EFFECT OF SEED DRESSING WITH FUNGICIDES ON DAMPING-OFF ROOT-ROT AND NODULATION IN SOYBEAN

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Abstract

Toxicity of five systemic fungicides on radial growth of *Macrophomina phaseolina* (Tassi) Goid and *Cephalophorium gregatum* Allington and Chamberl, the causal pathogens of damping-off and root-root diseases of soybean, were determined. In vitro, results showed that Benlate T, Quinolate CTS and Vitavax SP were the most inhibitory to fungal growth.

In greenhouse, fungicides (Benlate T, Quinolate CTS and Vitavax SP), applied as seed dressing, varied in their effect on nodulation, fresh weight and damping-off of soybean seedlings. Benlate T resulted in the least nodulation and seedling fresh weight and highly affected pre-and post-emergence damping-off of soybean seedlings, while Quinolate CTS and Vitavax SP differed in their effect on bacterial nodulation and seedlings fresh weight and were less effective on damping-off infection.

In field experiment, seed dressing with fungicides significantly decreased damping-off and increased healthy survivals in uninfested plants, which increased seed yield.

Studying the translocation of Benlate T, Vitavax SP and Quinolate CTS in soybean seedlings, indicated that three systemic fungicides remained active in stem tissues for at least 30 days.

INTRODUCTION

Damping-off, root-rot and seed root diseases of soybeans (Glycine max (L.) Merr.) are considered the most important diseases that affect plant stand causing great losses. Some of these diseases are caused by seed and soil-borne pathogens

such as *Macrophomina phaseolina* and *Cephalosporum gregatum* (Klage *et al.* 1978; Eresk 1979 and Abd El-Kader 1983).

Use of fungicides as seed treatments has always been the most practical and economic method in preventing soil-borne diseases (Meyer et al., 1974 and Abdel Lateef *et al.* 1984). *M.phaseolina* and *C.gregatum* are two of the causal pathogens frequently controlled by seed dressing with either protective or systemic fungicides (William 1983).

This work is aimed to evaluate the effect of five systemic fungicides on growth of the causal fungi in vitro and to (test their efficiency) in controlling the disease under greenhouse and field conditions.

MATERIALS AND METHODS

A- Isolates of the pathogens :

One isolate of each of *M.phaseolina* and *C.gregatum*, isolated from diseased soybean plants, were included in this study. These isolates proved to be pathogenic on Clark soybean cultivar (Arafa 1994).

B-Fungicides :

The fungicides used in the study were: **Benlate T** [50% Benomyl + 30% thiram], **Ridomil** [25% WP] Methyl N- (2-methoxyacetyl)-N-(2,6-xylyl) Dl-alaninate), Vitavax SP [75%]; 2,3 Dihydro-5-Carboxanilido-6-methyl-1,4 oxathiin, **Rizolex** T; 20% 0,0-dimethyl-0-2, 6-dichloro-p-toluine phosphothionate + 30% bis (dimethylthiocabamoyl) disulfide tetramethyl thuramidsulphide and **Quinolate CTS** [15%, Copper oxyquinolate + 7.5% carbendezim]. All fungicides were tested for their effect of growth of both fungi **in vitro**. Aliquots from stock solutions or suspensions of each fungicide were added aseptically to autoclaved PDA medium to give final concentrations of 5, 15, 25, 50, 100, 200, 400 and 600 ppm a.i. PDA medium free of fungicides was used as control.

C- Effect of the fungicides on radial growth of the pathogens :

A 5-mm diameter disc from the margin of an actively growing culture of each isolate was placed in the center of PDA medium in a Petri plate. Five replicates per concentration for each fungicide were used in each individual test. Radial growth was measured for two prependicular conlony diameter, 7 days after inoculation.

Percentage of toxicity was calculated according to Topps and Wain (1957).

D- Efficiency of fungicides in controlling damping-off and root-rot of Clark soybean seedlings :

In a greenhouse experiment, sterilized pots (30 cm diameter) were filled with 6 kg of autoclaved soil. Soil was infested, 7 days before planting, by mixing 5 g of the pathogen inoculum to 160 g of air-dried soil (i.e. 187. 5 g/pot) (Boosalis 1950). Inoculum was prepared by growing the isolated fungi, separately on sorghum grains for 3 weeks at 26°C (Whitehead 1957), then thoroughly mixed with the soil in each pot. Pots were watered every two days. Bacteria (*Rhizobium japonicum*) were added to soil in the pot and mixed thoroughly at rate of 0.01% (W/W) (Abd El-Momen and El-Sawah 1984) immediately after 4 days from infestation with tested fungi. Three fungicides, Benlate T, Quinolate CTS and Vitavax SP were applied as seed dressing at two rates of each. Ten treated seeds were planted per pot and replicated 4 times. Two control treatments were included; the first, non-treated seeds were planted in soils infested with tested fungi and *Rh.japonicum* (control 1) and the second, surface sterilized seeds were planted in soils infested with *Rh.japonicum* only (control 2). Percentage of pre and post-emergence damping-off were recorded, 15 and 30 days after planting, respectively. After 50 days plants were gently removed from the soil with their root system intact and washed by running water to remove adherent soil particles. Nodules were counted and the averages per plant were recorded. Fresh weight of each individual plant was weighed after the plants were air-dried for two hours.

A field experiment, was conducted during two seasons. Three replicated plots (3x3.5 m) for each of the three fungicides were arranged in a complete randomized block design. The field soil was infested with a mixture of the different pathogens, 15 kg mycelial growth/feddan, thoroughly mixed with enough amount of clay soil and distributed in furrows two weeks before planting. Clark soybean seeds were treated with the three tested fungicides at two rates of each and left to dry. Moreover, before sowing the above-treated seeds were inoculated with an active pure culture of *Rh.japonicum* and allowed to dry, then planted immediately in rows at 5 cm depth, 50 cm between rows and 20 cm within rows. Rhizobia - inoculated and fungicide-non treated seeds were used as control. Percentage of pre-and post-emergence damping-off were recorded after 15 and 30 days from planting and also percent of survival plants were recorded after 120 days. Yield of seed was weighed and adjusted to 13% moisture content.

E-Effect of the systemic fungicides translocated in soybean seedlings on C.gregatum growth :

In this experiment, seeds were treated with either of the three systemic fungicides (Benlate T, Quinolate CTS and Vitavax SP) at the rates 2, 3, 4 and 6 g/kg seeds (Peterson and Edgington 1970). Treated and untreated seeds were planted in 30 cm clay pots containing sterilized soil. Seedlings were uprooted 10, 15, 20,25, 30 and 35 days after planting, then washed carefully with sterilized water and dried between two sterilized filter papers. The basal part of the stems, each about 5 cm. long, were cut into 5 discs 1 cm length. Plant discs were set vertically soon after cutting on PDA medium in Petri-dishes previously seeded with spore suspension of *C.gregatum* (2.5 ml. of spore suspension /100 ml. agar medium containing 5.4x106 spores/ml.). Mean diameters of inhibition zones around these discs were recorded after 6-days growth at 26°C. The obtained data were statistically analysed and the least significant difference (LSD) at the 5% level was calculated.

RESULTS AND DISCUSSION

A- *In vitro* toxicity of fungicides :

Results in Table 1 show that Benlate T, Quinolate CTS and Vitavax SP were the most toxic fungicides against growth of both *M.phaseolina* and *C. gregatum in vitro*. Concentration of Benlate T, Quinolate CTS and Vitavax SP that completely inhibited the growth of *M.phaseolina* were 15, 25 and 100 ug/ml, respectively, while for *C.gregatum* were 25,50 and 50 ug/ml respectively. Benlate T was the most effective against *M.phaseolina* and *C.gregatum* compared with other fungicides. Moreover, Rizolex and Ridomil MZ were the least effective fungicides against both tested fungi.

These results are somewhat similar to those of Ilyas *et al* (1975), who concluded that Benlate and Tecto were more toxic to *M.phaseolina* than Vitavax and Thiram, and much more so than Captan. Moreover, in a comparative study of Benlate and Tecto, among other fungicides Goyal and Methrotra (1981) reported that Benlate gave more promising results than Tecto against *M.phaseolina*. Furthermore, both fungicides were more fungitoxic than Vitavax-Thiram, Vitavax Captan, Orthocide 75, Rovral and Vitavax 300 (Abou-Donia *et al.* 1983 and Abada (1995).

B- Green-house studies :

The three fungicides; Benlate T, Vitavax SP and Quinolate CTS were used, as

Table 1. Effect of sole cropping and intercropping of maize on the rhizosphere microfolra at different dates, from sowing to maturity, in 1.0 gm dry weight soil.

Fungi	Fungicide	Reduction in fungal growth (% to control) at different concentrations							
		5[a]	15[a)]	25[a)]	50	100	200	400	600
Macrophomina phaseolina	Benlate T	48.8b)	100 b)	100	100	100	100	100	100
	Vitavax SP	49.5b)	52.9	66.5	81.8	100	100	100	100
	Quinolate CTS	23.7	63.6	100	100	100	100	100	100
	Rizolex	27.6	40.8	56.4	69.2	82.4	82.4	100	100
	Ridomil MZ	24.5	46.2	64.2	70.4	70.4	78.0	100	100
Cephalosporium gregatum	Benlate T	50.9	68.4	100	100	100	100	100	100
	Vitavax SP	55.3	62.3	89.9	100	100	100	100	100
	Quinolate CTS	60.0	74.7	86.4	100	100	100	100	100
	Rizolex	39.4	44.0	60.2	66.6	68.4	77.8	84.2	100
	Ridomil MZ	26.2	41.6	54.0	62.4	74.0	80.6	95.4	100

a) All used rates are µg/ml a.i.
b) % reduction of fungal growth compared with control (untreated medium).

seed treatment at two levels, Seed were sown in soil infested with *Rhizobium* and infested or not infested with *C.gregatum* or *M.phaseolina*.

Results in Table 2 show that Benlate T, Vitavax SP and Quinolate CTS reduced percent of damping-off compared with untreated control 1. In case of *C.gregatum*, the lowest percentages of damping-off were obtained when seeds were treated with either one of the three tested fungicides. Benlate T was the most effective followed by Quinolate CTS and Vitavax SP, when applied at all tested rates. Results also revealed that the effect of these fungicides was more pronounced at the recommended rates. These results are in agreement with results of many earlier investigators, Sharan and Gupta (1974) suggested the use of seed treatment to control soybean Cephalosporium Collar-rot disease. On the other hand, Thiram, Vitavax and Captan gave significant increase in soybean emergence and reduced pre-emergence rot caused by *M.phaseolina* (Singh *et al.* 1974). Benomyl (Benlate) was also reported (Abdel-Lateef *et al.* 1984) to be absorbed by soybean roots and translocated to the stem, where it prevented *M. phaseolina*, previously added to soil, from attacking the plant.

Results also show that all the tested seed-dressing compounds did not adversely affect root nodulation process except for Benlate T; number of nodules/plant were 2.4 to 4.0 in soil infested with *M.phaseolina* or *C.gregatum* against 18.91 to 21.5 for the control 2 (soil not infested with fungi). Besides, the tested compounds did not sharply reduce number of nodules/plant. In general, data indicated that the total number of nodules per plant decreased, when tested fungi were combined with *Rh.japonicum* as compared with Rh. japonicum alone (control 2), while, no nodulation appeared when seed were not treated fungicides and planted in soil infested with fungi. Therefore, there is no obvious antagonism between these fungi and the nodule bacterium. Similar rusults were reported by Chou and Schmitthenner (1974) and Orellane *et al.* (1976).

Fungcides apparently interfered with the colonigation of roots by the pathogenic fungi, therefore allowing some nodulation to occur. However, it can be concluded that benlate thas some adverse effect on nodulation.

The adverse effect of Benlate T may be due to the presence of thiram which was reported by El-Sawah *et al.* (1975-1978) and Ziedan *et al.* (1979) as inhibitor to rhizobial growth and root nodulation.

It is obvious that fungicids tested showed different efficiencies in preventing

Table 2. Effect of seed dressing fungicides on damping-off and bacterial-root nodulation in Clark soybean plants, grown in potted soil inoculated with *M.phaseolina* or *C.gregatum*.

Fungi	Dose g/kg seed	Damping-off (%)[a]						Mean number of nodules/plant		Mean number of nodules/plant	
		M.phaseolina			*C.gregatum*						
		Pre-emerg	Post-emerg	Total	Pre-emerg	Post-emerg	Total	*M. phaseolina*	*M. gregatum*	*M. phaseolina*	*M. gregatum*
Benlate T	2	7.5[c]	5.0	12.5	5.0	2.5	7.5	3.3	4.0	9.14	10.88
	4	10.0	2.5	12.5	7.5	0.0	7.5	2.4	2.6	8.8	8.0
Quinolate CTS	2	12.5	7.5	20.0	10.0	5.0	15.0	14.8	13.4	21.28	20.42
	4	12.5	10.0	22.5	10.0	7.5	17.5	10.6	12.0	17.28	19.50
Vitavax SP	3	10.0	7.5	17.5	7.5	5.0	12.5	15.5	14.0	15.50	16.18
	6	15.0	12.5	27.5	12.5	2.5	15.0	14.2	12.6	14.80	13.16
Control (1)[d]	-	40.0	27.5	67.5	35.0	25.0	60.0	0.0	0.0	6.48	5.28
Control (2)[e]	-	0.0	0.0	-	0.0	0.0	-	21.5	18.91	24.8	23.16
L.S.D. at 5%		4.4	3.1		2.9	3.4		2.6	1.9	2.0	3.6

a) Pre-and post-emergence damping-off of seedlings recorded 15 and 30 days after planting, respectively.
b) On a fresh weight basis.
c) Each value is the average of 4 replicates with 100 seeds each.
d) Non-treated seeds planted, in soil infested with tested fungi and *Rh. japonicum*, control (1).
e) Surface sterilized seeds, planted in soil infested with *Rh. japonicum* only, control (2).

infection and disease development. However, higher doses were accompanied by a some what higher disease incidence (Pre and post emcrgavee damping-off). This can be partially attributed to a possible phytotoxic effect of the higher doses. This phytoloxicity was also reflected by lower weights of plants in different treatments as well as in a lower nodulation compared with the appopriate control. Consequenty, it is important to apply the suitable funsicide dosage. It should be mentioned that the pesence of pathogenic funsi has prevented the nodulation.

Benlate T treatment resulted in the lowest plant fresh weight as compared with other fungicides or soil infestation with *Rh.japonicum* alone (control 2). This may be due to the partial inhibition of nodule formation and consequently poor nitrogen fixation. On the other hand, Quinolate CTS and Vitavax SP were the most effective fungicides in increasing nodulation and fresh weight/plant, and seeds inoculated with *Rh.japonicum* and sown in sterilized soil gave the highest plant fresh weight as compared with all other treatments. This was probably due to the beneficial effect of the tested fungicides, which caused prolonged inhibitory effect to fungi around the seeds which resulted in increased healthy seedlings.

C-Field experiments :

Data in Table 3 clearly indicate that the pathogenic fungi *M.phaseolina* and *C.gregatum* varied as regards to the incidence of diseases in the control (damping-off, root-rot and wilt) which amounted to 78.98 for *C.gregatum*, and 81.50% for *M.phaseolina*.

Fungicides application in the field, as seed dressing was generally effective against soybean damping-off and and root-rot disease. All tested fungicides decreased percentages of damping-off and diseased plants, and increased number of survival plants as compared with their respective percent in soil infested with either of the pathogens. However, in case of *C.gregatum* and *M.phaseolina*, the lowest percent of pre-and post-emergence damping-off were obtained when seeds were treated with Benlate T. Therefore, it could be concluded that as far as disease control was concerned, all tested fungicides were satisfactory equal except for Benlate T or Quinolate CTS which were more effective. These results were in accordance with Gray and Sinclair (1970) and Yehia *et al* (1982), who stated that Vitavax 300 was among the systemic fungicides, which reduced brown stem rot of soybean caused by *C.gregatum*. Abdel-Lateef *et al.* (1984) reported that pre and post emergence damping-off of soybean caused by *M.phaseoli* was best controlled by Benlate, Vitavax-Captan and Vitavax Thiram. Also, Singh *et al.* (1974) also found to give a

Table 3. Effect of seed dressing fungicides on damping-off, healthy survival plants and yield of Clark soybean, gorwn in artifically infested field.

Fungi	Dose g/kg seed	Damping-off (%)[a]						Survival [a]		Survival [a]		Seed yield of soybean kg/fed.	
		M.phaseolina			*C.gregatum*			Diseased		Diseased			
		Pre-emerg	Post-emerg	Total	Pre-emerg	Post-emerg	Total	*M. phaseolina*	*C. gregatum*	*M. phaseolina*	*C. gregatum*	*M. Phaseolina*	*C. gregatum*
Benlate T	2	10.92[c]	7.56	18.48	12.60	7.14	19.74	9.24	5.88	72.28	74.38	1004	1002
	4	9.24	5.46	14.70	10.08	7.98	18.06	8.82	5.04	76.48	76.88	996	1000
Quinolate CTS	2	14.28	6.30	20.58	14.70	6.76	12.46	7.76	2.52	71.66	76.02	1115	1119
	4	12.18	5.04	17.22	13.86	5.44	19.30	4.20	3.36	78.58	77.34	1118	1116
Vitavax SP	3	15.96	8.40	24.36	17.64	9.66	27.30	6.30	4.62	69.34	68.08	1009	1110
	6	15.12	7.94	23.06	16.80	8.40	25.20	5.46	2.94	71.48	71.86	1008	1012
Control (1)[c]	-	30.25	39.07	69.32	27.73	37.39	65.12	12.18	13.86	18.50	21.02	640	605
L.S.D. at 5%		3.6	2.7		4.1	3.2		1.7	2.5	5.3	6.6	32.1	29.5

a) Damping-off (Pre-and post-emergence damping-off) 15 and 30 days after planting and percent survivals (disease and healtyy plants) 120 days after seeding. of seedlings recorded 15 and 30 days planting, respectively.
b) Each reading is the average of three replicates.
c) Soybean seeds not-inoculated with and un-treated with fungicides, planted in soil infested with tested fungi as control.

significant increase of soybean seedling emergence and reduce incidence of different fungi including *M.phasoli.*

Results of seed yield showed that all the tested seed-dressing fungicides resulted in significiant increase of yield. However, differences between fungicides were not significant.

Finally, we can conclude from field results that the best compounds and rates that have resulted significant decrease of damping-off disease and highest seed yield increase were: Quinolate CTS, Benlate T, and Vitavax SP used at a rate of 2,2, and 3 g/kg seed respectively.

D- Effect of the translocated systemic fungicides on *C.gregatum* growth :

The translocation of three systemic fungicides namely Benlate T, Vitavax SP and Quinolate CTS in soybean seedlings was studied *in vitro.*

Data presented in Table 4 indicate that the three fungicides remained effective in stem tissues for at least 20 days where *C.gregatum* inhibition zones were increased with the advance of plant age to 20 days, then gradually decreased, when plant parts of 35 days old were tested. It should be noted that mean diameter of inhibition zones in the presence of Quinolate CTS was more than those observed for Benlate T and Vitavax SP. This agreed with Peterson and Edgington (1969) who reported that Benlate was rapidly hydrolysed to methyl-2-benzimidazol carbonate (MBC) which was absorbed by roots and acropetally translocated through the stem. Forty five days after emergence, Benlate disappeared from the stems and then accumulated in leaves. In this respect, Nayak and Verma (1975) stated that Vitavax/ Thiram was gradually translocated up-wards and persisted in roots and hypocotyls for at least 4 weeks. The prolonged effect of disease control, resulting from the systemic fungicides having one of Benzimidazole derivatives such as Benlate T and Quinolate CTS or carboxin such as Vitavax 300 and Vitavax SP was due to their uptake and localization in the hypocotyl and the cotyledonary tissues and their persistence later in the epicotyl giving the plants a prolonged period of protection against the latent stages of infection (Thapliyal and Sinclair 1970 and 1971 and Sinclair 1974).

Table 4. Effect of three systemic fungicides translocated in soybean seedings a on the growth of *C.gregatum in vitro.*

Fungi	Rates g/kg seeds	Mean diameter of inhibiton zones (m.)					
		Plant age in days					
		10	15	20	25	30	35
Benlate T	2	11.5b)	12.5b)	13.78	9.5	8.0	0.0
	4	11.90	13.1	13.9	10.0	8.3	0.0
Quinolate CTS	2	12.4	14.0	17.0	11.0	6.5	0.0
	4	12.9	15.0	17.9	12.0	7.1	0.0
Vitavax SP	3	10.2	12.7	15.2	10.1	7.5	0.0
	6	13.7	13.9	16.0	12.0	9.0	0.0
Control (1)c)	-	0.0	0.0	0.0	0.0	0.0	0.0

a) Stem segments were taken immediately below the primary leaf node.
b) Data are the average of 4 replications.
c) stem segments on the PDA plates.

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تأثير مطهرات البذرة الفطرية فى مقاومة أمراض موت البادرات وتأثيرها على العقد الجذرية لفول الصويا

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أظهرت نتائج اختبار سمية خمس مبيدات جهازية على نمو الفطرين ماكروفومينا فاصولينا وسيفالوسيوريم جرجاتم على البيئات المغذية، أن المبيدات بنليت تى، كينولات سى تى أس ، فيتافاكس أس بى كانت أكثر المبيدات المختبرة فعالية ضد الفطرين محل الدراسة.

وعند تقييم هذه المبيدات كمعاملة بذرة قبل الزراعة كانت النتائج كالتالى :

أولا : فى الصوبة :

اختلفت المبيدات فى تأثيرها على تكوين العقد الجذرية والوزن الطازج ومدى الإصابة بأمراض موت البادرات فى فول الصويا بكلا الفطرين محل الدراسة، ووجد أن البنليت تى كانت أكثر هذه المبيدات تأثيرا فى تخفيض عدد العقد الجذرية والوزن الطازج لبادرات فول الصويا، بينما باقى المبيدات المختبرة وهى كينولات سى تى أس – فيتافاكس أس بى أعطت عكس النتائج السابق ذكرها، والتشابه بينهما كان فى تقليل نسبة موت البادرات سواء كان قبل أو بعد ظهورها فوق سطح الأرض.

ثانيا : فى الحقل :

المبيدات الثلاثة المختبرة أثرت معنويا فى تقليل كل من الإصابة بأمراض موت البادرات وكذا نسبة النباتات المصابة ولكن التاثير كان أكثر وضوحا فى زيادة نسبة النباتات السليمة مما أدى الى زيادة كمية محصول البذرة.

وفى دراسة انتقال المبيدات الفطرية الجهازية فى أنسجة بادرات فول الصويا (كلارك)، ظهر أن البنليت تى ، كينولات سى تى أس، الفيتافاكس أس بى ظلت فعالة فى أنسجة ساق البادرات لمدة لا تقل عن ٣٠ يوما.