

## EFFECT OF PLANT PROTECTION CHEMICALS ON *BRADYRHIZOBIUM* COWPEA (*VIGNA UNGUICULATA*[L] WALP)

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**Abstract:** Effect of selected plant protection chemicals were tested on *Bradyrhizobium* both in the laboratory and in the field. The fungicides Thiram, Bordeaux mixture and Fytolan showed inhibition of the bacteria under *in vitro* conditions. These fungicides caused an initial reduction in rhizosphere population of *Bradyrhizobium* in the field. But later a gradual increase in the bacterial count was noticed. The fungicide Carbendazim and insecticides Carbofuran, Phorate and HCH showed no inhibition of *Bradyrhizobium* under laboratory and field conditions. Combinations of all the three insecticides with Thiram, Bordeaux mixture and Fytolan showed inhibition of the bacteria in *in vitro*. The rhizosphere bacterial population showed an initial reduction when these were applied in the soil.

**Key words:** *Bradyrhizobium*, cowpea, fungicides, insecticides, *Vigna unguiculata*.

### INTRODUCTION

Inoculation of legumes with *Bradyrhizobium* is a well established and successful practice to ensure adequate nitrogen nutrition in place of fertilizer nitrogen. Legume-rhizobium symbiosis is the most promising one because it supplies approximately 80-90 per cent of the total nitrogen requirement of legumes. Cowpea is one of the most important legumes, forms an important crop in tropical regions. Cowpea-rhizobium symbiosis is well known for its high nitrogen fixing ability. Large number of plant protection chemicals have been used to control pests and diseases. Even though these will help a lot to protect the crop from pests and diseases, these may also affect the nod-target organisms including beneficial micro-organisms. Here some fungicides, insecticides and their combinations were tested on *Bradyrhizobium* both in the laboratory and in the field.

### MATERIALS AND METHODS

Native strain of *Bradyrhizobium* was isolated from root nodules of cowpea plants collected from the fields of the College of Horticulture, Vellanikkara, Trichur. Yeast extract mannitol agar medium (Allen, 1953) was used for isolation and purification of the bacteria. Morphology, Gram reaction and cultural characters were studied to confirm the identity of the organism. An *in vitro* study was conducted to find out the effect of plant protection chemi-

cals on *Bradyrhizobium*. It was tested by paper disc method. The treatments were as follows:

Fungicides	Conc., ppm
F <sub>0</sub> Control	
F <sub>1</sub> Carbendazim	1000
F <sub>2</sub> Thiram	2000
F <sub>3</sub> Bordeaux mixture	10000
F <sub>4</sub> Fytolan	3000
<i>Insecticides</i>	
I <sub>0</sub> Control	-
I <sub>1</sub> Carbofuran	12.5
I <sub>2</sub> Phorate	5
I <sub>3</sub> HCH	10

The above concentrations of fungicides, insecticides and their combinations were prepared in sterile distilled water. Sterile filter paper discs of 10 mm diameter were dipped in the prepared solutions and were placed at the centre of the YEMA medium seeded with 48 hour old culture of *Bradyrhizobium* in sterile petriplates. Sterile filter paper discs dipped in sterile water served as control. The plates were

Table 1. *In vitro* inhibitory effect of fungicides, insecticides and their combinations on the growth of *Bradyrhizobium*

Treatments	Diameter of inhibition zones, cm
F <sub>0</sub> I <sub>0</sub>	0
F <sub>0</sub> I <sub>1</sub>	0
F <sub>0</sub> I <sub>2</sub>	0
F <sub>0</sub> I <sub>3</sub>	0
F <sub>1</sub> I <sub>0</sub>	0
F <sub>1</sub> I <sub>1</sub>	0
F <sub>1</sub> I <sub>2</sub>	0
F <sub>1</sub> I <sub>3</sub>	0
F <sub>2</sub> I <sub>0</sub>	2.4
FA	2.3
F <sub>2</sub> I <sub>2</sub>	2.5
F <sub>2</sub> I <sub>3</sub>	2.2
F <sub>3</sub> I <sub>0</sub>	3.0
FA	2.6
F <sub>3</sub> I <sub>2</sub>	2.4
FA	2.4
F <sub>4</sub> I <sub>0</sub>	2.4
F <sub>4</sub> I <sub>1</sub>	2.7
F <sub>4</sub> I <sub>2</sub>	2.5
F <sub>4</sub> I <sub>3</sub>	2.8

incubated at room temperature and diameter of inhibition zones were recorded after three days.

The fungicides, insecticides and their combinations were tested in the field also. The experiment was conducted as 5 x 4 factorial experiment in RBD with three replications. Cowpea variety *Pusa Komal* was used for the study. Seed inoculation of *Bradyrhizobium* was done. Plant protection chemicals were applied

in the soil two weeks after planting. Carben-dazim (0.1%), Thiram (0.2%), Bordeaux mixture (1%), Fytolan (0.3%), Carbofuran (0.75 kg ai ha<sup>-1</sup>), Phorate (1 kg ai ha<sup>-1</sup>) and HCH (2 kg ai ha<sup>-1</sup>) were applied. Soil population of *Bradyrhizobium* was estimated by dilution plate technique using YEMA medium. Random samples of soil were taken from each plot and a composite sample of 1 g was taken for making 10<sup>-4</sup> dilution in sterile water. From this, 1 ml was pipetted out to sterile petriplate using a sterile pipette and to this 10-15 ml of the YEMA medium was poured and swirled to mix the soil suspension with medium uniformly. Petriplates were incubated at room temperature for observing the colonies of *Bradyrhizobium*. Natural soil population of the bacteria was estimated before sowing. The rhizosphere population of *Bradyrhizobium* was estimated after the inoculation and 7, 21 and 35 days after the application of plant protection chemicals.

Growth parameters like plant height, root length, total fresh weight, root dry weight and number of nodules were recorded. Nitrogen content was also estimated.

## RESULTS AND DISCUSSION

Native strain of *Bradyrhizobium* was isolated from root nodules of cowpea plant. Large gummy colonies with white colour was produced on YEMA medium. The bacterium was Gram negative and rod shaped. Growth on glucose peptone agar was scanty without any change in colour of the medium. These characters of *Bradyrhizobium* were described by Vincent (1977).

From the *in vitro* study, it was found that the fungicide Carben-dazim was not inhibitory to the growth of *Bradyrhizobium*. A similar result was obtained by Chakraborty *et al.* (1985), when Carben-dazim (0.2%) was tested on *Rhizobium* sp. from lentil, arhar, mung, groundnut and berseem. Thiram, Bordeaux mixture and Fytolan inhibited the growth of *Bradyrhizobium*. The inhibitory effect of Thiram on the growth of *R. phaseoli*, *R. japonicum*,

Table 2. Population changes of Bradyrhizobium in cowpea rhizosphere as influenced by fungicides, insecticides and their combinations

Sl. No.	Treatments	Soil population of Bradyrhizobium x 10 <sup>4</sup> cells per g of soil				
		Before sowing	14 days after sowing	After application of PP chemicals		
				7 days	21 days	35 days
1	F <sub>0</sub> I <sub>0</sub>	3.7	9.2	14.2	21.8	22.2
2	F <sub>0</sub> I <sub>1</sub>	3.3	10.6	16.8	20.3	23.8
3	F <sub>0</sub> I <sub>2</sub>	3.5	11.0	15.4	18.2	22.0
4	F <sub>0</sub> I <sub>3</sub>	3.8	9.3	14.8	19.7	26.9
5	FA	2.8	9.6	18.3	18.6	24.3
6	FA	2.6	10.1	17.5	23.5	26.4
7	"FA"	2.8	10.4	16.4	18.3	27.3
8	F <sub>1</sub> I <sub>3</sub>	3.0	11.4	18.9	19.5	21.4
9	FA	4.1	10.1	8.6	12.3	21.7
10	F <sub>2</sub> I <sub>1</sub>	3.8	9.9	9.0	14.6	26.5
11	FA	3.3	11.0	7.4	18.5	31.0
12	FA	3.7	8.8	8.0	11.0	27.3
13	F <sub>3</sub> I <sub>0</sub>	2.9	9.5	8.3	18.9	29.0
14	FA	3.1	10.0	7.4	23.4	27.6
15	FA	3.7	11.2	11.0	15.0	31.3
16	F <sub>3</sub> I <sub>3</sub>	2.7	10.4	9.0	17.5	30.6
17	F <sub>4</sub> I <sub>0</sub>	2.7	9.8	8.5	19.0	26.0
18	FA	2.5	9.4	9.0	20.3	26.0
19	FA	2.8	11.4	6.5	14.5	20.5
20	FA	3.2	11.2	7.4	18.3	21.2

Table 3. Number of nodules on Bradyrhizobium inoculated cowpea plants treated with fungicides, insecticides and their combinations

Fungicide treatments, F	Insecticide treatments, I				Mean
	I <sub>0</sub>	I <sub>1</sub>	I <sub>2</sub>	I <sub>3</sub>	
F <sub>0</sub>	63.333	56.000	58.667	54.667	58.167
F <sub>1</sub>	52.333	54.000	50.667	59.000	54.000
F <sub>2</sub>	44.000	50.667	28.333	35.333	39.583
F <sub>3</sub>	47.667	42.667	65.000	34.333	39.917
F <sub>4</sub>	37.000	32.333	39.000	38.667	36.750
Mean	48.867	47.133	42.333	44.400	-

CD (0.05) for F - 6.09; I - Not significant ; F x I - Not significant

*R. trifolii* and *R. meliloti* was reported by Mukewar and Bhide (1969). The *in vitro* inhibitory effect of copper fungicide Blue copper (0.5%) on *Rhizobium* isolated lentil, arhar and berseem was reported by Chakraborty *et al.* (1985). The insecticides Carbofuran, Phorate and HCH did not cause inhibition of *Bradyrhizobium*. The stimulatory growth of *Rhizobium* isolated from groundnut in culture by insecticide Carbofuran (15-30 ppm) and Phorate (4-10 ppm) was reported by Joshy and Kulkarni (1987). These insecticides when combined with Thiram, Bordeaux mixture and Fytolan caused inhibition of *Bradyrhizobium* (Table 1).

The rhizosphere population of *Bradyrhizobium* inoculated plants showed a gradual increase in control plot (Table 2). In the Carbendazim treated plot, no reduction in rhizosphere population of the bacteria was noticed. The performance of cowpea plants treated with Carbendazim was similar to that of control. No reduction in number of nodules (Table 3) and nitrogen content was observed. Ramadoss and Sivaprakasam (1989) reported that the cowpea seed treatment with Carbendazim was compatible with *Rhizobium* and had no inhibition in nodulation.

Rhizosphere population of *Bradyrhizobium* was not affected by insecticides Carbofuran, Phorate and HCH at their recommended doses. The fungicides, Thiram, Bordeaux mixture and Fytolan caused a reduction in *Bradyrhizobium* population after the application but later the bacterial count increased. A similar trend was noticed in the combination of insecticides with Thiram, Bordeaux mixture and Fytolan treated plants. The growth performance of cowpea plants treated with these fungicides did not show significant difference from control

plants. The number of nodules showed significant reduction. No reduction in nitrogen content was noticed.

From this study, it was clear that the recommended field doses of the fungicides and insecticides could be used without any adverse effect on *Bradyrhizobium* in cowpea.

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