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Azospirillum - a Possible Biocontrol agent for Seedvine Rot of Betelvine (*Piper betle* L.)

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ABSTRACT

Seedvine rot is one of the major problems of betelvine cultivation. Dipping of betelvine seedvine in *Azospirillum* suspension before planting resulted in 100 per cent rooting in 14 days of planting and reduced the seedvine rot by 84 per cent under field conditions. The association of *Azospirillum* with the roots of betelvine is established.

KEY WORDS: *Piper betle*, *Azospirillum*, seedvine rot

Betelvine (*Piper betle* L.), one of the commercial crops requiring high level of inputs, suffers due to several diseases causing concern to farmers (Marimuthu and Samiyappan, 1982). In Tamil Nadu, betelvine is raised through seedvines collected from one year-old garden. Normally two vines are planted near agathi (Sesbania), the support crop by inserting the vines into a hole formed by a peg. At least two nodes are burried in soil and the rest of the vines are allowed to lie flat on the bed and mud balls are placed at nodal regions. Under normal conditions the seedvine strikes roots after 3 weeks of planting. Seedvines often rot before striking roots due to *Phytophthora* and other saprophytic fungi. The incidence of seedvine rot ranges from 5 to 20 per cent. Dipping the seedvine in Bordeaux mixture (0.5%)+Streptocycline 500 ppm solutions upto 3-4 nodes for 30 min has been reported to contain seedvine rot (Marimuthu *et al.*, 1982). However, no information is available on the biological control of seedvine rot in betelvine.

Further, the field observations made by the author revealed that the seedvines normally escaped rotting after striking roots. The association of *Azospirillum* with the roots of betelvine has been reported by Marimuthu *et al.* (1986). *Azospirillum* is known to produce growth promoting substances which enhanced root, shoot and dry matter production in several crop plants (Tilak and Negi, 1987). Hence, the present study was undertaken to find out the usefulness of *Azospirillum* in hastening rooting and control of seedvine rot of betelvine.

MATERIALS AND METHODS

i) Isolation of Azospirillum: The local varieties of betelvine *viz.*, Karuppupachai kodi, Vellaipachai kodi and Karpoori were used for isolation of *Azospirillum* and further studies were carried out using Karpoori. The technique of isolation of *Azospirillum* from rhizosphere and rhizoplane is essentially the same as detailed by Dobereiner and Day (1976). The root samples were collected from six months-old betelvines from the fields and washed free of the soil. The roots were cut into small bits, surface-sterilized with 10 per cent sodium hypochlorite for one minute and washed with changes of sterile distilled water. One or two of such root bits were plunged into test tubes containing 5 ml of nitrogen-free semisolid malic acid medium (Okon *et al.*, 1976). The tubes were incubated at 28°C for 3 to 5 days by which time sub-surface, white pellicles were formed in the tubes. By following the standard methods of isolation, the cultures of *Azospirillum* were purified and maintained on soytrypticase agar slants.

Table 1. Occurrence of *Azospirillum* in the roots of betelvine

Varieties	Population of *Azospirillum*	
	Rhizosphere*	Rhizoplane@
Karuppupachaikodi	6.5×10^4	12.0×10^4
Vellaipachaikodi	7.8×10^4	3.4×10^4
Karpoorikodi	12.8×10^5	2.2×10^4

* Per g of soil on dry weight basis
@ Per g of root tissue on dry weight basis

ii) Quantitative determination of Azospirillum:

Soil samples along with the roots were brought to laboratory, rhizosphere and rhizoplane (plain root surface) samples were separated out and the samples were placed in sterile physiological saline for dilution purposes. Following the most probable number technique (MPN) the population of *Azospirillum* in the samples was enumerated (Cochran, 1950). Based on the taxonomic keys of Tarrand *et al.* (1978) the cultures of *Azospirillum* were identified.

iii) Pot culture studies:

Peat-based inoculum of *Azospirillum* prepared in packets (250 g) containing Ca. 3.6×10^8 cells per g of peat on dry weight basis was used for seedvine treatment. For comparison, bordeaux mixture (0.5%) + streptocycline (0.05%) which is a standard recommendation for seedvine treatment and a commercially available rooting medium Seradix-B were used. The cut end of seedvines were allowed to touch Seradix-B powder kept in Petri dish and tapped against the plate to remove excess Seradix-B. The vines were dipped in *Azospirillum* solution (one pkt of *Azospirillum* in 20 litres of water giving Ca. 14×16^6 cells/ml) or dipped in chemical or water control for 30 min. Soil collected from betel gardens were filled in 15 cm pots and each pot was planted with two vines. Each treatment had 50 vines. Pots were kept in humid chamber for 14 days and then the seedvines were removed. The number of seedvines showing root formations, the number of roots per vine and length of roots were recorded.

iv) Field trial:

Seedvines after treatment with chemicals and *Azospirillum* were planted in beds as per the standard method followed in the local area for trench type of cultivation. Each treatment had 50 hills (100 seedvines) and the experiment was replicated five times. One day before lifting of vines, the number of seedvines rotted were counted. Ten hills were selected at random from each treatment and the vine height and number of branches were recorded.

RESULTS AND DISCUSSION

All the three varieties of betelvine which are cultivated in Tamil Nadu *viz.*, Karpoori, Karuppupachai and Vellaipachai kodi had fairly high counts of *Azospirillum* in their rhizosphere. Karpoori contained relatively larger

Table 2. Effect of *Azospirillum* on rooting of seedvines (Karpoori)

Treatment	No. of roots/seedvine	Length of roots (mm)	Per cent root formation
Azospirillum - dip	5.2	30.0	100.0
Seradix - B - dip	3.3	15.0	85.0
Bordeaux mixture 0.5% + Streptocycline 0.05%	1.5	5.0	50.5
Control (water)	0.0	0.0	0.0
CD (P=0.01)	0.47	0.83	

Table 3. Effect of *Azospirillum* on seedvine rot of betelvine (Karpoori).

Treatment	One day before lifting of vine		
	Seedvine rot (%)	Vine height (cm)	Branches (Nos.)
Azospirillum - dip	5.7	56.4	6.4
Seradix - B - dip	15.6	50.9	5.0
Bordeaux mixture 0.5% Streptocycline 0.05%	12.8	49.8	4.9
Control (Water)	35.9	49.7	4.5
CD (P=0.01)	1.20	1.81	0.68

population of *Azospirillum* in the rhizosphere while the other two recorded higher population in the rhizoplane (Table 1). The data on the effect of *Azospirillum* on rooting of seedvines are presented in Table 2. The seedvines dipped in *Azospirillum* showed 100 per cent rooting on 14th day with an average of 5 roots per vine which were 30 mm in length while control treatment showed no rooting. The data on seedvine rot recorded in the field (Table 3) revealed 84 per cent reduction of seedvine rot due to *Azospirillum* treatment followed by Bordeaux mixture + Streptocycline (64.3%) and Seradix-B (56.5%) over control. *Azospirillum* treatment also significantly increased the number of productive branches and vine height.

The present study clearly showed that dipping of seedvines in the *Azospirillum* suspension could greatly enhance the rooting in two weeks while untreated normal cuttings took nearly three weeks to strike roots. The hastening of rooting, probably because of the hormonal effect, greatly helped in containing the seedvine rot. As seedvine rot largely occurs due to *Phytophthora* and other saprophytic fungi, early rooting avoided this problem. Interestingly, the vine height and number of productive branches were also increased which meant a positive influence on leaf yield as reported by Marimuthu *et al.* (1986).

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