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EFFECT OF BENTONITE AND TEMPERATURE ON VIABILITY OF ARBUSCULAR MYCORRHIZAL FUNGI (*GLOMUS intraradices*) SPORES USED IN BIOFERTILIZER

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ABSTRACT Arbuscular Mycorrhizal Fungi and bentonite both are used as biofertilizer. The spores of Arbuscular Mycorrhizal Fungi lose their viability over a period of time due to factors like storage, temperature and the carrier material used for the preparation of bio-fertilizers. So, it is very important to check the viability of spores in carrier material which is going to be used for the preparation of biofertilizer at different temperatures and to find out means to increase their shelf life. The aim of our experiment was to check the effect of bentonite as a carrier of biofertilizer on the viability of AMF spore at two different temperatures. Bentonite was purchased from the local vendor. AMF spores (*Glomus intraradices*) were kept at two different treatments of bentonite i.e. saturated bentonite and semi saturated bentonite at two different temperatures that is room temperature (30±20C) and at low temperature (15±20C) for the period of 30 days. Spore viability can be determined quickly by vital stain technique using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide(MTT) (An & Hendrix 1988).After 1, 10, 20 and 30 days the viability was checked by using MTT stain. The highest no. of viable spores was observed in semi saturated bentonite at low temperature i.e., 23.00±2.00 and the lowest no. of viable spores was observed in saturated bentonite at room temperature i.e., 16.00±1.00 and other treatments showed average viability i.e 17.67±1.15 and 18.67±0.53. The observed viable spore showed colors ranging from brick red, purplish blue, dark purple and pinkish purple. Non- viable spores remained unstained or black.

KEYWORDS : Bentonite, *Glomus intraradices*, Carrier, Biofertilizer, MTT stain.

INTRODUCTION

Arbuscular Mycorrhizal Fungi is one of the soil organisms which show the beneficial relation with the plants. AMF infect the plant in four different stages i.e. formation of hyphae, arbuscules, vesicles, and the spore formation, it also provide all the macro and micro nutrients phosphorus, zinc, copper and sulfur to the plants and help in its proper growth by increasing the length in root by forming hyphae, therefore, Arbuscular Mycorrhizal Fungi is used as bio-fertilizer (Vishwakarma et.al, 2023; Bücking et.al 2012). But the viability of Arbuscular Mycorrhizal Fungi is affected the factors like temperature and carrier which is used in bio fertilizers. Therefore, Maintenance of the viability of AMF is biggest task. So it is very important to check the viability of spore in carrier material which is used for preparation of bio fertilizer at different temperatures and to find out different ways to increase their shelf life. For increase the life of organism present in the bio-fertilizer suitable carrier is required. The carrier is the delivery material of live microorganisms from the processing plant to the field. It represents as a major element of the inoculants and has a crucial significance in the delivery of the correct number of viable cells in good physiological condition. The carrier should be cost effective and eco-friendly and also improve viability during storage. Bentonite clay is one of the naturally occurring clay which shows the best absorption property, and it is also used in chemical free bio-fertilizers (R. S. Smith 1992). It also helps the plant for their proper growth.

MATERIALS AND METHODS

Experimental Set-up:

The bentonite based AMF were tested at two different treatments of bentonite i.e. saturated bentonite and Semi saturated bentonite with spore of *Glomus intraradices*, which was identified from the rhizospheric soil of coleus plant which was previously cultivated in green house of N.E.S Ratnam College. Treatments were stored for the period of 30 days at two different temperatures that is room temperature (30±2^{0}C) and at low temperature (15±2^{0}C). Three replicates were made for each treatment.

Preparation Of Saturated Bentonite:

For preparing the saturated Bentonite, 30g of autoclaved bentonite powder was mixed with an equal amount of distilled water i.e. 30 ml.

Preparation Of Semi Saturated Bentonite:

30g of autoclaved bentonite powder was mixed with 15ml of distilled water in container and closed the container and kept for 2 days to retain the water contain and then crushed it in mortar and pestle to make powder.

Viability Test:

Viability of spore was checked by MTT staining method (Singh & Chahar, 2021; An & Hendrix 1988; Walley & Germida 1995). 30 spores from each replicate were treated with MTT 3-(4, 5 dimethylthiazol-yl-2, 5-diphenyl-2H-tetrazolium bromide) for 48 hours and observed under electron microscope.

RESULTS

Table no. 1 shows the viable spore count stored for 30 days in two treatments of bentonite and at two temperatures.

The no. of viable spores in the saturated bentonite were 27.33±2.08 and 28.67±1.53 on first day and 16.00±1.00 and 17.67±1.15 on 30th day at room temperature (30±2^{0}C) and low temperature (15±2^{0}C) respectively.

Which was very much less than the semi saturated bentonite i.e. 28.33±0.58 and 29.67±0.58 on first day and 18.67±0.53 and 23.00±2.00 on 30th day at room temperature (30±2^{0}C) and low temperature(15±2^{0}C) respectively.

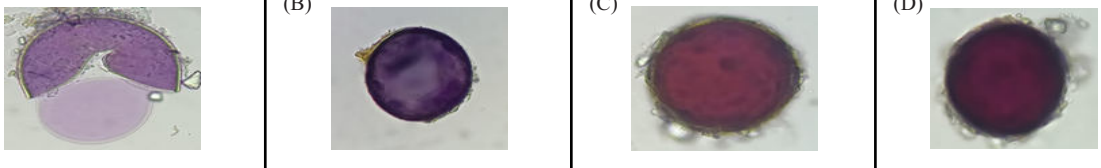
As per our study the semi saturated bentonite shows maximum count of viable spores. In Fig. 1, after the treatment of MTT the viable spores show brick red, purplish blue, dark purple and pinkish purple and non-viable spores remain unstained or black.

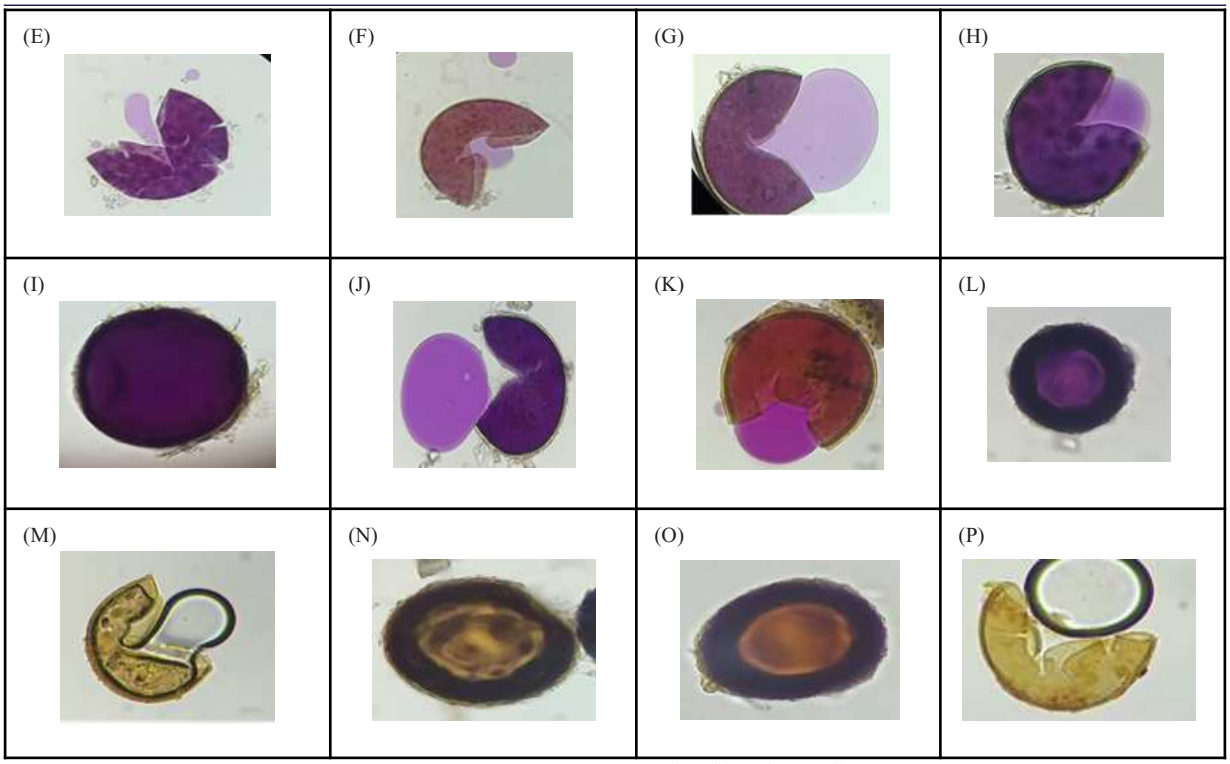
Table 1: Influence Of Bentonite and temperature On The Viability Of AMF Spores.

Treatments of Bentonite		Day 1	Day 10	Day 20	Day 30
Saturated Bentonite	R.T	27.33±2.08	23.00±1.73	17.67±1.53	16.00±1.00
	L.T	28.67±1.53	24.67±1.53	21.00±1.00	17.67±1.15
Semi saturated Bentonite	R.T	28.33±0.58	23.00±1.00	19.00±2.00	18.67±0.53
	L.T	29.67±0.58	27.33±0.58	24.67±1.15	23.00±2.00

*R.T-Room temperature (30±2°C) and L.T - Low temperature (15±2°C), Mean ± STD.

(A) (B) (C) (D)





- **Viable spores: Brick red, purplish blue, dark purple and pinkish purple (A-L)**
- **Non- viable: spores remained unstained or black (M-P).**

Fig1. MTT Stained Spores Showing Viability.

DISCUSSION

For preparing AM based biofertilizer the first aspect which we should understand is viability of AMF spores. For preparing the biofertilizer different carriers were used. The carriers which prepare biofertilizer should be ecofriendly and have good effects on plants as well as on viability of organism which was stored in the same carrier material (Singh & Chahar, 2021). It was also recorded that bentonite is the best carrier for the AMF with the small amount of water, but the amount of water is not known for better viability of Arbuscular Mycorrhizal Fungi. This study was carried out by using two different treatments based on water content and temperature i.e., saturated bentonite and semi saturated bentonite at two different temperatures that is room temperature 30±2^{0}C and low temperature 15±2^{0}C. As per our experiment bentonite is good carrier for the viability of Arbuscular Mycorrhizal Fungi. It shows 23±2 viable spores (in semi saturated bentonite at low temperature 15±2°C.) and the same results were also recorded by Ritu et al. 2012; Kremer et al 1983; Paczkowski et al 1979. As per our results the treatment which is stored at low temperature showed more no. of viable spores compared to room temperature 30±2^{0}C. According to B. A. Daniels &J. A. Menge 1980, bentonite stored at low temperature i.e.15±2^{0}Cshows best viability.

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