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In vitro interaction between fungicides and beneficial plant growth promoting Rhizobacteria

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Laboratory experiments were conducted to study the effects of five fungicides on the growth of *Bradirhizobium japonicum* and Phosphate solublizing bacteria (PSB) at different concentrations that is, (0, 0.1, 0.2, 0.3, 0.4 and 0.5%). Fungicide effects on bacteria were determined on the basis of their growth in respective medium. The population of *B. japonicum* declined significantly while PSB population showed non-significant growth reduction. Minimum (13.5 × 10^4 CFU ml^{-1}) of *B. japonicum* were recorded at 0.5% concentration. However, reduction in PSB at 0.5% thiram was non-significant. The mean population of microorganisms declined significantly by carbendazim. Maximum reduction in *B. japonicum* and PSB population was recorded at 0.5 and 0.4% carbendazim concentration respectively. Vitavax exhibited maximum population reduction at 0.5% concentration where (6.5 × 10^4 CFU ml^{-1}) of microorganisms were recorded. The mean population of microorganisms declined significantly with increasing carboxin concentration and 5.6 × 10^4 CFU ml^{-1} of *B. japonicum* was recorded at 0.5% concentration of carboxin. The effective mean population irrespective of the organisms showed significant decrease with increasing concentration of thiomethaxam. Among all the fungicides, minimum bacterial growth was observed at 0.5% thiomethaxam concentration. Experimental results revealed that, higher concentration of all the fungicides exerted toxic effect on beneficial soil microflora.

Key words: *Bradirhizobium japonicum*, Phosphate solublizing soil bacteria (PSB), fungicides, growth.

INTRODUCTION

Soil application of mineral fertilizers, bio-inoculants, organic amendments and pesticides are some of the commonly adopted strategies with the sole aim of optimizing crop productivity and economic return. Use of chemical fertilizers can enhance the system productivity and crop residue return, but their continuous use has deprived the soil of its fertility, organic matter and soil organisms. Current practices used for legume production include inoculation of seeds with rhizobia to ensure effective nodulation and subsequent Nitrogen (N) fixation and treatment of the seed with fungicides to reduce seed rot and seedling damping-off resulting from infection by soil-borne pathogens. However, the adverse effect of fungicides on agriculturally important microorganisms such as nitrogen fixers and phosphate solubilizers, resulting in poor performance of applied microbial inoculants, is a subject of great concern; several studies have conclusively shown that, some of these chemicals are incompatible with *Rhizobium* (Welty et al., 1988; Ramos and Ribeiro, 1993).

Chemically treated seeds are inoculated with rhizobia which may be adversely affected by these chemicals. Fungicides differ in their effects on the growth and survival of *Rhizobium* and *Bradyrhizobium,* depending on the concentration of the fungicide (Hashem et al., 1997). Toxic effects of carboxin, folpet and captan on *Rhizobium*

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Table 1. Effect of different fungicides on the growth of *B. japonicum* ($\times 10^4$ CFU ml^{-1}).

Concentration	Fungicides				
	Thiram	Carbendazim	Carboxin	Vitavax	Thiomethaxam
0	33.5	33.5	33.5	33.5	33.5
0.1	27.3	26.2	27.6	25.7	30.1
0.2	23.2	20.5	22.1	20.7	25.2
0.3	18.8	15.3	15.5	15.1	15.8
0.4	16.1	10.4	10.7	10.4	10.6
0.5	13.5	6.36	5.6	6.5	5.4
Mean ($\times 10^4$ CFU ml^{-1})	22.1	18.7	19.7	18.6	20.1
SEm$\pm$	0.50	0.56	0.61	0.58	0.58
CD (5%)	1.47	1.65	1.79	1.69	1.71

CD, Critical difference; NS, non significant; SEm, standard error of mean n=3.

sp. have also been reported; (Heinonem-Tanski et al., 1982) but there is no literature that deals with free living N fixers and phosphate solubilizers. . Therefore, the knowledge relating to the effect of commonly used fungicides on phosphate solubilizers and non-symbiotic N fixers is of uniqne importance in order to avoid the deleterious effect of fungicides on beneficial microorganisms. The number of the counts in the solid media may not give a true measure of toxicity of fungicide to *Rhizobium* and *Bradirhizobium japonicum* since the test depends not only on the sensitivity of the chemical but also its ease of diffusion through the agar (Johnen and Drew, 1977). Keeping in view the above facts, present investigation was undertaken to examine the effects of some frequently used fungicides on *B .japonicum* and Phosphate solublizing soil bacteria (PSB) population in their respective medium.

MATERIALS AND METHODS

In order to study the impact of different fungicides concentrations on *B. japonicum* and PSB growth, a laboratory study was conducted on two factorial completely randomized design with three replications. The effect of different fungicides on PSB and B. japonicum was studied by growing these bacteria on yeast extract mannitol (YEM) agar described by Cleyet-Marel (1993) with 10 ml congo red per liter and Pikovskaya's medium by serial dilution pour plate method (Subba, 1986) using different concentrations of fungicides. The growth of the above bacteria at 0% concentrations was compared with the growth at other concentrations to study the effect of fungicides on these bacteria. Five different fungicides (thiram, carbendazin, carboxin, vitavax, and thiomethaxam) with six concentrations (0, 0.1, 0.2, 0.3, 0.4, and 0.5%) were selected to test their influence on *B. japonicum* and PSB population. The petri plates were incubated at 27 ± 2°C and all the treatments were replicated in thrice.

RESULTS AND DISCUSSION

The data illustrated in the Tables 1 and 2 indicate the effect of different concentrations of thiram on *B. japonicum* and PSB population in respective medium. The population of both the microorganisms showed significant effect of thiram. The population of *B. japonicum* significantly declined by addition of thiram from 0 to 0.5%. Minimum number of *B. japonicum* colonies (13.5 × 10^4 CFU ml^{-1}) was found with 0.5% concentration which was 59.7% less than control. However, reduction in PSB population was non-significant but population showed gradual declination with increasing concentration. The minimum population (4.8 × 10^4 CFU ml^{-1}) of PSB was recorded with 0.5% of thiram which was 76.0% less than control. The mean population irrespective of microorganism was significantly reduced by the addition of thiram from 0 to 0.5%.

Tables 1 and 2 showed the effect of carbendazim concentrations on the multiplication of *B. japonicum* and PSB. The mean population, irrespective microorganism registered significant declination with added concentration of carbendazim. Minimum population was of *B. japonicum* (6.36 × 10^4 CFU ml^{-1}) and PSB 2.2 × 10^4 CFU ml^{-1} recorded with 0.5% concentration of carbendazim. The mean population of *B. japonicum* was 88.7% more than PSB irrespective of carbendazim concentrations. Carbendazim significantly reduced the population of both, *B. japonicum* and PSB at each concentration. The population of *B. japonicum* decreased significantly with increasing concentration of carbendazim while PSB showed significant declination up to 0.4% of carbendazim.

The mean population irrespective of microorganisms significantly declined with increasing concentration of carboxin. The lowest population of *B. japonicum* (5.6 × 10^4 CFU ml^{-1}) was recorded with 0.5% carboxin which was 83.2% less than control irrespective of carboxin concentration. The mean *B. japonicum* population was significantly higher (99.8%) than PSB. *B. japonicum* population was significantly higher than PSB at all concentrations of carboxin. *B. japonicum* showed in

Table 2. Effect of different fungicides on the growth of PSB (×10^4 CFU ml^{-1}).

Concentration	Fungicides				
	Thiram	Carbendazim	Carboxin	Vitavax	Thiomethaxam
0	20.0	20.0	20.0	20.0	20.0
0.1	16.5	14.9	14.4	15.6	16.4
0.2	13.0	11.3	10.7	11.8	12.6
0.3	9.4	7.06	7.7	8.0	10.5
0.4	7.1	4.4	4.7	5.4	7.7
0.5	4.8	2.2	2.1	3.0	3.6
Mean (×10^4 CFU ml^{-1})	11.8	9.9	9.9	10.6	11.8
SEm$\pm$	0.29	0.32	0.35	0.33	0.34
CD (5%)	NS	NS	NS	NS	NS

CD, Critical difference; NS, non significant; SEm, standard error of mean n=3.

significant reduction in population with each increasing concentration of carboxin PSB also showed reduction population but the effect was non-significant with the increasing concentration of carboxin, lowest population of 2.1 × 10^4 CFU ml^{-1} was recorded at 0.5% concentration.

The effect of vitavax on the survival of *B. japonicum* and PSB was significant. The mean population of microorganisms significantly reduced at each concentration of vitavax. The lowest mean population of *B. japonicum* 6.5 × 10^4 CFU ml^{-1} was recorded with 0.5% vitavax. The mean population of *B. japonicum* was significantly higher (75.5%) than PSB irrespective of vitavax concentration. The population of *B. japonicum* was significantly more than PSB at each concentration. Similarly the population of both the microorganisms was significantly reduced at all the concentrations of vitavax. Reduction in population was of 80.0 and 85.0%r espectively, in comparsion to control was recorded at 0.5% of vitavax which showed 6.5 and 3.0 × 10^4 CFU ml^{-1} respectively, of *B. japonicum* and PSB.

The effective mean population irrespective of the organism showed significant decrease with increasing concentration of thiomethaxam. The mean number of *B. japonicum* was significantly higher than PSB by 70.3%. *B. japonicum* population declined significantly with increasing concentration of thiomethaxam. The lowest cell number of *B. japonicum* (5.4× 10^4 CFU ml^{-1}) was recorded with 0.5%of thiomethaxam. The population of PSB also showed decreasing trend with increasing concentrations of thiomethaxam, but the decreases were non-significant. The least population 3.6 × 10^4 CFU ml^{-1} was recorded with 0.5% of thiomethaxam.

The results showed that, *B. japonicum* and PSB population decreases consistently with increasing concentrations of all the fungicides in comparison to control. These findings were matching with Isoi and Yoshida (1988) who found that, when 50 ppm thiram was added to an in vitro *Rhizobium* culture (*B. japonicum* and *Rhizobium leguminosarum*), the growth of *B. japonicum* was completely inhibited while *R. leguminosarum* was unaffected. Similar findings were also reported by Ahmed et al. (2007) who described the effect of different fungicide concentrations on inhibition of growth and the size of R*hizobium* and B*radyrhizobium* colony and then concluded that, captan at the concentrations of 100 and 1000 µg/L was the most toxic, followed by thiram, milcurb, luxan then frernasan-D. Increasing the concentration of each fungicide reduced the colony size of rhizobial and *bradyrhizobial* strains with different degrees of sensitivity. Decrease in PSB population with fungicides was also reported by Gaind et al. (2007) who concluded that, the PSB showed decline in their viable population on prolonged contact with fungicides. Niewiadomska (2004) also reported that, the herbicides and pesticides applied also inhibit the growth and multiplication on microbes in soil.

Conclusion

Lower doses of fungicides did not have any adverse effect on the growth of *B. japonicum* and PSB, whereas, higher doses reduced the growth of both the PGPRs. Different fungicides have different mode of action due to which reduction in population was variable among them. This could also be correlated with the cytotoxic effect of used fungicides. Thus, it can be concluded that, the use of higher fungicide doses is harmful for their growth and functions.

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