

**FUNGICIDE TOLERANCE AGAINST STRAINS OF *BRADYRHIZOBIUM JAPONICUM***  
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**ABSTRACT**

Ten strains of *Bradyrhizobium japonicum* were isolated from ten localities from Pravara area and labeled as B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>4</sub>, B<sub>5</sub> ..... B<sub>10</sub>. These isolates were tested for different fungicides combination. From the finding it was interesting to note that the *B. japonicum* strains showed variation in resistance to different fungicides combination.

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KEY WORDS : *B. japonicum*, Fungicides combination, *Glycine max* strains

**Introduction**

Soybean (*Glycine max*) is an important crop plant belonging to family Papilionaceae now called as golden bean contain 40% protein, 20% carbohydrates, 3.80% crude fibers, vit.A, vit. B and vit. C. in India. It is cultivated during kharif season in Maharashtra it is cultivated in different district. Nitrogen fixation in soybean takes place with nodule formation. Nodule development on soybean is a sort of communication between host root and bacteria i.e. release of isoflavonoids by host and recognition by expressing nod factor and invade root hair and multiply and form nodule.

Nitrogen fixation process depends upon successive symbiosis between bacteria and host plant i.e. size, number of root nodules as well as biomass. Significant increase in number and size of nodule increases crop productivity of soybean, earlier flowering, and number of seed<sup>1,5,9</sup>.

Besides nitrogen fixation, *B.japonicum* produce extracellular substances like vitamins, growth promoting substances which enhance the seed germination, quick initial growth of tap root and early plant development<sup>1,3</sup>.

Using fungicides crop diseases control in legume fields has contributed to increase yield and improved quality<sup>4</sup>. Fungicides not only affect plant growth but also have a detrimental effect on soil

microorganisms growth and metabolism<sup>2,6</sup>. Some studies have been evaluated on the effect of different fungicides on Rhizobium bacteria growth and nitrogen fixation capacity<sup>10,11</sup>. Rhizobium isolates have different wide resistance to fungicides<sup>7,8</sup>, therefore efforts were taken to investigate the effects of fungicides on growth of *B.japonicum* isolates

**Materials and Methods**

Uprouted soybean plants from ten localities at Pravaranagar (Dist. Ahmednagar) were brought to the laboratory, roots were washed, and pink nodules were collected in beaker and washed with distilled water. The nodules were surface sterilized in 0.1% HgCl<sub>2</sub> for 5 min, rinsed four times with sterilized distilled water, treated with 70% ethyl alcohol for 3 min. and repeatedly washed with sterilized distilled water. Nodules were crushed with 1 ml. water and suspensions of *B. japonicum* were made. Serial dilutions of nodule extract were prepared and 1ml of 10<sup>-6</sup> dilution was spread on sterile Congo red Yeast Extract Mannitol Agar (CREYEMA) plates. The plates were incubated at 26<sup>o</sup>C ± 2<sup>o</sup>C for 4 days. Large gummy colonies of *Rhizobia* appeared on CREYEMA plates within 3 days. Ten strains of *Rhizobia* were isolated as B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>4</sub>, B<sub>5</sub> ..... B<sub>10</sub>, strains were maintained on CREYEMA slants. The efficiency of isolates was studied against different fungicides like Bavistin, Mancozeb, SAAF+Mancozeb at different ppm

TABLE - 1 : Resistance of *B. japonicum* strains against SAAF fungicide

Strain	Concentrations in ppm								
	20	40	50	100	1000	2000	3000	4000	Control
B <sub>1</sub>	+++	++	-	-	-	-	-	-	+++
B <sub>2</sub>	+++	++	-	-	-	-	-	-	+++
B <sub>3</sub>	+	-	-	-	-	-	-	-	+++
B <sub>4</sub>	+++	++	-	-	-	-	-	-	+++
B <sub>5</sub>	+++	+++	+++	++	++	++	+	-	+++
B <sub>6</sub>	+++	++	++	-	-	-	-	-	+++
B <sub>7</sub>	+++	++	-	-	-	-	-	-	+++
B <sub>8</sub>	+++	++	++	-	-	-	-	-	+++
B <sub>9</sub>	+++	++	-	-	-	-	-	-	+++
B <sub>10</sub>	+++	++	-	++	++	++	-	-	+++

TABLE- 2 : Resistance of *B. japonicum* strains against Bavistin fungicide

Strain	Concentration in ppm								
	100	250	500	1000	2000	2500	3000	3500	Control
B <sub>1</sub>	+++	+++	++	++	+	-	-	-	+++
B <sub>2</sub>	+++	+++	++	++	+	-	-	-	+++
B <sub>3</sub>	+++	+++	++	++	+	-	-	-	+++
B <sub>4</sub>	+++	+++	++	++	+	-	-	-	+++
B <sub>5</sub>	+++	+++	++	++	++	-	-	-	+++
B <sub>6</sub>	+++	+++	++	++	++	+	+	-	+++
B <sub>7</sub>	+++	+++	++	++	+	-	-	-	+++
B <sub>8</sub>	+++	+++	++	++	++	-	-	-	+++
B <sub>9</sub>	+++	+++	++	++	+	-	-	-	+++
B <sub>10</sub>	+++	+++	++	++	+	-	-	-	+++

**TABLE - 3 : Resistance of *B. japonicum* strains against Mancozeb fungicide**

Strain	Concentration in ppm							
	500	1000	2000	2500	3000	3500	4000	Control
B <sub>1</sub>	+	-	-	-	-	-	-	+++
B <sub>2</sub>	+++	+++	+++	+++	++	++	-	+++
B <sub>3</sub>	+++	++	-	-	-	-	-	+++
B <sub>4</sub>	+	-	-	-	-	-	-	+++
B <sub>5</sub>	+	-	-	-	-	-	-	+++
B <sub>6</sub>	+	-	-	-	-	-	-	+++
B <sub>7</sub>	+++	+	-	-	-	-	-	+++
B <sub>8</sub>	+++	+	-	-	-	-	-	+++
B <sub>9</sub>	+++	-	-	-	-	-	-	+++
B <sub>10</sub>	+	-	-	-	-	-	-	+++

TABLE - 4 : Resistance of *B. japonicum* strains against SAAF+ Mancozeb Fungicide

Strain	Concentrations in ppm							
	25	50	100	200	400	800	1000	Control
B <sub>1</sub>	+++	+	-	-	-	-	-	+++
B <sub>2</sub>	+++	++	++	-	-	-	-	+++
B <sub>3</sub>	+++	+	-	-	-	-	-	+++
B <sub>4</sub>	+++	++	++	++	+	+	-	+++
B <sub>5</sub>	+++	++	++	++	+	+	-	+++
B <sub>6</sub>	+	-	-	-	-	-	-	+++
B <sub>7</sub>	+++	++	++	++	+	-	-	+++
B <sub>8</sub>	+++	++	++	++	+	-	-	+++
B <sub>9</sub>	+++	+++	++	++	-	-	-	+++
B <sub>10</sub>	+++	-	-	-	-	-	-	+++

concentrations by food poisoning techniques. Equal volume of sterile double strength nutrient and double strength concentration of fungicides were mixed in petridishes. After solidification of plates a loopful isolate were inoculated on plates aseptically and incubated at room temp. The observations were recorded in the form of growth of colonies.

### Results and Discussions

Resistance against different fungicides (Tables 1 - 4) indicates that all the strains except B<sub>3</sub> were resistant to 20 ppm conc. of SAAF. However, B<sub>5</sub> strain was proved to be highly resistant to 50 ppm SAAF conc. For Bavistin (Table - 1) B<sub>5</sub>, B<sub>6</sub>, and B<sub>8</sub> proved their resistance up to 2000 ppm conc., to

Mancozeb fungicide (Table - 2) only B<sub>2</sub> strains proved their resistance upto 3500 ppm conc. However, the remaining strains have proved their resistance up to 5000 ppm., to SAAF + Mancozeb combination (Table - 4) B<sub>4</sub> and B<sub>5</sub> showed their resistance up to 800 ppm conc. However B<sub>7</sub> and B<sub>8</sub> up to 400 ppm conc. and others were to the range 25 to 100 ppm conc. The variation in resistance in *R. japonicum* strains have pointed out that mercury based fungicides inhibits growth of *Rhizobium*. Worker<sup>11</sup> supported that carbendazim pyroxychlore and quintozene were least toxic and thiram the most toxic followed closely by captan, zineb, chlorothalonil, dodine, folpet and benomyl.

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