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EFFECT OF FUNGICIDES ON THE EFFICACY OF *RHIZOBIUM MELILOTI* AND *BRADYRHIZOBIUM* SP., IN THE CONTROL OF ROOT INFECTING FUNGI ON CHICKPEA

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Abstract

Bradyrhizobium sp., with Benlate or Bavistin and *Rhizobium meliloti* isolates with Captan or Topsin-M showed better control of *Fusarium solani* infection on chickpea roots than their separate use. *Bradyrhizobium* sp., (Mungbean isolate) with Bavistin showed complete control of *Macrophomina phaseolina* infection, whereas Captan was found more effective against *Rhizoctonia solani* infection when used alone or with rhizobia. Maximum number of nodules per plant was produced by *Bradyrhizobium* sp., (TAL-620, chickpea isolate) used with Benlate or Bavistin. Some rhizobial isolates also significantly ($p<0.05$) enhanced plant height when used with Benlate, Bavistin or Topsin-M.

Introduction

Chickpea (*Cicer arietinum* L.) an important pulse crop, which is infected by blight disease (Hafeez, 1986) is also known to be attacked by root infecting fungi and root knot nematode (Saxena *et al.*, 1996; Ehteshamul-Haque & Ghaffar, 1994). Seed treatment with fungicides is a routine practice to ensure good emergence and better crop stand (Nene & Thapliyal, 1979; Ramos & Ribeiro, 1993). However, seed treatment with fungicides affect the rhizobial survival on seeds and consequently nodulation and nitrogen fixation (Graham *et al.*, 1980; Kecskes & Vincent, 1973). In pea, Ceresan adversely affects nodulation (Milthorpe, 1945) whereas Captan and Thiram had no adverse effect under field conditions (Nene *et al.*, 1969). The present report describes the effect of fungicides on the efficacy of *Bradyrhizobium* sp., and *Rhizobium meliloti* in the control of root rot disease caused by *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium* spp., on chickpea.

Materials and Methods

Cultures of *Bradyrhizobium* sp., R_1 (KUCC 811, Chickpea isolate TAL 620), R_2 (KUCC 823, mungbean isolate), R_3 (KUCC 819, mashbean isolate) and *R. meliloti* R_4 (KUCC 816, Lucerne isolate), R_5 (KUCC 139, Lucerne isolate - 3DOA1) and R_6 (KUCC 827, Fenugreek isolate) multiplied on yeast extract mannitol agar were used in this study. Seeds of chickpea (*Cicer arietinum* L.) were i) dipped in a suspension of rhizobia containing 2.8×10^9 cfu ml^{-1} in 1% gum arabic used as sticker, ii) treated with fungicides viz., Captan, Benlate, Bavistin and Topsin-M in small container (@ 2g/kg) and iii) seeds after treatment with fungicides were treated with rhizobia. Population of

rhizobia per seed are given in Table 1. The seeds were sown in 8 cm diam., plastic pots, each containing 250 gm of soil. The soil had a natural infestation of 3-9 sclerotia of *M. phaseolina* g^{-1} of soil as found by wet sieving and dilution technique (Sheikh & Ghaffar, 1975), 4-11% colonization of *R. solani* on sorghum seeds used as baits

Table 1. Effect of fungicides on the efficacy of *Rhizobium meliloti* and *Bradyrhizobium* sp. in the control of root infecting fungi of chick pea.

No.	Treatment	Infection %		
		M. phaseolina	*F. solani*	*R. solani*
1.	Control	58.33	91.66	16.66
	Bradyrhizobium spp.			
2.	R1 (Tal-620)	33.33 (-42.85)	58.33 (-36.36)	41.66 (+150.06)
3.	R2 (Mungbean isolate)	41.66 (-28.57)	100 (+9.09)	0.00 (-100.00)
4.	R3 (Mashbean isolate)	58.33 (-0.00)	83.33 (-9.08)	0.00 (-100.00)
	Rhizobium meliloti			
5.	R4 (lucerne isolate)	33.33 (-42.85)	83.33 (-9.08)	8.33 (-50.00)
6.	R5 (3 DOA-1)	25.00 (-57.14)	91.66 (-0.00)	8.33 (-50.00)
7.	R6 (Fenugreek isolate)	33.33 (-42.85)	83.33 (-9.08)	0.00 (-100.00)
8.	Captan	33.33 (-43.85)	100 (+9.09)	0.00 (-100.00)
9.	Benlate	41.66 (-28.57)	75.00 (-18.17)	47.22 (+183.43)
10.	Bavistin	44.33 (-24.00)	66.66 (-27.27)	8.33 (-50.00)
11.	Topsin-M	16.66 (-71.43)	91.66 (-0.00)	8.33 (-50.00)
12.	Captan + R1	41.66 (-28.57)	83.33 (-9.08)	25.00 (-50.00)
13.	Catan + R2	44.33 (-24.00)	75.00 (-18.17)	0.00 (-100.00)
14.	Captan + R3	25.00 (-57.14)	66.66 (-27.27)	0.00 (-100.00)
15.	Captan + R4	58.33 (-0.00)	75.00 (-18.17)	0.00 (-100.00)
16.	Captan + R5	41.66 (-28.57)	83.33 (-9.08)	0.00 (-100.00)
17.	Captan + R6	33.33 (-43.85)	66.66 (-27.27)	0.00 (-100.00)
18.	Benlate + R1	33.33 (-43.85)	33.33 (-63.63)	83.33 (+400.00)
19.	Benlate + R2	41.66 (-28.57)	33.33 (-63.63)	66.66 (+300.12)
20.	Benlate + R3	50.00 (-14.28)	25.00 (-72.72)	58.33 (+250.12)
21.	Benlate + R4	30.33 (-48.00)	22.00 (-75.99)	58.33 (+250.12)
22.	Benlate + R5	33.33 (-42.85)	41.66 (-54.54)	66.66 (+300.12)
23.	Benlate + R6	16.66 (-71.43)	58.33 (-36.36)	75.00 (+350.18)
24.	Bavistin + R1	33.33 (-42.85)	33.33 (-63.63)	25.00 (+50.06)
25.	Bavistin + R2	0.00 (100.00)	33.33 (-63.63)	66.66 (+300.12)
26.	Bavistin + R3	33.33 (-42.85)	50.00 (-45.45)	66.66 (+300.12)
27.	Bavistin + R4	58.33 (-0.00)	41.66 (-54.54)	66.66 (+300.12)
28.	Bavistin + R5	25.00 (57.14)	33.33 (-63.63)	66.66 (+300.12)
29.	Bavistin + R6	16.66 (-71.42)	41.66 (-54.54)	66.66 (+300.12)
30.	Topsin -M + R1	33.33 (-42.85)	66.66 (-27.27)	16.66 (-0.00)
31.	Topsin - M + R2	50.00 (-14.28)	50.00 (-45.45)	50.00 (+200.12)
32.	Topsin - M + R3	44.33 (-24.00)	75.00 (-18.17)	19.33 (+16.02)
33.	Topsin - M + R4	50.00 (-14.28)	66.66 (-27.27)	33.33 (+100.06)
34.	Topsin - M + R5	33.33 (-42.85)	66.66 (-27.27)	33.33 (+100.06)
35.	Topsin - M + R6	50.00 (-14.28)	66.66 (-27.27)	25.00 (+50.06)

LSD = 0.05, Treatment = 34.78, Pathogen = 10.18

[Values in parenthesis represent percentage increase (+ve) or decease (-ve) over control]

(Wilhelm, 1955) and 3500 cfu g^{-1} of soil of a mixed population of *F. oxysporum* and *F. solani* as assessed by soil dilution technique (Nash & Snyder, 1962). There were 4 replicates of each treatment and pots were randomized on a screen house bench and kept at 50% W.H.C. (Keen & Raczkowski, 1921).

To determine the incidence of fungi on roots, the method used by Short *et al.*, (1980) was modified where plants were uprooted after 6 weeks growth. After washing with tap water, five one cm long root pieces from each plant were cut, surface sterilized with 1% $Ca(OCl)_2$ for 3 minutes and transferred onto PDA plates containing penicillin (100000 units/litre) and streptomycin (0.2 gm/litre). After incubation for 5 days at 28°C incidence of root infecting fungi viz., *M. phaseolina, R. solani* and *Fusarium* spp., were recorded. Data were analysed and subjected to Factorial ANOVA (FANOVA) followed by least significant differences (LSD) according to Gomez & Gomez (1984).

Results

More than 50% control in *M. phaseolina* infection was observed where *Bradyrhizobium* strain R_1 and Topsin-M were used alone or Captan was used with R_3 and Bavistin was used with R_4. *R. meliloti* (R_5) and Topsin-M when used alone or where *R. meliloti* (R_6) was used with Captan, Benlate with R_4 and R_6 and Bavistin with R_5 and R_6 showed more than 50% control of *M. phaseolina* infection. Bavistin used with R_2 and R_6 and Benlate with R_6 produced greater reduction in *M. phaseolina* infection than either used alone. Use of rhizobia or fungicides were less effective against *F. solani* infection when used alone, except *Bradyrhizobium* isolate R_1 (chickpea isolate). Combined use of Benlate or Bavistin with *Bradyrhizobium* sp., and *R. meliloti* isolates significantly ($p<0.05$) reduced *F. solani* infection. A complete control of *R. solani* infection was observed where *Bradyrhizobium* isolates viz., R_2, R_3 and *R. meliloti* isolate R_6 and Captan were used alone or where Captan was used with rhizobia (Table 1). Greater plant height (31.0 cm) was produced as compared to untreated control (24.0 cm) by *R. meliloti* isolate R_6 followed by *Bradyrhizobium* isolate R_1 (29.6 cm) used with Bavistin. Maximum fresh weight of shoot was produced by R_3 (3.4 g) as compared to untreated control (2.7 g) followed by Captan (3.3 g). Use of R_1 with Benlate resulted in maximum number of nodules per plant followed by R_4 used with Benlate or Bavistin (Table 2).

Discussion

There are diverse reports on the effect of fungicides on rhizobia. Vitavax had no significant effect and Benlate greatly reduced rhizobial survival on bean seeds (Ramos & Ribeiro, 1993). Simultaneous inoculation of seeds with rhizobia and fungicides affected the rhizobial survival on bean seeds (Lopes & Portugal, 1986). In the present study use of *Bradyrhizobium* sp., and *R. meliloti* with Benlate or Bavistin resulted in better control of *M. phaseolina* and *F. solani* infection than either used alone. The activity of rhizobia are stimulated by Dithane whereas Benlate, Bavistin and Sicarol were inhibitory to rhizobia (Narayana *et al*, 1981). Similarly Heneberg *et al.*, (1983), reported Benlate as least and Radotiram as most lethal to rhizobia. Use of *B. japoni-*

Table 2. Effect of fungicides on the effecacy of *Bradyrhizobium* sp., and *Rhizobium meliloti* on Rhizobial population, plant growth and root nodulation in Chickpea.

Treatment	Population Rhizobia per seed	Plant Height (cm)	Shoot Weight (gm)	Nodules per plant
Control	0	24.08	2.76	1.5
Bradyrhizobium sp.				
R1 (Tal-620)	6.9×10^6	25.41 (+5.23)	2.83 (+2.47)	1.16 (-29.30)
R2 (Mung bean isolate)	9.0×10^6	26.33 (+8.54)	2.77 (+0.36)	2.66 (+43.60)
R3 (Mash bean isolate)	8.4×10^6	25.66 (+6.15)	3.41 (+19.06)	0.08 (-1775)
Rhizobium meliloti				
R4 (Lucerne isolate)	7.3×10^6	26.41 (+8.82)	2.71 (-1.81)	4.33 (+65.35)
R5 (3DOA 1)	8.6×10^6	26.71 (+9.84)	2.99 (+7.69)	1.16 (-29.31)
R6 (Fenugreek isolate)	7.3×10^6	26.50 (+9.13)	2.58 (-6.97)	2.66 (+43.60)
Captan	0	24.66 (+2.35)	3.36 (+17.85)	0
Benlate	0	21.83 (-10.30)	2.66 (-3.75)	0
Bavistin	0	25.58 (+5.86)	2.79 (+1.07)	0
Topsin - M	0	22.66 (-6.26)	2.81 (+1.77)	0
\Captan + R1	3.6×10^6	25.83 (+6.77)	2.47 (-11.74)	7.66 (+80.41)
Captan + R2	3.6×10^6	22.50 (-7.02)	2.56 (-7.81)	0.16 (-837.50)
Captan + R3	7.6×10^6	24.16 (+0.33)	2.40 (-15.00)	1.25 (-20.00)
Captan + R4	1.3×10^6	22.83 (-5.47)	2.27 (-21.58)	0.81 (-80.72)
Captan + R5	2.1×10^6	24.91 (+3.33)	2.34 (-17.94)	1.83 (+18.03)
Captan + R6	2.3×10^6	22.25 (-8.22)	2.51 (-9.96)	0.91 (-64.83)
Benlate + R1	2.5×10^6	25.26 (+4.67)	2.14 (-14.52)	22.00 (+93.18)
Benlate + R2	5.8×10^6	24.16 (+0.33)	2.36 (-16.49)	6.08 (+75.32)
Benlate + R3	3.0×10^6	26.00 (+7.38)	2.11 (-30.81)	4.83 (+68.94)
Benlate + R4	1.9×10^6	24.75 (+2.70)	2.19 (-26.02)	19.58 (+92.33)
Benlate + R5	3.0×10^6	27.41 (+12.14)	2.59 (-6.56)	14.66 (+89.76)
Benlate + R6	2.8×10^6	29.83 (+19.27)	2.19 (-26.02)	475 (+68.42)
Bavistin + R1	4.5×10^6	29.66 (+18.81)	2.41 (-14.52)	18.75 (+92.00)
Bavistin + R2	2.2×10^6	25.58 (+5.86)	2.55 (-8.23)	16.91 (+91.12)
Bavistin + R3	2.9×10^6	27.08 (+11.07)	2.36 (-16.94)	7.00 (+78.57)
Bavistin + R4	4.1×10^6	25.16 (+4.29)	2.29 (-20.52)	17.25 (+91.30)
Bavistin + R5	1.8×10^6	29.91 (+19.49)	2.68 (-2.98)	8.41 (+84.05)
Bavistin + R6	3.5×10^6	31.00 (+22.32)	2.40 (-15.00)	13.41 (+88.81)
Topsin - M + R1	2.6×10^6	20.91 (-15.16)	2.37 (-15.23)	11.58 (+88.07)
Topsin - M + R2	2.0×10^6	26.41 (+8.82)	2.38 (-15.96)	6.41 (+76.59)
Topsin - M + R3	3.6×10^6	23.50 (-2.46)	2.74 (-0.72)	4.33 (+65.35)
Topsin - M + R4	4.7×10^6	27.41 (+12.14)	2.56 (-7.81)	10.66 (+85.92)
Topsin - M + R5	1.6×10^6	29.16 (+7.42)	2.46 (-12.19)	7.91 (+81.03)
Topsin - M + R6	3.5×10^6	28.16 (+14.48)	2.82 (2.12)	6.33 (+76.30)

LSD = 0.05

[Values in parenthesis represent percentage increase or decease (-ve) over control]

cum with Bavistin showed better control of root infecting fungi and produced better nodulation in soybean (Ehteshamul-Haque & Ghaffar, 1995). Combined use of rhizobia with Benlate and Bavistin also produced greater numbers of nodules than used alone. There are also reports where seed treatment with fungicides can cause an increase (Lopes & Portugal, 1986), a reduction (Jones & Giddens, 1984; Rennie & Dubetz, 1984) or no effect (Kucey & Bonetti, 1988) on nodulation. Our results on chickpea are similar to the findings of Gupta *et al.*, (1985), where seed treated with Bavistin and *Rhizobium* showed highest number of nodule per plant and highest yield of chickpea. Presumably fungicides reduce competitive rhizospheric microorganisms and may provide easy chance for rhizobial activity. The use of rhizobial strains naturally tolerant to fungicides can increase nodulation and maintain the survival of inoculated strains on seeds (Lennox & Alexander, 1981) which may also provide better protection of roots from invasion by root infecting fungi thus resulting in healthy plant growth.

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