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#### SURVIVAL OF *AZOSPIRILLUM BRASILENSE* IN DIFFERENT CARRIERS

NITROGEN fixation by *Azospirillum brasilense*<sup>1</sup> is maximum at 25°–35° C<sup>1</sup> and hence it is important to test the efficacy of this organism on various crops under tropical field conditions. One of the prerequisites for large scale field studies is the selection

of a suitable carrier material for this organism. It has been reported<sup>2</sup> that soil and farm yard manure (FYM) in the ratio of 1 : 1 is a good carrier and at room temperature (upto a maximum of 35° C), the organism survived upto seven months in this carrier. No quantitative information is available on the proliferation and survival of *A. brasilense* in different carriers at different periods of storage. An attempt has been made to assess the survival of the organism in different carriers quantitatively and the results are reported here.

Six carrier materials, soil, FYM, [soil + FYM (1 : 1)], [soil + FYM + clay (vermiculite) (5 : 3 : 2)], [FYM + clay + charcoal (5 : 2 : 3)] and [soil + FYM + clay + charcoal (1 : 5 : 2 : 2)] were dried to 3% moisture at 80° C and ground to pass through 200 mesh sieve, and the pH of the carriers adjusted to 7.0 with calcium carbonate or with 1 N sulphuric acid. They were sterilized at 15 lb pressure for 4 h in an autoclave for three consecutive days.

A mixture of three-day-old broth cultures of *A. brasilense* (one isolated from the roots of rice var. 'Madhu' and another from *Cynodon dactylon*, a weed grass, grown individually in Okon's medium) was used for inoculation, adding just enough broth to adjust the moisture level to 50% of their water holding capacity. The carriers after mixing with the culture of *A. brasilense* were packed in polyethylene bags and incubated at room temperature (30°–35° C).

TABLE I

*Survival of Azospirillum brasilense in different carriers at room temperature (30°–35° C)*

Treatments	Days of sampling (mean of four replications)							
	Initial	15	30	60	90	120	150	180
	(× 10 <sup>8</sup> )	(× 10 <sup>10</sup> )	(× 10 <sup>10</sup> )	(× 10 <sup>10</sup> )	(× 10 <sup>10</sup> )	(× 10 <sup>10</sup> )	(× 10 <sup>8</sup> )	(× 10 <sup>8</sup> )
Soil	57.7	1.2	0.001	—	—	—	—	—
Farm yard manure (FYM)	66.0	18.8	31.8	3.5	—	—	—	—
Soil + FYM (1 : 1)	44.3	217.0	700.0	120.0	60.8	10.5	32.0	4.0
Soil + FYM + clay (vermiculite) (5 : 3 : 2)	46.0	351.3	50.8	8.2	—	—	—	—
FYM + clay + charcoal (5 : 2 : 3)	5.3	83.8	29.8	0.7	—	—	—	—
Soil + FYM + clay + charcoal (1 : 5 : 2 : 2)	33.3	28.0	18.8	0.6	—	—	—	—

— : no count at this dilution.

The viability of the organism in different carriers was monitored at regular intervals thereafter, by plating dilutions of the different carriers on a modified Okon's medium<sup>3</sup> containing bromothymol blue (Lakshmi-Kumari *et al.*—unpublished).

The population of *Azospirillum* in different carriers reached a maximum by 15 days of incubation at room temperature with the exception of soil + FYM where it reached a maximum at 30 days (Table I). The combination of the soil and FYM gave higher (*Azospirillum* count than the individual carriers. Besides holding high moisture, the obvious virtues of FYM lie in its ability to improve the surface area and porosity of the carriers<sup>5</sup> when used in combination with soil so as to facilitate increased *Azospirillum* growth. Addition of FYM also enhances the organic matter content of the carrier which in turn is reflected in the survival of *Azospirillum* as well.

In most of the cases, the population of *Azospirillum* was maintained upto a level of  $10^{10}$  in different carriers even after 120 days of storage except in the case of the soil, where the count was considerably reduced by 30 days of incubation at room temperature (Table I). However, it is interesting to note that the combination of soil and FYM helped to retain the population of *Azospirillum* ( $400 \times 10^7$ ) even upto six months' storage. These results are in conformity with our earlier findings<sup>2</sup> on the survival of the organism in different carriers upto 31 weeks.

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### BOLL ROT OF COTTON FROM AGRA

DURING September–November 1977, a large number of cotton bolls were found rotted in the fields exhibiting mycelial growth and discolouration. The present communication deals with the nature and extent of such boll rots.

For isolation of associated fungi, small bits of surface sterilized, diseased bolls were plated on PDA and Czepek's media and incubated for a week at 28°C ( $\pm 2^\circ$ C). Five fungi, viz., *Aspergillus flavus* Link, *A. niger* van Tieghem, *Cladosporium cladosporioides* (Fres) de Vries, *Cephalosporium roseogriseum* Saksena and *C. asperum* Marchal were found consistently associated with the diseased bits.

The pathogenicity tests were conducted with all the isolates in the laboratory and in the field. During field trials the atmospheric temperature ranged between 22–23.5°C and relative humidity between 60–65%; whereas in the laboratory, the inoculated bolls were incubated at 60 (approximately near field level) and 90% relative humidity (approximately the average level available during rainy season).

The inoculations were made by three methods; (1) pricking the sterilized boll surface with a fine needle and immediately after the injury, the inoculations were made with each of the above fungi separately; (2) the respective fungi were inoculated on uninjured healthy boll surfaces; and (3) the bolls were pressed so that their valves were separated slightly at the top and immediately after, the inoculum was placed on the edge of the valves. Subsequently, the inoculated bolls, in each case were covered with sterilized polythene bags. In the field tests, however, the bags were removed after three days to provide the natural conditions. Ten replicates were used for each fungus under each treatment. Respective controls were maintained. Reisolations from inoculated bolls were made, following the standard procedures. The maintenance of relative humidity and calculation of per cent rot was done by the procedure and formula suggested by Prasad and Bilgrami<sup>2</sup>.

Under field tests only *C. roseo-griseum* caused some rotting when inoculated on injured fruits but not on uninjured while others failed to infect.

In the laboratory tests, only *A. flavus* and *C. roseo-griseum* caused 4 and 8.5% rot, respectively, at 60% relative humidity. However, at 90% relative humidity all the isolates except *C. cladosporioides* were pathogenic and displayed increased virulence as compared to that at 60% relative humidity. The differential symptoms and severity of rots induced by the respective pathogen at 90% relative humidity may be summarized as follows: (a) *A. flavus* produced dry, brown rots to the extent of 10 and 23% on uninjured and injured fruits respectively. *A. niger*