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In vitro compatibility of *Bradyrhizobium japonicum* with systemic fungicides

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Abstract
The bacterium *B japonicum* was successfully isolated from functional root nodules of soybean plants. *In vitro* evaluation, by inhibition zone technique revealed all of the seven each systemic and non-systemic at their recommended field dosages (100% RD), 50% and 125% of the RD showed both synergistic and toxic effects against *B. japonicum.* Out of the seven systemic fungicides tested carbendazim 50% WP and hexaconazole 5% EC tested at all three concentrations were found highly compatible with *B. japonicum* and rest of the five fungicides *viz.*, tebuconazole 25% WG, thiophanate methyl 75% WP, difenconazole 25% EC, propiconazole 25% EC and pyraclostrobin 20% WG were non-compatible with the test bacterium.

Keywords: Soybean, *Bradyrhizobium japonicum*, *in vitro*, compatibility

Introduction
Soybean (*Glycine max* (L.) Merrill) is one of the commercially important grain legumes extensively grown in the world, under varying soil types and climatic conditions. Soybean has contributed significantly to "Yellow revolution" in India and as a pulse-cum-oilseed crop forms an integral part of daily diet of Indian peoples. The family Rhizobiaceae, currently includes the genera *viz.*, *Rhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium* etc., which are collectively referred as Rhizobia (Kumar and Raghuram, 2016) [2]. Soybean, being a leguminous crop, can be fix atmospheric nitrogen through symbiotic association with effective and competitive strains of Rhizobia (*Bradyrhizobium japonicum / Rhizobium japonicum*) and thereby improve soil fertility as well as productivity of subsequently grown cereal crops. However, symbiotic association between *Rhizobium*-legumes, amount of biological nitrogen fixation, plant growth and thereby crop yield potential are generally affected due to adverse environmental conditions such as drought stress, salt stress, acidity / alkalinity, nutrients deficiency, heavy metals and various pesticides (fungicides, insecticides, herbicides) used to combat diseases, insect-pests and weeds. Under intensive cultivation of soybean and other grain legumes, along with various inputs a wide range of fungicides (seed treatment, foliar sprays, soil drench) are often used on large scale. These agrochemicals used exert either synergistic or antagonistic and/or both kinds of effects on the soil resident or introduced *Rhizobium* spp., thereby influence the crop growth as well as yield potentials (Zaidi *et al*., 2005; Muthomi *et al.*, 2007; Kunal and Sharma, 2012; Deshmukh *et al.*, 2014) [1, 3-5].

The fungicides applied to leguminous plants either as seed dressing or soil drenching may affect symbiotic relationship and may persist for longer time. A broad variation regarding susceptibility of individual rhizobial strains to agrochemicals was found in different rhizobial species, as well as significantly higher susceptibility of fast growing than slow growing rhizobia (Deshmukh *et al.* 2014) [1].

Materials and Methods
Various fungicides (systemic were evaluated *in vitro*, each at three different dosages i.e. recommended field dose RD, 50% of 100% RD and 125% of RD to access their compatibility with *B. japonicum*, by employing paper disc / inhibition zone technique and using YEMA as basal culture medium. One ml YEMA broth culture of *B. japonicum* (24-48 hrs. aged) was poured in sterile glass Petri plates (90 mm dia.). To these plates, autoclaved and cooled (45 ˚C)

YEMA was poured (20 ml/plate) and rotated the plates gently in clock-wise and anti-clock-wise directions, for uniform mixing of the bacterium with the medium.

Whatman's filter paper (Whatman Filter paper No. 42) discs (1 mm dia.) pre-sterilized in autoclave were soaked/impregnated for 5 min, in the test concentrations of the test agrochemicals, separately. A single disc was placed at centre on *B. japonicum* seeded solidified YEMA medium in Petri plates. Three Petri plates per treatment per concentration per replication were maintained. The Petri plates containing test bacterium seeded YEMA medium and inoculated with Whatman's filter paper disc soaked in distilled water were maintained as untreated control. Both treated and untreated Petri plates were incubated at 28±2 ˚C.

Results and Discussion

The results (Table1, Fig.1 a, b) revealed that all of the seven systemic fungicides tested at various concentrations, exhibited significant differences in the amount of inhibition zone (mm) recorded at 48 and 72 hrs. of incubation. Further, the zone of inhibition was found to be increased steadily with increase in concentrations of the test fungicides.

At 48 hrs. of incubation, the amount of inhibition zone produced with the test systemic fungicides at the dosages of 50% RD (D_1), 100% RD (D_2) and 125% RD (D_3) was ranged from 00.00 – 17.42 mm, 00.00 – 19.23 mm and 00.00 – 19.39 mm, respectively, with average inhibition zone in the range of 00.00 – 18.68 mm.

Table 1: *In vitro* compatibility of *B. japonicum* with systemic fungicides at 48 and 72 hrs.

Tr. No.	Treatments	Inhibition zone* (mm) at 48 hrs. and dosages			Av. inhibition zone	Inhibition zone* (mm) at 72 hrs. and dosages			Av. inhibition zone
		D_1	D_2	D_3		D_1	D_2	D_3	
T_1	Carbendazim 50% WP	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
T_2	Tebuconazole 25% WG	16.25	16.39	17.33	16.65	20.3	20.33	22.25	20.96
T_3	Hexaconazole 5% EC	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
T_4	Thiophanate methyl 75% WP	14.13	14.42	15.13	14.56	16.31	17.45	17.59	17.11
T_5	Difenconazole 25% EC	16.04	17.15	17.51	16.90	21.45	22.18	23.36	22.33
T_6	Propiconazole 25% EC	17.32	17.48	18.3	17.73	23.30	23.52	24.21	23.67
T_7	Pyraclostrobin 20% WG	17.42	19.23	19.39	18.68	22.42	24.14	25.39	23.98
T_8	Control	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
S.E ±		0.36	0.27	0.31	-	0.24	0.26	0.30	-
C.D (*P*=0.01)		1.05	0.79	0.93	-	0.72	0.78	0.90	-

* = Mean of three replications, D_1 = 50% RD, D_2 = 100% RD, D_3 = 125% RD

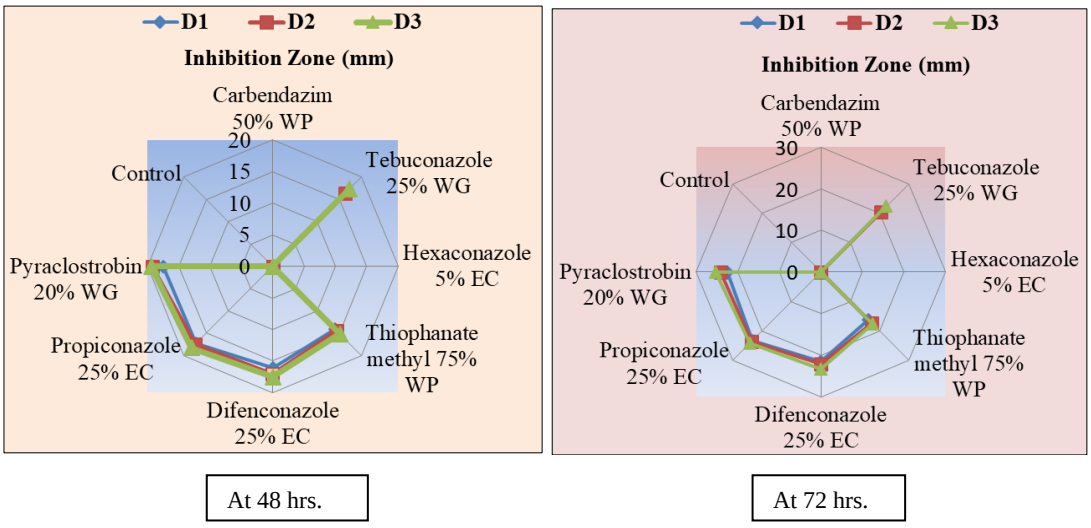

Fig 1: *In vitro* compatibility of *B. japonicum* with systemic fungicides at 48(a) and 72(b) hrs.

Whereas, at 72 hrs. of incubation, the amount of inhibition zone produced was found to be increased steadily at all three dosages and it was ranged from 00.00 – 22.42 mm, 00.00 – 24.14 mm and 00.00 – 25.39 mm, respectively at D_1, D_2 and D_3 dosages, with average inhibition zone in the range of 00.00 – 23.98 mm.

The fungicides, carbendazim 50% WP and hexaconazole 5% EC at all three dosages were found highly compatible with *B. japonicum*, as they didn't showed any zone of inhibition, both at 48 and 72 hrs. of incubation. Whereas, rest of the five fungicides *viz.*, tebuconazole 25% WG, thiophanate methyl 75% WP, difenconazole 25% EC, propiconazole 25% EC and pyraclostrobin 20% WG tested at three various concentrations were found non-compatible with the test bacterium, as they expressed significant inhibition zones, at both 48 and 72 hrs of incubation.

Thus, of the seven systemic fungicides tested, carbendazim 50% WP and hexaconazole 5% EC were found highly compatible with *B. japonicum* and rest of the five fungicides *viz.*, tebuconazole 25% WG, thiophanate methyl 75% WP, difenconazole 25% EC, propiconazole 25% EC and pyraclostrobin 20% WG were non-compatible with the test bacterium.

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