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In vitro compatibility of *B. japonicum* with fungicides

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Abstract
Laboratory experiment was conducted to study the test of compatibility with systemic, non-systemic / contact, combi-fungicides at 50%, 75%, 100% and 125% of the Reommended doses were evaluated *in vitro* against *B. japonicum* i.e. Bj-9 (effective isolate out of 25 isolates) of soybean, by inhibition zone technique. The fungicides carbendazim 50% WP and mancozeb 75% WP at all four dosages were found compatible with *B. japonicum*, as they didn't showed any zone of inhibition, at 72 hrs of incubation. Whereas, rest of the three fungicides *viz.*, captan 75% WP, carboxin 75% WP, vitavax power 75% WP tested at four various concentrations were found inhibited by the test bacterium and produced average inhibition zone of around 00.00-19.66 mm, 8.56mm and 10.52mm respectively, at 72 hrs of incubation.

Keywords: Economics, B:C ratio, net monetary, GMR, agroforestry

Introduction

Soybean (*Glycine max* (L.) Merill) the "Golden bean" has played a significant role in the contribution of yellow revolution in India. It is an important oilseed as well as pulse crop, the most likely solution for overcoming the world's protein hunger as it contains 20% edible oil, 40% protein, 21% carbohydrates and 11.5% iron. Symbiotic nitrogen fixation resulting from mutual beneficial interaction between soybean and root nodule bacteria *Rhizobium* provides a significant role of N fertilization.

Under intensive cultivation of soybean and other grain legumes, along with various inputs a wide range of fungicides, insecticides (seed treatment, foliar sprays, soil drench) and herbicides (pre and post-emergence) are often used on large scale. These agrochemicals used exert either synergistic or antagonistic or both kinds of effects on the soil resident or introduced *Rhizobium* spp., thereby influence the crop growth as well as yield potentials. The extensive use of N-fertilizers is both harmful to the environment and results in the depletion of fossil fuels. Continuous depletion of nitrogen (N) from the soil pool by processes such as volatilization and leaching results in the decline of soil nitrogen reserves in agricultural soils (Abdullahi *et al.*, 2008) [1]. The economic and environmental costs of the heavy or wrong use of inorganic nitrogen fertilizers in agriculture are a global concern and mandates that alternatives be urgently sought.

Fungicides affects microbial population as equally as pathogens present in the soil. The percent inhibition and its duration vary with chemical, environmental conditions and soil type. Fungicides differ in their effects on growth and survival of *Rhizobium* and *Bradyrhizobium* strains depending on the characteristics of that strain and concentration of fungicides (Hashem *et al.*, 1997) [4]. The agrochemicals 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 fungicides was found in different rhizobial species, as well as significantly higher susceptibility of fast growing than slow growing rhizobia (Deshmukh *et al.*, 2012) [3]. So, the present investigation is planned to study the compatibility of *Bradyrhizobium japonicum* with fungicides for the higher productivity of soybean with effective management of seed borne as well as soil borne pathogens.

Materials and Methods

Twenty five isolates of *Bradyrhizobium japonicum* and *Rhizobium* sp. were isolated from effective root nodules of soybean collected from different locations of Maharashtra and Madhya Pradesh. The isolation was made on YEMA with congored as per standard procedure. Among all these isolates only Bj-9 (effective isolate) was then tested for their compatibility with commonly used fungicides in soybean cultivation.

Compatibility of *Bradyrhizobium japonicum* to different concentrations of fungicides.

Various fungicides (systemic, contact/ non-systemic and combi-) were evaluated *in vitro*, each at four different dosages i.e. recommended field dose (RD), 50%, 75%, 100% 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 YEM broth culture of *B. japonicum* (24-48 hrs. aged) was poured in sterile glass petri plates (90 mm dia.). YEMA media was then poured in the plates and rotated clockwise and anticlockwise for uniform mixing. Whatman's filter paper (Whatman filter paper No. 42) discs 5 mm dia. pre-sterilized in autoclave were soaked / impregnated for 5 min, in the test concentrations of the test fungicides, separately. Four discs of different concentrations and one disc soaked in distilled water was kept in centre as untreated / control were placed on rhizobia seeded solidified YEMA medium in petri plates. Three petri plates per treatment per replication were maintained and incubated at 28±2 °C (Ahmed *et al.*, 2007).

Table 1: Compatibility of *B. japonicum* with different concentrations of fungicides

Tr. no.	Treatments	Concentrations (ppm)			
		50% RD	75% RD	100% RD	125% RD
1	Carbendazim 50% WP	500	750	1000	1250
2	Captan 75% WP	1500	2250	3000	3750
3	Mancozeb 75% WP	1250	1875	2500	3125
4	Carboxin 75% WP	1250	1875	2500	3125
5	Carboxin 37.5% WP + Thiram 37.5P% WP	1250	1875	2500	3125

Results and Discussion

In vitro compatibility of *B. japonicum* with fungicides

The results revealed that systemic, contact / non- systemic and combi-fungicides evaluated *in vitro*, exhibited varied inhibition zone which indicated the degree of compatibility of *B. japonicum* (effective strain *i.e.* Bj-9) with the test fungicides. Fungicides tested at various concentrations (dosages), exhibited significant differences in the amount of inhibition zone (mm) recorded at 72 hrs of incubation. Further, the zone of inhibition was found to be increased steadily with increase in concentrations of the test fungicides.

At 72 hrs of incubation, the amount of inhibition zone produced with the test fungicides at the dosages of 50% RD, 75% RD, 100% RD and 125% RD by captan was 15.12 mm, 17.56 mm, 20.89 mm and 25.10 mm, respectively, with average inhibition zone in the range of 19.66 mm (Plate I-[b]). Carboxin produced 3.43 mm, 7.89 mm, 8.50 mm, 8.50 mm, and 14.45mm respectively, with an average inhibition zone of 8.56 mm (Plate I-[d]). Whereas, vitavax power formed the inhibition zone of 5.00 mm, 9.15 mm, 10.43 mm and 17.50 mm respectively with an average zone of 10.52 mm (Plate I-[e]). As the hrs of incubation exceeded, the amount of inhibition zone produced was found to be increased steadily at all four dosages.

Thus, the fungicides carbendazim (Plate I-[a]) and mancozeb (Plate I-[c]) at all fosur dosages were found compatible with rhizobia, as no zone of inhibition was observed at 72 hrs of incubation. Whereas, rest of the three fungicides *viz.*, captan, carboxin, vitavax power tested at four various concentrations were found non-compatible with the test bacterium, as they expressed significant inhibition zones over control at 72 hrs of incubation (table 2, fig. 1).

Table 2*:* *In vitro* compatibility of *B. japonicum* with fungicides

Tr. No.	Treatment	Inhibition zone (mm)				
		50% RD	75% RD	100% RD	125% RD	Av. Inhibition zone
1	Carbendazim 50% WP	00.00	00.00	00.00	00.00	00.00
2	Captan 75% WP	15.12	17.56	20.89	25.10	19.66
3	Mancozeb 75% WP	00.00	00.00	00.00	00.00	00.00
4	Carboxin 75% WP	3.43	7.89	8.50	14.45	8.56
5	Carboxin 37.5% WP + Thiram 37.5% WP	5.00	9.15	10.43	17.50	10.52
6	Control (uninoculated)	00.00	00.00	00.00	00.00	00.00

*RD= Recommended Dose

Singh and Lodha (1997) [6] observed the compatibility of five chemicals seed protectants *viz.*, thiram, dithane M-45, captan, ridomil-MZ and bavistin with *Bradyrhizobium japonicum* in soybean and reported that ridomil MZ and bavistin were found to be compatible. The findings reported by Deshmukh *et al.* (2012) [3] that the test fungicides *viz*., thiram 75% WS, carbendazim 50% WP, copper oxychloride, mancozeb 75% WP and ridomil 68% WP were compatible with the *Bradyrhizobium japonicum*. Zilli *et al*. (2009) [7] are also on the similar line of present investigation.

The results of present study were corroborates with findings of Panwar *et al*. (2015) [5] who studied the *in vitro* compatibility of *Rhizobium* with fungicides *viz.*, carbendazim 50% WP, captan 50% WP, thiram 75% WS and metalaxyl 35% WS (@ 50, 100, 200, 300 and 500 ppm), by poison food technique. They reported that metalaxyl and thiram even at lower concentration are non-compatible and rest of the fungicides are compatible with the bacterium, even at higher concentration.

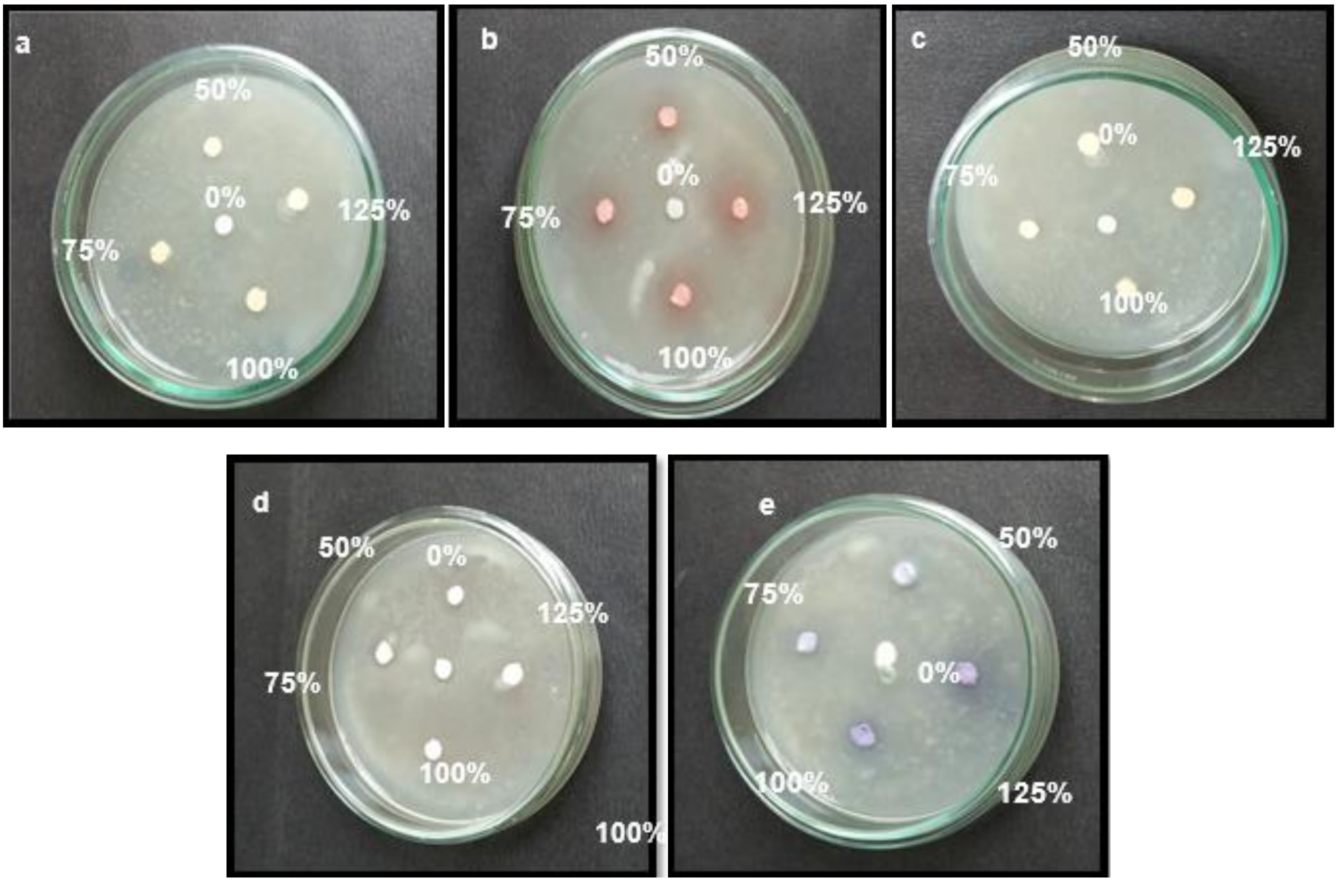

Plate I: *In vitro* compatibility of *B. japonicum* with fungicides a) Carbendazim b) Captan c) Mancozeb d) Carboxin e) Vitavax power

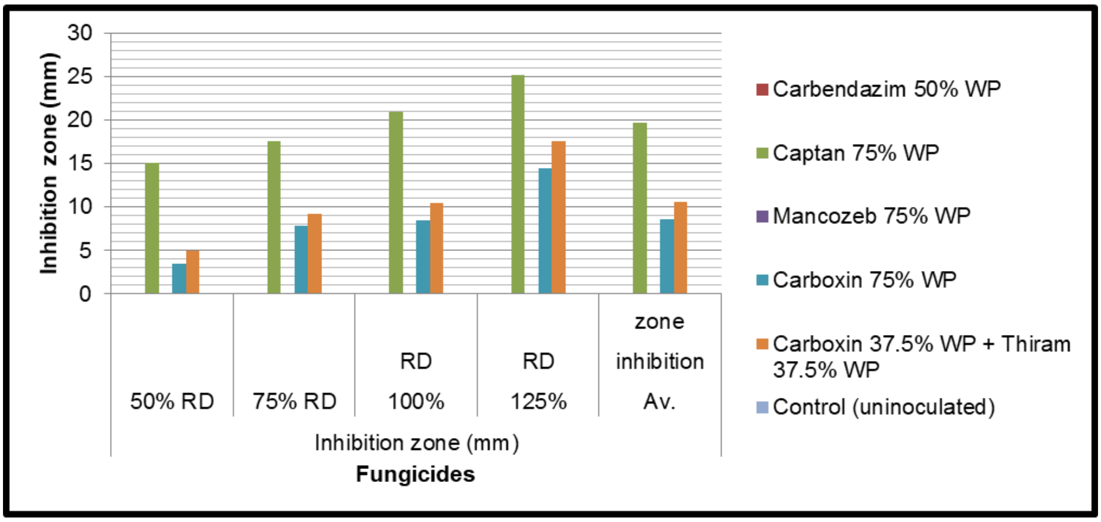

Fig 1: *In vitro* compatibility of *B. japonicum* with fungicides

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