

## Short Communication

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# EFFECT OF STORAGE TEMPERATURE ON BIOFERTILIZER PREPARATION FOR TREE-LEGUME

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The viability of two rhizobia cultures on lignite based biofertilizer under different storage conditions, was studied. The optimum temperature for storage of the biofertilizer was found to be  $28\pm 1^\circ\text{C}$ .

**Key Words :** Biofertilizer, storage temperature, tree-legumes.

In view of fast depleting energy resources reliance is increasing on biological nitrogen fixation, scientists have harnessed the process of biological nitrogen fixation at commercial level in the form of biofertilizers in agricultural field but its use in forestry has been limited (Hedge, 1986). Successful applications of legume inoculation depends upon reorganizing and establishing the complementary conditions required by both the partners in the symbiosis. The selection of suitable strain of rhizobia is one of the most important criteria in producing effective legume inoculant (Biswal and Mishra, 1988). It is well established fact that effective inoculant based on its capacity to fix nitrogen fixation for the host for which the culture was recommended, under a wide range of field conditions.

*Dalbergia sissoo* and *Leucaena leucocephala* are the most promising multipurpose fast growing tree legumes have been used in the present investigation and the effect of temperature for the storage of biofertilizer (*Rhizobium* inoculant preparations) for survival of *Rhizobium loti* symbiotic with *Leucaena leucocephala* and *Bradyrhizobium* sp. symbiotic with *Dalbergia sissoo* has been investigated.

Two cultures namely, *Rhizobium loti* 2378 and *Bradyrhizobium* sp. 2380 isolated from *Leucaena leucocephala* and *Dalbergia sissoo*, respectively, were used in this study.

Lignite was used as carrier for biofertilizer. It was finely ground (100 mesh) and the pH adjusted to 6.8. About 200 gm of lignite was filled in polypropylene bags (size 127-X 1780mm & 0.076-mm thickness and sterilizable); and sealed. The carrier sterilized at  $121^\circ\text{C}$  for 2 h on three successive days in an

autoclave before inocula preparation and its supplementation. Sterility of the carrier was checked by plating on yeast extract mannitol agar medium (YEMA) containing congo red as described by Muniruzzaman and Khan (1992).

Both the bacterial cultures were grown in YEM broth in a rotary shaker at 200 rpm and incubated at  $28\pm 1^\circ\text{C}$  for 5 days. The number of cells per ml was observed by spread plate method. An amount of 75 ml ( $2 \times 10^8$ ) of broth was added with carrier and mixed aseptically for individual strain. The inoculant packages were incubated for 1 week at  $28^\circ\text{C}$  and then kept under three different temperature  $5\pm 1^\circ\text{C}$  for 5 days. The number of cells per ml was observed by spread plate method. An amount of 75 ml ( $2 \times 10^8$ ) of broth was added with carrier and mixed aseptically for individual strain. The inoculant packages were incubated for 1 week at  $28^\circ\text{C}$  and then kept under three different temperature  $5\pm 1^\circ\text{C}$ ,  $28\pm 1^\circ\text{C}$  and  $38\pm 1^\circ\text{C}$  for 17 weeks. The number of viable rhizobia under each temperature was determined by plate count every 2 weeks in triplicate on YEM agar plates supplemented with congo red (25 g/ml) (Muniruzzaman and Khan 1992).

Both *R. loti* and *Bradyrhizobium* species were applied in such a way that viable cells count remain  $2 \times 10^8 \text{ ml}^{-1}$ . Under all the three different temperature, it was observed that mean growth of isolates was maximum on room temperature. The count of viable cells  $\text{ml}^{-1}$  of *R. loti* 2378 recorded higher at  $28\pm 1^\circ\text{C}$  temperature, the viable cell number of *R. loti* 2378 increased sharply up to 5th week and thereafter a gradual decline was observed until 17th week. The highest count in number of cells was recorded during

Table 1. Viability of *Rhizobium loti* 2378 (*Leucaena leucocephala*) and *Bradyrhizobium* sp. 2380 (*Dalbergia sissoo*) under different temperature.

Temperature	Culture	Viability (Log CFU ml <sup>-1</sup> )								
		Duration (weeks)								
		1	3	5	7	9	11	13	15	17
5±1°C	2378	7.50	7.80	8.10	8.50	8.60	8.40	8.10	8.00	7.40
	2380	7.40	7.70	7.90	8.20	8.10	7.75	7.55	7.51	7.15
28 ±1°C	2378	8.10	9.60	9.80	9.70	9.40	9.10	8.70	7.90	7.10
	2380	8.20	9.80	9.50	8.95	8.90	8.85	7.87	7.61	7.10
38±1°C	2378	7.10	7.00	7.90	6.80	6.50	6.40	6.30	6.20	5.50
	2380	7.20	6.90	6.80	6.60	6.50	6.27	5.70	4.10	4.00

Values mean of three replicates.

Values highly significant at 5% probability level.

storage of inoculants at 30±2°C by Muniruzzaman and Khan (1992) in case of *Sesbania* rhizobia.

Most reports have indicated better survival of rhizobia under refrigeration (Chao and Alexander, 1984; Rodriguez-Navarro, 1991). Since large-scale refrigeration facility for storage of the inoculants is quite expensive, good survival of inoculant cultures at room temperature constitutes a desirable property for developing countries.

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