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Effect of fungicides and bioagents on number of microorganisms in soil and yield of soybean (*Glycine max*)

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Abstract. *Mishra G, Kumar N, Giri K, Pandey S, Kumar R. 2014. Effect of fungicides and bioagents on number of microorganisms in soil and yield of soybean (*Glycine max*). Nusantara Bioscience 6: 45-48.* In field experiments, the effect of selected fungicides and bioagents on number of soil microorganisms and yield of soybean (*Glycine max* L. Merill) was investigated. The results showed that some of the crop protections preparations applied in the experiment (as seed dressing) increased the populations of the examined microorganisms after the harvest of crops. Maximum counts of bacteria were recorded with Thiamethoxam at 3 g kg^{-1} while *Pseudomonas at* 3 g kg^{-1} showed the highest population of fungi, Actinomycetes, *Bradyrhizobium japonicum*, PSB and *Pseudomonas*. The highest straw and grain yields of 3241.6 and 1439.4 kg ha^{-1}, respectively, were recorded with *Pseudomonas* at 3 g kg^{-1}.

Keywords: Carbendazim, carboxin, *Pseudomonas,* thiram, thiamethoxam, *Trichoderma viride,* vitavax.

INTRODUCTION

Many scientists have conducted research to explore the effect of fungicide seed dressing on number of soil microorganisms and yield of various crops. Here, an attempt has been made to analyze the effect of some fungicide as well as some bioagents on soil microflora and yield with special reference to soybean (*Glycine max* L. Merill). Soybean is an important oilseed crop of the 21st century with high protein (40-45%) and oil content (20-22%). It has an important place in the agriculture and oil economy of India by currently occupying 9.67 million ha with an estimated production and productivity of 10.22 million tons and 1006 kg ha^{-1} respectively. The soybean has a wide range of geographical adaptation, unique chemical composition, and good nutritional value, functional health benefits and versatile end uses (food, feed and non-edible). It has good adaptability towards a wide range of soil and climate and fetches good returns to farmers even with low level of agricultural inputs. It has also played a significant contribution to the yellow revolution in India and as a food plant, it forms an important part of routine diet of people in India. Today, soybean belongs to one of most important economic crops in the subcontinent. Soybean occupied a unique position in agriculture by virtue of the ability to fix atmospheric nitrogen (300 kg N ha^{-1}) with the help of root nodule bacteria *Bradyrhizobium japonicum* (Bezdicek et al. 1978).

Soybean is a legume and generally does not need to be fertilized with nitrogen in case where effective homologous strains of bradyrhizobia are present in soil. This is because of a symbiotic relationship with soil bacteria called bradyrhizobia which attach themselves to the soybean root and form nodules. The efficiency of biological nitrogen fixation is markedly dependent on the mutual compatibility of both partners, and is influenced by a number of environmental factors (Sprent and Minchin 1983; Vincent 1980). Biological nitrogen fixation with the soybean crop can be improved by seed inoculation with superior *Bradyrhizobium* strains, but factors that reduce the population of inoculated bradyrhizobia on the seed will directly affect the efficiency of the process.

Seed treatment with fungicides has been broadly practiced as cheap insurance against seed and soil-borne pathogens, but the toxicity of most fungicides to bradyrhizobia has often been underestimated. Fungicidal seed dressing used to improve the early plant emergence are often damaging to *Rhizobium* applied as inoculants to legume seed. Some reports claim little damage, which may reflect the considerable variation within and in between different groups of *Rhizobium* in their sensitivity to fungicides (Curley 1975). Nodulation, nitrogen fixation and growth of various legumes can be inhibited by fungicides. Therefore, keeping the above points in view, the present investigation was carried to study the influence of applied fungicides and bioagents on soil microflora and yield of soybean.

MATERIALS AND METHODS

A field experiment was conducted during 2009-2010 at Norman. E. Borlaug Crop Research Center, Pantnagar, India to study the influence of selected fungicides and bioagents on soil microflora and yield of soybean variety JS 335. The soil of the experimental site was silty clay loam of pH 7.64 having 0.76% organic carbon, 254 kg ha^{-1} available nitrogen, 23.26 kg ha^{-1} available phosphorus and 145 kg ha^{-1} available potassium (Table 1). The experiment was conducted in randomized block design with three replications in 4 x 5 m^2 plots. Soybean seed (JS 335 variety) was sown with a spacing of 45 cm between rows, at 5cm depth and 14 to 15 seeds per meter. Various field operations from preparatory tillage to the harvesting of the crop viz. ploughing and leveling, manure and fertilizer applications, irrigations, sowing and weeding were undertaken as per requirements.

Table 1. Characterization of some soil physicochemical properties

Soil level	Texture	Bulk density	pH	SOC (%)	Avail. N (kg ha^{-1})	Avail. P (kg ha^{-1})	Avail. K (kg ha^{-1})
(0-30 cm)	Silty clay loam	1.37	7.6	0.76	254	23.26	145

The crop was uniformly fertilized with a basal dose of nitrogen (urea), phosphorus (SSP) and potassium (MOP) at 20, 60, 40 kg ha^{-1}, respectively at the time of sowing. Plant population was maintained to 40 plants per square meter area. Soybean seed was treated with fungicide concentrations, ranging from 2 g kg^{-1} to 3 g kg^{-1} of seed. Treatments were Thiram (T_1), Thiram + Carbendazim (T_2), Carboxin (T_3), Vitavax (T_4), *Trichoderma viride* (T_5), *Pseudomonas* (T_6), Thiamethoxam (T_7) and control (T_8).

Pour plate serial dilution method was used for estimating the population of total bacteria, fungi and Actinomycetes in soil. The soil was serially diluted and aliquots of suitable dilutions were plated with the appropriate culture medium in triplicate. The culture media used were nutrient agar for bacteria, “Martin’s Rose Bengal Streptomycin Agar” media for fungi, “Ken Knight Agar” medium for Actinomycetes, yeast extract mannitol (YEM) agar for *B. japonicum*, Pikovskaya’s media for phosphate solubilizing bacteria (PSB) and “King’s B” media for *Pseudomonas* (Cleyet-Marel 1993). The population of these microorganisms in soil was computed by multiplying the mean colonies with the dilution factor used for computing population.

$$\text{Viable counts (g}^{-1}\text{ soil)} = \frac{\text{(number of colony x dilution factor)}}{\text{Weight of soil}}$$

RESULTS AND DISCUSSION

Population of soil microbes

The effect of applied fungicide and bioagents on total bacterial population in the soil was not significant in comparison to control (Table 2). Bacterial count in soil ranged from 7.5 to 8.8 x 10^6 CFU g^{-1} of soil at crop harvest. Thiamethoxam at 3 g kg^{-1} showed the maximum population of bacteria in soil. Seed inoculation with Thiram + Carbendazim 2:1 at 3 g kg^{-1}, Vitavax at 3 g kg^{-1}, *T. viride* at 5 g kg^{-1} and Thiamethoxam at 3 g kg^{-1} increased bacterial population in the soil of 2.7, 1.6, 2.6 and 11.1% over control treatment. Seed inoculation with *Pseudomonas* at 3 g kg^{-1} showed the maximum number of fungi (12.27 x 10^4 CFU g^{-1} of soil), 13.6% more over control. All the treatments except *Pseudomonas* at 3 g kg^{-1} showed numerically decrease in the fungal population in comparison to control treatment. At harvest, the effect of applied fungicide and bioagents was also non-significant in comparison to control treatment. Seed inoculation with *Pseudomonas* at 3 g kg^{-1} showed the maximum number of Actinomycetes counts (11.4 x 10^5 CFU g^{-1} of soil) and showed 13.9% more Actinomycetes population over control. All the treatments showed numerically increase in Actinomycetes population in comparison to control. At harvest, the effect of applied fungicide and bioagents was not-significant in comparison to control treatment.

Table 2. Effect of fungicides and bioagents on bacteria (×10^6), fungi (×10^4) and actinomycetes (×10^5) population at harvest of soybean

Treatments	Bacteria (×10^6)	Fungi (×10^4)	Actinomycetes (×10^5)
Thiram at 3g kg^{-1}	7.88	10.52	10.74
Thiram+Carbendazim 2 1 at	8.14	10.61	10.06
3g kg^{-1}	7.55	9.65	10.64
Carboxin at 2g kg^{-1}	8.05	6.95	10.06
Vitavax at 2g kg^{-1}	8.13	8.88	10.52
Trichoderma viride at 5g kg^{-1}	7.88	12.27	11.42
Pseudomonas at 3g kg^{-1}	8.80	9.65	10.57
Thiamethoxam at 3g kg^{-1}	7.92	10.80	10.02
Control			
CD 5%	NS	2.27	NS

At the time of harvest *B. japonicum* count in soil ranged from 27.7 to 35.2 x 10^4 CFU g^{-1} of soil at crop at harvest (Table 3). *Pseudomonas* at 3 g kg^{-1} showed the maximum population of *B. japonicum* in soil. Seed inoculation with Thiram at 3 g kg^{-1}, Vitavax at 3 g kg^{-1}, *T. viride* at 5 g kg^{-1} and *Pseudomonas* at 3 g kg^{-1} increased *B. japonicum* population in soil by 10.8, 0.6, 4.0 and 19.5% over control treatment. The effect of different fungicides and bioagents was significant in comparison to control treatment at harvesting. PSB population in soil ranged from 11.6 to 23.8 x 104 CFU g^{-1} of soil. Treatments having *Pseudomonas* at 3 g kg^{-1} and Thiamethoxam at 3 g kg^{-1} showed a significant increase of 73.1 and 54.7%, while and *T. viride* at 5g kg^{-1} showed a decrease of 17.7 in the PSB population in comparison to control treatment. The effect of applied fungicide and bioagents was also non-significant in comparison to control treatment.

Table 3. Effect of fungicides and bioagents on *B. japonicum* (×10^4), PSB (×10^4) and *Pseudomonas* (×10^4) counts at harvest of soybean

Treatments	*B. japonicum* (×10^4)	PSB (×10^4)	*Pseudo-monas* (×10^4)
Thiram at 3g kg^{-1}	32.66	13.8	6.9
Thiram+Carbendazim 2 1 at 3g kg^{-1}	28.56	16.4	7.9
Carboxin at 2g kg^{-1}	27.73	16.1	7.5
Vitavax at 2g kg^{-1}	29.64	16.3	6.5
Trichoderma viride at 5g kg^{-1}	30.63	11.6	7.4
Pseudomonas at 3g kg^{-1}	35.23	23.8	8.0
Thiamethoxam at 3g kg^{-1}	29.13	21.3	6.7
Control	29.46	13.7	7.3
CD 5%	NS	3.2	NS

Pseudomonas population in soil ranged from 6.5 to 8.02 x 104 CFU g^{-1} of soil. Seed-inoculation with *Pseudomonas* at 3 g kg^{-1} gave the maximum number of *Pseudomonas* population and showed the numerical increase of 8.5% in comparison to control treatment. The treatments Thiram + Carbendazim 2:1 at 3 g kg^{-1}, Carboxin at 2 g kg^{-1}, *T. viride* at 5 g kg^{-1} showed the numerically more number of *Pseudomonas* count in comparison to control treatment.

Grain and straw yield

The given treatments significantly affected the grain yield as compared with control. The highest grain yield (1446.4 kg ha^{-1}) was recorded in the treatment with Carboxin at 2 g kg^{-1} while lowest 1244.92 kg ha^{-1} in control. Treatments having Carboxin at 2 g kg^{-1} and *Pseudomonas* at 3 g kg^{-1} seed inoculation showed significant increases of 16.2 and 15.6%, respectively over control. All the treatments except Thiamethoxam at 3 g kg^{-1} gave a significantly higher grain yield than control. The applied treatments showed significant effect on the straw yield in comparison to control (Table 4). The highest straw yield of 3241.6 kg ha^{-1} was obtained with inoculation of *Pseudomonas* at 3 g kg^{-1} which was 16.5% higher than the control. All the treatments showed numerical increases in the straw yield over the control. Treatments with Carboxin at 2 g kg^{-1} and *Pseudomonas* at 3 g kg^{-1} were significantly better than control.

Table 4. Effect of fungicides and bioagents on Grain and Straw yield of soybean

Treatments	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})
Thiram at 3g kg^{-1}	1428.08	3010.56
Thiram + Carbendazim 2 1 at 3g kg^{-1}	1435.54	3062.16
Carboxin at 2g kg^{-1}	1446.45	3230.50
Vitavax at 2g kg^{-1}	1385.60	2946.56
Trichoderma viride at 5g kg^{-1}	1373.19	2877.56
Pseudomonas at 3g kg^{-1}	1439.46	3241.66
Thiomethaxam at 3g kg^{-1}	1316.63	2790.20
Control	1244.92	2782.30
CD 5%	108.77	295.54

The fungicide and bioagents influenced the population of bacteria in soil after harvest of crops. The highest bacterial population was shown by Thiamethoxam at 3 g kg^{-1}. Wainwright and Pugh (1974) concluded that field application of fungicides at twice the normal rate resulted in increases in bacterial population. Fungal population in soil was significantly affected by the use of fungicide and bioagents over control treatment. All fungicide treatments showed lower fungal population in comparison to control. Several studies showed that the fungal population was being reduced with the application of fungicides (Ingham 1985). Treatment with *Pseudomonas* at 3 g kg^{-1} showed the highest population of fungi in soil. This might be because inoculation of *Pseudomonas* enhanced phosphate supply in the soil and created conducive environment in soil for fungi. The reduction in fungal population with *T. viride* was due to its parasitic action on other fungi in soil. The population of Actinomycetes was not affected by the use of fungicides and bioagents. The effect of fungicides and bioagents on *B. japonicum* population was found not-significant. Gianasi et al. (2000) also found that fungicide seed treatment did not affect nitrogen fixation by *B. japonicum* in soybean. Highest population *B. japonicum* was found with *Pseudomonas* at 3 g kg^{-1}. Treatments with Thiram + Carbendazim 2:1 at 3 g kg^{-1}, Carboxin at 2 g kg^{-1} and Thiamethoxam at 3 g kg^{-1} showed lower counts of *B. japonicum* in comparison to control treatment. Kaur et al. (2007) reported that Carbendazim is toxic to the nodule bacterium. Thiram concentration beyond 500 µg mL^{-1} was observed to be highly toxic with respect to plant growth factors and rhizobial infection to the *G. max* (Bikrol et al. 2005). The results also showed that fungicides and bioagents inoculation significantly influenced PSB population in soil over control treatment. *Pseudomonas* at 3 g kg^{-1} gave the highest PSB population. All the fungicide treatment showed a lower population of PSB in comparison to *Pseudomonas* treatment. 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.

Seed treatment with fungicides and bioagents have increased grain yield of soybean. Soares et al. (2004) also reported that fungicide treatments showed higher yield than non-treated plants, varying from 14.5 to 27.3%. Carboxin at 2 g kg^{-1} numerically increased grain yield over control treatment. Revellin et al. (1993) found that Vitavax 200FF (Carboxin and Thiram), had a small effect or no effect on the survival of *B. japonicum* and on the nodulation and yield of soybean. It was possibly due to the reduced infection by soil pathogens (*Fusarium* spp., *Pythium* spp. and *Rhizoctonia* spp.) which was significantly controlled in fungicide-treated seeds compared with untreated control and might be due to improvement in soil health. The numerical increase in straw yield was found with fungicides and bioagents over control treatment. Ekundayo (2003) also observed that fungicides did not prevent seed germination. Greater seedling emergence was obtained with fungicide-treated and inoculated seeds compared with fungicide-untreated but inoculated control. Maximum straw yield was recorded with *Pseudomonas* at 3 g kg^{-1}. Zaidi and Singh (2001) observed that inoculation with *B. japonicum* strain SB-12 and different isolates of *fluorescent Pseudomonas* as well as their possible combinations significantly increased yield of soybean over control. This might be due to the reduction in infection by soil pathogens and greater seedling emergence.

CONCLUSION

The study is able to clarify the apparent confusion regarding the response of the seed dressing with plant protection measures for the soybean crop. Agriculturally, the expectation is the increase in yield which should be judged by the success of the dressing of the selected fungicides and bioagents. The value of this study has been to highlight the importance of identifying the crop protection measure through seed dressing and it has been found that seed dressing with *Pseudomonas* is very helpful in maintaining the soil microbial population and achieving the maximum yield of this valuable oilseed crop. The finding suggests soybean seed treatment with *Pseudomonas* should be followed as a routine.

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