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Effect of systemic fungicides on the efficacy of *Pseudomonas aeruginosa* (Schroeter) migula in the control of root-infecting fungi of wheat

I. A. SIDDIQUI, S. S. SHAUKAT, S. EHTESHAMUL-HAQUE AND S.A.J. KHAN*

Soilborne Diseases Research Laboratory, Department of Botany, University of Karachi, Karachi-75270, Pakistan.
*Crop Diseases Research Institute Pakistan Agricultural Research Council, Karachi-75270, Pakistan.

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Summary

Effect of various fungicides on the efficacy of *Pseudomonas aeruginosa*, a plant growth-promoting rhizobacterium in the control of root-infecting fungi such as *Macrophomina phaseolina*, *Fusarium oxysporum* and *F. solani* on four different varieties of wheat was evaluated under field conditions. Bayleton (a.i. triadimephon), Bavistin (a.i. carbendazym) and Topsin-M (a.i. thiophanate-methyl) reduced bacterial survival on wheat seeds whereas Benlate (a.i. benomyl) was not effective in this respect. *P. aeruginosa* used in combination with Benlate showed effective control of soilborne root-infecting fungi along with the enhancement of growth and grain yield of wheat.

Key words: *Pseudomonas aeruginosa*, fungicides, root-rot, soilborne fungi

INTRODUCTION

Use of chemical pesticides is a routine practice to suppress soilborne plant-pathogens. Little attention is given to the possibility that they may also be toxic to plant-associated beneficial microorganisms such as those that are known to protect

crops against plant diseases (W e l l e r, 1988). A wide range of free-living bacteria, when applied as inoculants, are able to enhance crop productivity and are currently undergoing studies at development and commercialization (K l o e p p e r et al., 1989). One group of beneficial bacteria, the plant growth-promoting rhizobacteria (PGPR), has been shown to enhance emergence of canola and soybeans (K l o e p p e r et al., 1986) and to improve yield of canola under field conditions (K l o e p p e r et al., 1989). One of the potential limitations to the commercial utility of seed-applied bacterial inoculants is the simultaneous use of various agrichemicals as seed treatments in various crop production systems. However, one „fungicide-compatible" PGPR, *Bacillus subtilis* strain A-13 (Quantum 4000), marketed by Gustafsson, is currently registered and being used commercially on peanut in the Southern United States (T u r n e r, B a k m a n, 1991).

Of the various rhizosphere bacteria, *Pseudomonas aeruginosa* a plant growth-promoting rhizobacterium has shown promising results in the control of soilborne root-infecting-fungi on chickpea and chilli (I z h a r et al., 1995; S i d d i q u i et al., 1999). An experiment was therefore carried out to examine the effects of different systemic fungicides on the efficacy of *P. aeruginosa* in the control of soilborne root-infecting fungi such as *Macrophomina phaseolina, Fusarium oxysporum* and *F. solani* in four varieties of wheat (*Triticum aestivum* L.).

MATERIALS AND METHODS

P. aeruginosa strain IE-6 isolated from the rhizosphere of cotton, multiplied on King's B medium for 5 days at 30°C was used in the present study. Aqueous cell suspension of the bacterium was prepared by scrapping the culture from medium surface with the help of sterilized bent glass rod after adding 10 ml sterile distilled water. The suspension obtained was stored in a refrigerator at 6°C prior to use. Seeds of different varieties of wheat viz., Kiran, Srasabz, Zardana and Parwaz were i) treated with a cell suspension of *P. aeruginosa* containing 3.0 x 10^9 cfu ml^{-1}; ii) treated with various fungicides such as Benlate, Bayleton, Bavistin and Topsin-M at the rate of 2g/kg seeds, iii) treated with both *P. aeruginosa* and the fungicides. Seeds treated with sterile distilled water were used as control. Population of *P. aeruginosa* per seed was determined 48 h after treatment.

The experiment was conducted in 2 x 1 meter microplots in a field located near the Department of Botany, University of Karachi in a complete randomized block design. Thirty seeds of each wheat variety were sown separately in 1.5 meter furrows. After germination only eight seedlings were maintained per plot. The soil was naturally infested with 3-9 sclerotia g^{-1} of *M. phaseolina* as found by a wet sieving and dilution technique (S h e i k h, G h a f f a r, 1975), and 2500 cfu g^{-1} of soil of mixed population of *Fusarium* spp., as determined by soil dilution technique (N a s h, S n y d e r, 1962). Treatments were replicated three times and plots were watered daily up to first 15 days and thereafter every second day.

Plants were uprooted after 45 and 90 days interval and growth parameters such as plant height and fresh weight of shoot were recorded. Grain yield per plant was

recorded at 90 days. Roots were thoroughly washed in running tap water, cut into small segments, surface sterilized with 1% $Ca(OCl)_2$ for three minutes and 5 pieces were plated onto PDA plates containing penicillin (100, 000 units/l.) and streptomycin sulfate (0.2g/l.). The plates were incubated at 30°C for 5 days and root infection caused by root-infecting fungi (*M. phaseolina, Fusarium oxysporum* and *F. solani*) was recorded as follows:

$$\text{Infection } \% = \frac{\text{Number of plants infected by a pathogen}}{\text{Total number of plants}} \times 100$$

RESULTS

Effects of systemic fungicides on seed population of *Pseudomonas aeruginosa*

Most of the fungicides used had an inhibitory effect on population of *P. aeruginosa* as the bacterial population was significantly ($p<0.05$) reduced. Bavistin was most toxic, causing a drastic decline in bacterial populations while, Benlate was least toxic to *P. aeruginosa* (Table 1).

Table 1

Population of *Pseudomonas aeruginosa* per seed od different cultivars of wheat after treatment whit systemic fungicides

Treatments	Log_{10}(cfu x+1)			
	Kiran	Sarsabz	Zardana	Parwaz
Pseudomonas aeruginosa	6.58a	6.42b	6.53a	6.51a
P. aeruginosa + Benlate	6.55a	6.51a	6.54a	6.49a
P. aeruginosa + Bayleton	6.38c	6.28c	6.25b	6.36b
P. aeruginosa + Topsin-M	6.46b	6.31bc	6.28b	6.32bc
P. aeruginosa + Bavistin	6.19d	5.93d	6.01c	6.21c

Mean values with same letters are not significantly different;
Duncan´s Multiple Range Test; ($p<0.05$)

Effects of systemic fungicides on the efficacy of *Pseudomonas aeruginosa* in the suppression of root-infecting fungi in wheat

P. aeruginosa used alone or in combination with various fungicides significantly ($F= 7.28$; $p<0.001$) reduced root-infection caused by *M. phaseolina*. Cultivars also differed significantly ($F= 9.97$; $p<0.001$) as a wheat cv. Zardana was most infected with *M. phaseolina* whereas Sarsabz was least attacked by this fungus. In plants uprooted after 90 days, *P. aeruginosa*-Benlate combination was most effective in the suppression of *M. phaseolina* showing complete inhibition of the fungus in Kiran and Sarsabz cvs. *P. aeruginosa* used alone in Sarsabz, also showed complete suppression of *M. phaseolina* infection (Fig. 1).

Similarly, *F. oxysporum* infection was significantly ($F=2.74$; $p<0.01$) reduced after treatment with *P. aeruginosa* alone or used in conjunction with the fungicides. *F. oxysporum* infection was significantly higher in Kiran cv. whereas Zardana and Parwaz were less affected. At final harvest, *P. aeruginosa* used alone or mixed with Benlate in Kiran, Benlate or Topsin-M used separately in Sarsabz, Benlate used alone or mixed with *P. aeruginosa* in Zardana and Bayleton used singly or in conjunction with *P. aeruginosa* showed more than 50% inhibition of *F. oxysporum* infection (Fig.1).

Treatments showed significant ($F=3.31$; $p<0.001$) differences in root infection caused by *F. solani*. Cultivars also showed significant differential response ($F= 5.52$; $p<0.01$). *F. solani* infection was relatively higher in Kiran cv. After the second harvest, Benlate or *P. aeruginosa* used individually in Sarsabz, and Bayleton used alone in Parwaz showed complete inhibition of *F. solani* infection. Similarly, Benlate and *P. aeruginosa* either used separately or in combination in cv. Kiran, and Bavistin used alone in Sarsabz showed more than 50% reduction in *F. solani* infection (Fig. 1).

Effects of systemic fungicides on the efficacy of *Pseudomonas aeruginosa* on growth and grain yield of wheat

The four fungicides (Benlate, Bayleton, Topsin-M and Bavistin) alone or in combination with *P. aeruginosa* had no significant effect on plant height ($F=1.13$ n.s.). However, cultivars did differ significantly in plant height ($F=2.68$, $p<0.05$). Shoot weight was significantly ($F=4.62$, $p<0.01$) influenced by the treatments (fungicides alone or mixed with *P. aeruginosa*). *P. aeruginosa* greatly increased the shoot weight of wheat in particular that of cvs. Kiran and Sarsabz where shoot weight was almost doubled over that of controls at the final harvest (Table 2). Grain yield was significantly influenced by the treatments ($F=7.97$; $p<0.001$). The four cvs. also differed significantly in grain yield with Sarsabz giving the highest yield (Table 2). Among the fungicides, Topsin-M application resulted in a significant increase in the grain yield in cvs. Sarsabz, Zardana and Parwaz (p at the most 0.05), while, Benlate significantly elevated the yield in Sarsabz and Parwaz ($p<0.01$). In all four cvs., *P. aeruginosa* alone significantly increased the yield over the controls (p at the most 0.05). Benlate in conjunction with *P. aeruginosa* significantly elevated the yield in cvs. Kiran, Sarsabz and Parwaz (p at the most 0.05) while Topsin-M in conjunction with *P. aeruginosa* significantly elevated the yield in all four cultivars ($p<0.001$, for cv. Zardana $p<0.05$). On the other hand, grain yield in the combination of Benlate and *P. aeruginosa* was significantly increased in cvs. Zardana and Parwaz ($p<0.001$).

Table 2.

Effects of systemic fungicides on the efficacy of *Pseudomonas aeruginosa* on growth and grain yield production of four wheat varieties

Treatments	Plant height (cm) Varieties								Grain yield per plant (g) Varieties								Shoot weight (g) Varieties			
	Kiran		Sarsabz		Zardana		Parwaz		Kiran		Sarsabz		Zardana		Parwaz		Kiran	Sarsabz	Zardana	Parwaz
	Harvest time (Days)																			
	45	90	45	90	45	90	45	90	45	90	45	90	45	90	45	90	90	90	90	90
Control	36,5	65,6	45,1	71,8	44,6	72,1	39	65,4	7,7	10,7	6,6	7,8	9,1	10,2	7,4	9,3	8,38	9,38	7,86	8,67
Benlate	42,2	76,7	47,5	77	44,5	70,3	41	65,6	9,8	17,2	7,1	11,6	7,2	20,2	8,6	14,9	8,47	9,85	7,56	8,93
Bayleton	36,4	63,3	44,9	83	41,9	71,2	37,8	65,2	9,3	14,3	8	18,8	9,4	14,9	7,1	14,7	8,21	9,27	7,87	9,08
Topsin-M	42,7	72,7	44,5	78,9	43,9	73,4	32,2	65,6	12,1	15,6	7,2	9,9	8,4	14,1	4,2	8,7	8,46	10,41	7,93	8,28
Bavistin	38,6	73,5	46,4	71,8	39,8	71,9	37,5	61,7	6	7,6	6,4	7,7	10,5	14,6	8,6	13,9	8,38	10,33	8,23	8,91
P. aeruginosa (Pa-7)	45,4	77,8	48,8	74,2	42,2	70,1	37,7	65,6	10,7	23,8	11,3	20	7,9	15,2	7,3	15,9	9,22	9,62	8,04	8,98
Benlate + Pa-7	43,5	75,4	51	79,7	50,1	68,9	40,8	66,8	10,1	19,7	11,7	22,2	12,9	16,2	9,9	16,8	10,17	9,78	7,92	9,74
Bayleton + Pa-7	37,3	71,9	45,1	76,9	40,3	69,7	34,1	59,2	7,7	16,6	5,5	19,3	5,5	16,1	4,8	11,2	8,86	9,61	9,12	8,96
Topsin-M + Pa-7	41	70,1	48,3	41,2	45,8	75,5	38,3	73,4	7,8	14,9	12,2	11,6	14,9	18,5	7,6	17,1	9,16	10,13	8,15	9,08
Bavistin + Pa-7	44,1	73,7	46,2	82,9	42,7	74,6	35,4	69,2	10,2	14,4	12,1	15,5	5,8	21,4	5,3	20,4	8,49	9,65	8,35	9,01
F values for																				
Treatment (T)	1.13 NS								4.62 ***								7.97 ***			
Varieties (V)	2.68 *								1.97 NS								453.81 ***			
Day (D)	36.32 ***								110.29 ***								Not applicable			
T x V	0.97 NS								1.27 NS								3.39 ***			
T x D	0.96 NS								2.73 **								Not applicable			
V x D	1.09 NS								0.60 NS								Not applicable			
T x V x D	1.01 NS								1.01 NS								Not applicable			

* p <0.05; *** p<0.001; NS, non-significant

DISCUSSION AND CONCLUSION

Fungicides had variable effects on the seed survival of *Pseudomonas aeruginosa*. Of the fungicides used, Bavistin was most toxic, followed by Bayleton and Topsin-M, while Benlate had no significant effect on the survival of the bacterium. So far no report exists on the effects of the fungicides used on the seed survival of *P. aeruginosa* though reports exist on the survival of some other plant growth-promoting or nitrogen-fixing bacteria in combination with fungicides. Simultaneous inoculation of seeds with rhizobia and fungicides affected the survival of *Rhizobium* spp. (Lopes, Portugal, 1986). Similar to our findings, Heneberg et al. (1983), reported Benlate as least toxic to rhizobia.

In general, *P. aeruginosa* either used alone or in conjunction with fungicides afforded some degree of protection against root-infecting fungi namely *M. phaseolina*, *F.oxysporum* and *F. solani*. Ehteshamul-Haque, Ghaffar (1995) found better control of root-infecting fungi with Bavistin in combination with *Bradyrhizobium japonicum*. Similarly, Siddiqui et al. (1998), found effective control of root-infecting fungi (*M. phaseolina* and *F. solani*) using combinations of rhizobial strains and the fungicides Benlate, Bavistin and Topsin-M. It has been demonstrated by Leavy et al. (1988) that the application of an antagonistic fluorescent *Pseudomonas* markedly reduced the symptoms and the growth of certain fungi on wheat while the growth of the bacterium remained uninfluenced by a variety of fungicides. Presumably the chemicals adversely affected resident rhizospheric colonists thereby giving the fungicide tolerant agent a competitive advantage (Ahmed, Baker, 1987).

A fungicide-tolerant biological control agent such as *Pseudomonas aeruginosa* can provide better control of root-infecting fungi when used in combination with a compatible fungicide thereby giving better protection to the crop and increasing plant growth.

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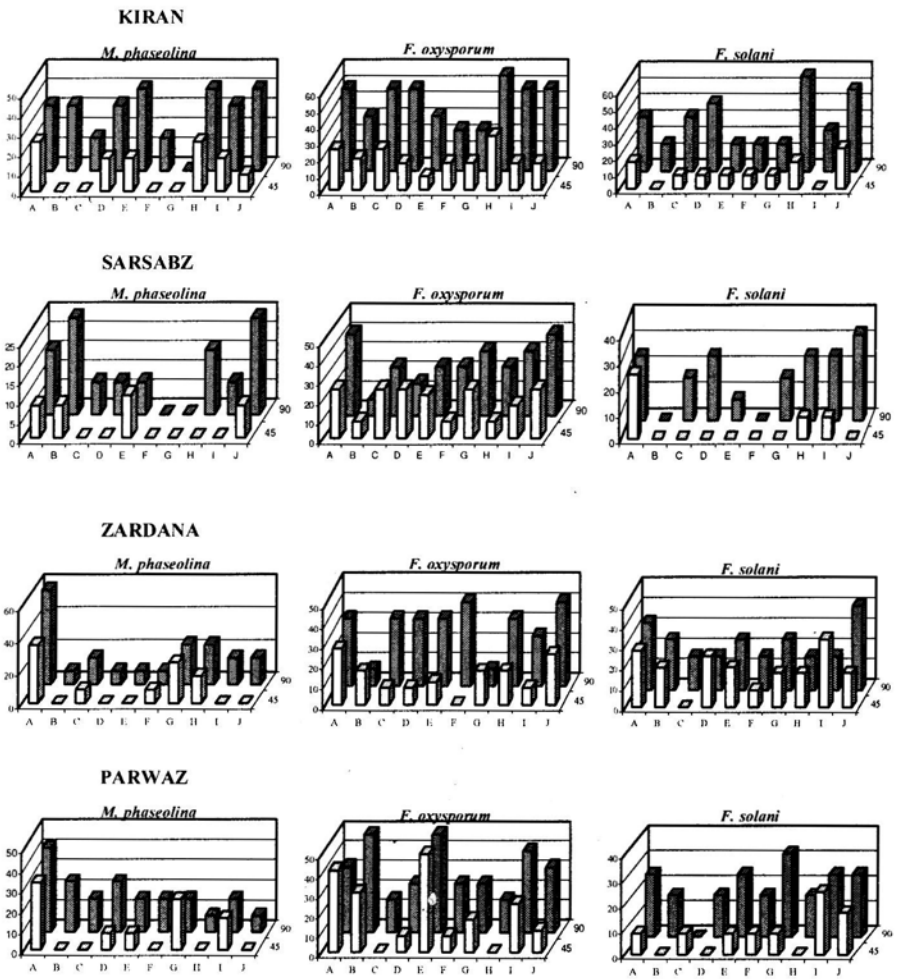
Wpływ systemicznych fungicydów na skuteczność *Pseudomonas aeruginosa* (Schroeter) migula w ochronie przed porażeniem korzeni pszenicy

Streszczenie

W warunkach polowych badano wpływ różnych fungicydów na skuteczność *Pseudomonas aeruginosa*, bakterii ryzosferowej korzystnie oddziaływującej na wzrost roślin, w ochronie przed grzybami porażającymi korzenie takimi jak *Macrophomina phaseolina, Fusarium oxysporum* i *F. solani* dla czterech różnych odmian pszenicy. Fungicydy Bayleton (s.a. triadimephon), Bavistin (s.a. carbendazym) i Topsin – M (s.a. thiophanate-methyl) ograniczały żywotność bakterii na ziarnie pszenicy, natomiast Benlate (s.a. benomyl) nie ograniczał. Bakteria *Pseudomonas aeruginosa* zastosowana w kombinacji z Benlate okazała się skuteczna w ochronie przed grzybami przeżywającymi w glebie i porażającymi korzenie oraz przyczyniła się do wzrostu plonu ziarna pszenicy.

Fig. 1 Effects of systemic fungicides on the efficacy of *Pseudomonas aeruginosa* in the control of *Macrophomina phaseolina, Fusarium oxysporum* and *Fusarium solani* on four different wheat varieties



A= Control; B= Benlate; C= Bayleton; D= Topsin-M; E= Bavistin; F=*Pseudomonas aeruginosa*;
G= F + B; H= F + C; I = F + D; J = F + E