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EFFECT OF *BRADYRHIZOBIUM JAPONICUM* AND FUNGICIDES IN THE CONTROL OF ROOT ROT DISEASE OF SOYBEAN

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Abstract:

Seed dressing of soybean with *Bradyrhizobium japonicum* alone or mixed with different concentration of fungicides viz., benlate, dithane, bavistin and vitavax showed significant ($p<0.001$) protection of roots from infection of *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium* spp. Use of *B.japonicum* with benlate or bavistin against *Fusarium* spp., and *M.phaseolina* and with dithane against *Fusarium* spp., were more effective than their separate use. *B.japonicum* used with bavistin (@ 2500 a.i.) showed highest number of nodules per plant. Plant height increased over untreated control where seeds were treated with *B.japonicum*, benlate, bavistin or dithane or fungicides mixed with *B.japonicum*.

Introduction

Seed treatment with microbial antagonists or fungicides protect the seed from infection by seedborne and soilborne pathogens, enables the seed to germinate and establish as a healthy seedling (Chang & Kommedahl, 1968; Henis & Chet 1975; Windels, 1981). Seed treatment is therefore a routine practice to ensure good emergence and better crop stand (Nene & Thapliyal, 1979; Ramos & Ribeiro, 1993). There are diverse opinion whether legume seeds should be treated with fungicides and whether seed dressing materials might adversely affect the *Rhizobium* spp., and hence the nodulation. In pea, ceresan adversly affect nodulation (Milthorape, 1945) whereas captan and thiram had no adverse effect under field conditions (Nene *et al.*, 1969). In recent past use of rhizobia as microbial antagonists has enhanced the importance of rhizobia as seed dressing (Tu, 1978 Chakraborty; & Purkayastha, 1984; Ehteshamul-Haque & Ghaffar, 1993). No information is available on the use of *Bradyrhizobium japonicum* with fungicides on the control of root rot disease caused by *Macrophomina phaseolina* (Tassi) Goid, *Rhizoctonia solani* KÜhn and *Fusarium* spp. The present report describes the effect of seed dressing with *B. japonicum* alone or with different concentration of fungicides viz., benlate, dithane, vitavax and bavistin to see the combined effect of the fungicides with rhizobia on root rot disease and root nodulation of soybean.

Materials and Methods

A 7 day old culture of *B.japonicum* (TAL 102) on yeast extract mannitol agar was used. Seeds of soybean (*Glycine max* (L.) Merr.) were dipped in a suspension of 1) *B.*

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Table 1. Population of *Bradyrhizobium japonicum* per seed.

No.	Treatments	Rhizobia per seed
Dithane		
1.	Control	0
2.	*B. japonicum*	$6.3x10^7$
3.	Dithane 5000 ppm	0
4.	Dithane 2500 ppm	0
5.	Dithane 500 ppm	0
6.	*B. japonicum* + Dithane 5000 ppm	$6x10^6$
7.	*B. japonicum* + Dithane 2500 ppm	$2.6x10^6$
8.	*B. japonicum* + Dithane 500 ppm	$2.3x10^6$
Vitavax		
1.	Control	0
2.	*B. japonicum*	$6.3x10^7$
3.	Vitavax 5000 ppm	0
4.	Vitavax 2500 ppm	0
5.	Vitavax 500 ppm	0
6.	*B. japonicum* + Vitavax 5000 ppm	$0.3x10^3$
7.	*B. japonicum* + Vitavax 2500 ppm	$3.9x10^4$
8.	*B. japonicum* + Vitavax 500 ppm	$2.2x10^4$
Benlate		
1.	Control	0
2.	*B. japonicum*	$6.3x10^7$
3.	Benlate 5000 ppm	0
4.	Benlate 2500 ppm	0
5.	Benlate 500 ppm	0
6.	*B. japonicum* + Benlate 5000 ppm	$0.6x10^6$
7.	*B. japonicum* + Benlate 2500 ppm	$3.4x10^6$
8.	*B. japonicum* + Benlate 500 ppm	$1.6x10^6$
Bavistin		
1.	Control	0
2.	*B. jaoponicum*	$6.3x10^7$
3.	Bavistin 5000 ppm	0
4.	Bavistin 2500 ppm	0
5.	Bavistin 500 ppm	0
6.	*B. japonicum* + Bavistin 5000 ppm	$0.3x10^6$
7.	*B. japonicum* + Bavistin 2500 ppm	$1.2x10^6$
8.	*B. japonicum* + Bavistin 500 ppm	$3.2x10^7$

japonicum containing 2.8 x 10^9 cfu ml^{-1} in 1% gum arabic ii) different concentration of fungicides viz., dithane, benlate, vitavax and bavistin @ 5000, 2500 and 500 ppm a.i. and iii) different concentration of fungicides mixed with *B. japonicum* suspension giving 5000, 2500, and 500 ppm a.i. final concentration. Population of rhizobia per seed were recovered by transferring the 10 treated seeds in test tubes containing 10 ml sterilized distilled water (i.e. 1 seed in 1 ml). The test tubes were shaken and dilution series transferred on to YMA plates and cfu calculated by using the formula:

cfu per seed = No. of colonies of rhizobia x dilution factor

Population of rhizobia per seed are given in Table 1. Eight seeds were sown in 8 cm diam., plastic pots, each containing 250 gm of soil and after germination 5 seedlings were kept/pot. The soil had a natural infestation of 3-8 sclerotia g^{-1} of soil of *M. phaseolina* as found by wet sieving and dilution technique (Sheikh & Ghaffar, 1975), 12% colonization of *R. solani* as assessed by baiting technique (Wilhelm, 1955) and 3500 cfu g^{-1} of soil of *Fusarium* spp., (a mixed population of *F. solani* and *F. oxysporum*) as assessed by soil dilution technique (Nash & Snyder, 1962). The soil was adjusted at 50% W.H.C. (Keen & Raczkowski, 1921) while each treatment was replicated three times and pots were randomized on a screen house bench.

Plants were uprooted after 6 weeks growth and 5 one cm long root pieces were cut from each plant, washed in tap water, surface sterilized with 1% $Ca(OCl)_2$ for 3 minutes and transferred on to PDA plates containing penicillin (100000 unit/litre) and streptomycin (0.2 gm/litre). Plates were incubated for 5 days at 28°C and incidence of root infecting fungi viz., *M.phaseolina, R.solani* and *Fusarium* spp., were recorded. Observation on nodulation, fresh weight of shoot and plant height were also determined. Data were analysed and subjected to factorial ANOVA followed by least significant difference (LSD) according to Gomez & Gomez (1984).

Results

Of the fungicides used, vitavax was found more inhibitory to rhizobia since the number of rhizobial cells recovered from seed were less as compared to other rhizobial treatments (Table 1). No significant difference was found in any treatment in seed germination.

More than 50% reduction in *M. phaseolina* infection was found in treatments where *B. japonicum*, dithane (@ 2500 ppm), vitavax (@ 5000 & 2500 ppm), bavistin (@ 5000 ppm) and where *B. japonicum* was used with benlate (@ 5000, 2500 & 500 ppm), vitavax (@ 2500 ppm) and bavistin (@ 5000 & 2500 ppm). Complete control of *R. solani* infection was observed where *B. japonicum,* dithane (@ 5000 ppm), bavistin (@ 5000 ppm) and *B. japonicum* mixed with dithane (@ 5000 & 2500 ppm), vitavax and bavistin (@ 5000 ppm) were used. More than 50% reduction in infection of *Fusarium* spp., was found where *B. japonicum* and different concentration of dithane, benlate, vitavax and bavistin or *B. japonicum* was mixed with dithane, benlate, vitavax and bavistin (@ 5000, 2500 and 500 ppm). Use of *B.japonicum* with benlate and bavistin against *Fusarium* and *M.phaseolina* and dithane against *Fusarium* were found

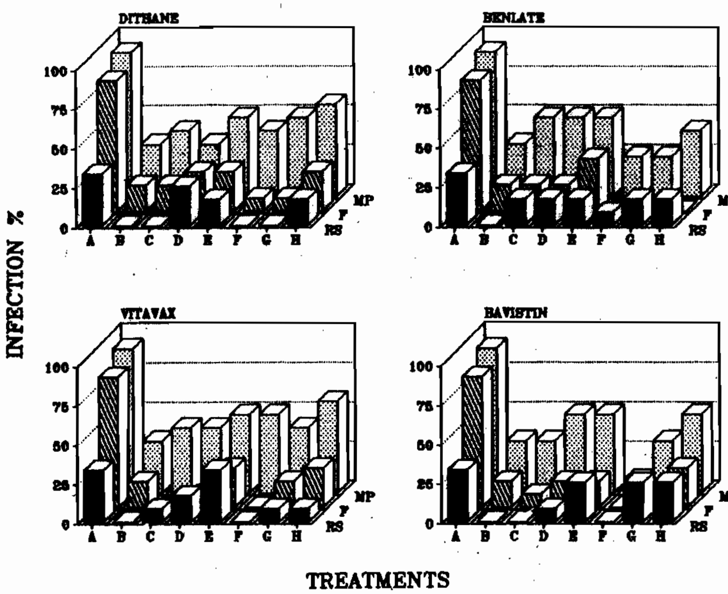

Fig. 1. Effect of seed dressing with *Bradyrhizobium japonicum* used alone or with different concentration of fungicides on infection of MP = *Macrophomina phaseolina*, F = *Fusarium* spp., and RS = *Rhizoctonia solani* on soybean roots.

A = Control, B = *B. japonicum*, C = 5000 ppm a.i. D = 2500 ppm a.i., E = 500 a.i., F = B+C, G = B+D, H = B+E.

$LSD_{0.05}$ (Fungicides) = 4.59, $LSD_{0.05}$ (Pathogens) = 3.97, $LSD_{0.05}$ (Treatments) = 6.49

more effective than their separate use (Fig. 1). Highest number of nodules per plant were produced by *B.japonicum* with bavistin (@ 2500 ppm) followed by *B.japonicum* with bavistin and benlate (@ 5000 ppm). Greater fresh weight of shoot was found in treatments where *B.japonicum* was mixed with bavistin (@ 5000 ppm). All the treatments showed an increase in plant height over untreated control (Fig. 2).

Discussion

There are diverse reports on the affect of fungicides on rhizobia where the activity of rhizobia are stimulated by dithane whereas benlate, bavistin and sicarol were inhibitory to rhizobia (Narayana *et al*, 1981). Similarly , benlate was least and radotiram most lethal to rhizobia (Heneberg *et al.*, 1983), whereas captan, PCNB, dithane and thiram had no adverse effect on *B.japonicum* (Jauhri & Agarwal, 1984). Patel *et al.*, (1989) found that dithane and bavistin had no adverse effect on rhizobia. In the present study *B. japonicum* used with benlate and bavistin showed better protection of roots from infection of *M. phaseolina* and *Fusarium* spp., than their separate use, whereas use of *B. japonicum* with bavistin showed better nodulation per plant, greater plant height and fresh weight. It is interesting to note that such similar reports have been made by Gupta *et al.*, (1985), where seed treated with bavistin and *Rhizobium* showed highest number of nodule per plant and highest yield of chick pea. Presumably

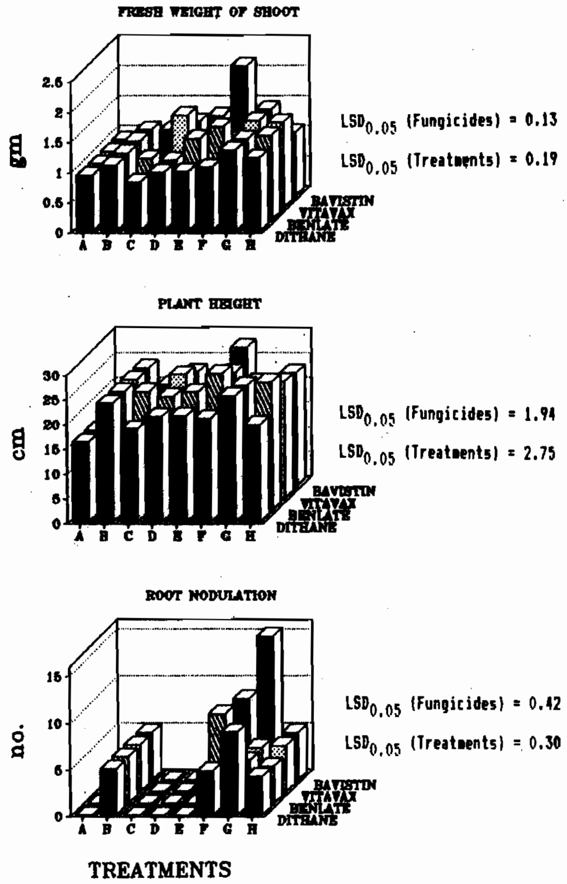

Fig. 2. Effect of seed dressing with *Bradyrhizobium japonicum* used alone or with different concentration of fungicides on fresh weight of shoot, plant height and root nodulation of soybean
A = Control, B = *B. japonicum*, C = 5000 ppm a.i. D = 2500 ppm a.i., E = 500 ppm a.i., F = B+C, G = B+D, H = B+E.

fungicides provide free chance for rhizobial activity by inhibiting other rhizosphere competitive microorganisms. It would suggest that a combination of *Rhizobium* and a suitable fungicide may provide better protection of roots from invasion by root infecting fungi and also enhance root nodulation thus resulting in healthy plant growth.

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References

Chakraborty, U., and R.P. Purkayastha. 1984. Role of Rhizobitoxine in protecting soybean roots from *Macrophomina phaseolina* infection. *Can. J. Microbial.*, 30: 285-289.

Chang, I. and T. Kommedahl 1968. Biological control of seedling blight of corn by coating Kernels with antagonistic microorganisms. *Phytopath.*, 58: 1395 - 1401.

Ehteshamul-Haque, S. and A.Ghaffar. 1993. Use of rhizobia in the control of root rot disease of sunflower, okra, soybean and mungbean. *J.phytopathology,* 138: 157-163.

Gomez, K. A. and A.A. Gomez. 1984. *Statistical procedures for agricultural research.* 2nd. ed. Wiley, NewYork. pp. 680.

Gupta, R.P, R.P. Katiyar and D.P. Singh. 1985. Effect of seed treatment with bavistin and *Rhizobium* on wilt incidence, nodulation and yield of chick pea. *Indian Phytopath.*, 38: 596 (Abst.).

Jauhri, K.S. and D.K. Agarwal. 1984. Effect of seed dressing fungicides on soybean inoculated with *rhizobium japonicum* in charcoal rot infested soil. *Pesticides,* 18: 47-48

Heneberg, R, B. Cvjetkovic and D. Sertic. 1983. Influence of the fungicides on formation of nodules on soybean roots. *Polyo privredea Znanstvenor smotra,* 61: 309-317.

Henis, Y., and I. Chet 1975. Microbiological control of plant pathogens. *Adv. Appl. Microbiol.*, 19: 85 - 111.

Keen, B.A. and H. Raczkowski. 1921. The relation between clay content and certain physical properties of soil. *J. Agric. Sci.,* 11: 441-449.

Milthorape, F.L. 1945. The compatibility of protectant seed dusts with root nodule bacteria. *J. Austr. Inst. Agric. Sci.*, 11: 89-92.

Narayana, Y.D., D. Radhakrishna and P.V. Rai. 1981. Effect of certain fungicides on nodulation of cowpea (*Vigna unguiculata* L.). *Current Research,* 10: 47-48.

Nash, S..M. and W.C. Snyder. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soil. *Phytopath.*, 52: 567-572.

Nene, Y.L., K. Agarwal and S.S.L. Srivastava. 1969. Influence of fungicidal seed treatment on the emergence and nodulation of soybean. *Pesticides,* 3: 26-27.

Nene, Y.L. & P.N. Thapliyal. 1979. *Fungicides in plant disease control.* Oxford & IBH Publishing Co., NewDelhi, Bombay, Calcutta. pp. 507.

Patel, G.Z., P.C. Lodha and I.S. Patel. 1989. *In vitro* effect of antibiotics and fungicides on *Rhizobium* sp., isolated from green gram (*Vigna radiata* Wilc.). *Pestology,* 13: 28-30.

Ramos, M.L.G. and W.O. Ribeiro. 1993. Effect of fungicides on the survival of *Rhizobium* on seed and the nodulation of bean (*Phaseolus vulgaris* L.) *Plant and Soil,* 152: 145-150.

Sheikh, A.H. and A. Ghaffar. 1975. Population study of sclerotia of *Macrophomina phaseolina* in cotton fields. *Pak. J. Bot.*, 7: 13-17.

Tu, J.C. 1978. Protection of soybean from severe *Phytophthora* root rot by *Rhizobium. Physiol. Plant Pathol.*, 12: 233-240.

Wilhelm, S. 1955. Longevity of the *Verticillium* wilt fungus in the laboratory and field. *Phytopath.*, 45: 180-181.

Windels, C. E. 1981. Growth of *Penicillium oxalicum* as a biological seed treatment on pea seed in soil. *Phytopath,* 71: 929- 933.

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