307.71	40	32.69	0.50	Ns
Error	11675.33	180	64.86		
Total	174542.27	269			

and was still better in storage at 4°C. The results also indicated that storage at 5°C was good as compared to 20°C under natural conditions. During the present study storage of VAM culture under natural condition where temperature was harsh and fluctuated a great deal, showed a severe decline in the viability of spores which support the results of Daft *et al.*, (1987) who found that VAM spores from different countries differed with respect to the longevity when exposed to different environment. This indicates a correlationship between viability of VAM spores and environmental conditions. The results of the present study would suggest that VAM spores in the form of axenic soil base culture could be maintained at low temperature of 5°C for upto 720 days with little loss in viability since it showed much sensitivity to high temperature fluctuations. The reason for the reduction in viability of VAM spores with the passage of time needs investigation.

### Acknowledgement

The research work is a part of Ph.D thesis of the senior author. Research grants received by the junior author from the National Scientific Research and Development Board (NSRDB) of the University Grants Commission and Pakistan Atomic Energy Commission (PAEC), Islamabad, Pakistan, are gratefully acknowledged.

## References

- Daft, M.J., D. Spencer and G.E. Thomas. 1987. Infectivity of vesicular arbuscular mycorrhizal inocula after storage under various environmental conditions. *Trans. Brit Mycol. Soc.*, 88: 21-27.
- Daniels, B.A. and H.D. Skipper. 1982. Methods for the recovery and quantitative estimation of propagules from soil. pp.29-35. In: *Methods and Principles of Mycorrhizal Research*. (Ed.) N.C. Schenck. Am. Phytopath. Soc., St., Paul. Minnesota, U.S.A.
- Ferguson, J.J. and S.H. Woodhead. 1982. Production of Endomycorrhizal inoculum. A. Increase and maintenance of vesicular arbuscular mycorrhizal fungi. pp. 47-54. In: *Methods and Principles of Mycorrhizal Research*. (Ed.) N.C. Schenck. Am. Phytopath. Soc., St., Paul. Minnesota, U.S.A.
- Haris, J.A., D. Hunter, P.Brich and K.C. Short. 1987. Vesicular arbuscular mycorrhizal population in stored top soil. *Trans. Br. Mycol. Soc.*, 89: 600-603.
- Harley, J.L., 1989. The significance of mycorrhiza. *Mycol. Res.*, 92: 129-139.
- Harley, J.L. and S.E. Smith. 1983. *Mycorrhizal Symbiosis*. Academic Press, London, pp. 483.
- Jenkins, W.R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Dis. Rep.*, 48: 692.
- Khan, A.G. 1972. The effect of vesicular arbuscular mycorrhizal association on growth of cereals. I. Effect on maize growth. *New Phytol.*, 71: 613-619.
- Louis, I. and G. Lim. 1988. Effect of storage of inoculum on spore germination of a tropical isolate of *Glomus clarum*. *Mycologia*, 80: 157-161.
- Menge, J.A. and L.W. Timmer. 1982. Procedure for inoculation of plants with vesicular arbuscular mycorrhizae in the laboratory, Green house and Field. pp. 59-68. In: *Methods and Principles of Mycorrhizal Research*, (Ed.) N.C. Schenck. Am. Phytopath. Soc. St. Paul Minnesota, U.S.A.
- Mohankumar, V. and A. Mahadevan. 1988. Viability of VAM spores in a tropical forest soil. *Mycorrhizae for Green Asia*. Proceedings of the First Asian Conference on Mycorrhizae (Eds.). A Mahadevan, N. Raman and K. Natarajan, Centre of Advanced Studies in Botany, University of Madras, Guindy Campus, Madras, India, pp. 89-90.
- Mosse, B. 1981. *Vesicular Arbuscular Mycorrhizal Research for Tropical Agriculture*. Res. Bulletin, p. 194.

(Received for publication 24 September, 1997.)