Zagazig J.Agric. Res., Vol. 32 No. (2) 2005 471 - 490

EFFECTIVENESS OF TWO *Bradyrhizobium japonicum* STRAINS AND SOIL APPLICATION WITH SOME COPPER FUNGICIDES ON SOYBEAN DAMPING-OFF DISEASE AND PLANT BIOLOGICAL ACTIVITY

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Accepted 8 / 3 / 2005

ABSTRACT: Laboratory and greenhouse experiments were conducted to evaluate the effect of four copper fungicides namely, coprous oxide, copper hydroxide, copper oxychloride and copper sulfate in the presence of two different strains of *B. japonicum* on soybean damping-off disease and soybean plants. The introduced strains were HH-303 (fungicides resistant) and 3407 (fungicides sensitive). The reaction of copper fungicides on bradyrhizobial strains was studied *in vitro* (on the growth in culture) and *in vivo* (on nodule performance and N_2-fixation). Also, pre- and post emergence damping-off, phosphorus and chlorophyll contents, enzymes activity and total counts of microorganisms in used soil were estimated. Copper oxychloride was the most inhibitor to both bradyrhizobial strains at 50 ppm., In the meantime, it significantly decreased the infection percentage with each of *Macrophomina phaseolina*, *Fusarium oxysporum* and *Sclerotium rolfisii* in comparison with either the control treatment or the other tested fungicides with the two inoculated bradyrhizobial strains. Copper fungicides reduced nodulation, shoot dry weight and nitrogen content. There was a negative correlation between enzymes activities, total counts of microorganisms in soybean rhizosphere and copper fungicides application. Hence, a great reduction in N_2-ase, dehydrogenase

activities and total number of fungi, bacteria and actinomycetes was observed. The results confirmed that copper fungicides had opposite effect on biological activity in both plants and rhizosphere.

Key words:*Bradyrhizobia, copper fungicides, soybean, plant biological activity, damping-off disease.*

INTRODUCTION

Soybean (*Glycine max* (L.) *Mer.*) is one of the main either oil or protein crops all over the world. So, the seeds are characterized by high nutritional values; 20 % oil and 40 % protein (Kassem, 1982). Different pathogens are known to attack soybean seeds, seedlings and roots causing serious damage (Mosa, 1982; Hassanien, 1985 and Sinclair & Backman, 1989). Pre- and post-emergence damping-off was controlled using seed dressing with different fungicides (Ellis *et al.*, 1979 and El-Gantiry *et al.*, 1989). These antifungal agents prevent seed and seedling roots damping-off and other fungal diseases (Gruzdyev *et al.*, 1988), and consequently the rate of seed germination and crop yield will increase. However, the recommended rates of fungicides applied are often inhibitory to root-nodule bacteria.One approach that has been considered is to screen different strains of *B. japonicum* for their resistance ability to various fungicides. Chahal and Sidhu (1992) found that inoculation with thiram-resistant strains of *Bradyrhizobium spp.* (*Aracis*) increased the number and nitrogenase activity of nodules as well as dry weight and N-content of peanut shoots compared to parental strains nodulated plants. Also, the effect of some fungicides on root nodules of cowpea (*Vigna sinensis*) was studied by El-Bahrawy and Ghazal (1989). They reported that the tested fungicides showed no inhibitory effect at any of the tested concentrations. Moreover, increases of symbiotic nodule performance and plant dry weights were observed. On the other hand, Lal (1988) reported that fungicides have direct effects on survival of *Rhizobium* and *Bradyrhizobium* on soil and may indirectly affect plant infection or nodule formation process. Whenever, Tesfai and Mallik (1986) showed deference in the effect of fungicides on soybean- rhizobia symbiosis. PCNB and fenaminsulf were more compatible than captan and captafol. The use of peat inocula containing fungicide-resistant strains of rhizobia is recommended.

The objectives of this study were to investigate: (1) the influence of soil treatment with some copper fungicides on the incidence of damping-off disease. (2) the effectiveness of some *B. japonicum* strains on the biological activity of soybean plants grown in soil treated with the recommended levels of the tested fungicides. and (3) the fungicides reaction on soil microorganisms.

MATERIALS AND METHODS

I. Rhizobial Strains, Soybean Cultivar and Fungicides :

Bradyrhizobium japonicum liquid culture strains HH303 and Rothamested 3407 were obtained from BNF Unit, Microbiology Dept., Soils, Water and Environment Res.Inst., Agric. Res. Center (ARC), Giza, Egypt. The strains were maintained on yeast extract mannitol agar medium (YEM) supplemented with 0.3% calcium carbonate (Vincent, 1970) and stored at 4°C.

Soybean seeds Giza 35 cultivar were kindly provided by Field Crops Res. Ins., ARC, Giza, Egypt.

Four types of copper fungicides were obtained from Plant Pathology Res. Ins., ARC, Giza, Egypt. The commercial and common name of the tested fungicides are shown below :

Commercial name	Common name
Kaprus Kz 50% wp	Coprous oxide
Kocide 101 77% wp	Copper hydroxide
Unicopper 50% wp	Copper oxychloride
Paracop 98% wp	Copper sulphate

Fungicides were applied to soil as 0.19, 0.19, 0.3 and 0.15 g/pot respectively before sowing. Seeds of Giza 35 cv. were coated with a single strain peat inocula using Arabic gum (16% w.v. in sterile distilled water) as an adhesive material.

II. Effect of Four Copper Fungicides on the Growth of *B. japonicum* Strains *in vitro*:

Four copper fungicides were screened for their relative toxicity towards rhizobial growth on YEM agar plates containing the desired fungicide. Subsamples of 10 □l of a freshly grown rhizobial culture to early Log phase were spotted on YEM agar plates containing different concentrations of each of the tested fungicides, i.e., 1, 5, 10, 25, 50 and 100 ppm, four replications of each were used.

Plates were incubated at 28°C for 7 days, then rhizobial growth was compared to control plates (untreated with fungicides) and the data were recorded.

III. Greenhouse experiments

A. Effect of the tested fungicides and *B. japonicum* on damping-off disease of soybean :

Pot experiment was carried out to evaluate the effect of soil treatments using the four tested fungicides at the on the incidence of pre- and post- emergence damping –off of Giza 35 soybean cultivar grown in soil artificially infested with three different fungi, each alone.

Pathogenic isolates of the causal organisms; *Macrophomina phaseolina*, *Sclerotium rolfsii* and *Fusarium oxysporum* were tested separately throughout the present study. The fungal inocula were grown on sterilized barely grains medium for 12 days at 25±1 °C. Each pathogen was added to the soil at the rate of 3 % (w/w). Each treatment was replicated four times, and five treated seeds with each of the two different strains of bradyrhizobia were sown in each pot containing infested soil to serve as control. Pre-and post-emergence damping-off was taken after 15 and 30 days after sowing.

B. Effect of the tested fungicides and *B. japonicum* on nodulation, shoots dry weight, and nitrogen and phosphorus shoot contents:

The effect of the four fungicides on nodulation, shoots dry weight, and shoot contents of nitrogen and phosphorus was determined from the greenhouse experiment. Soybean seeds were surface sterilized (Vincent, 1970), and then coated with a single bradyrhizobial strain peat inocula of either HH303 or 3407. Seeds were sown in soil treated with the desired fungicide at 1000 and 2000 ppm concentrations in sterile plastic pots of 30 cm diameter containing sterile soil and moistened with N-free nutrient solution (Norris, 1964). Plants were watered with N-free nutrient solution when needed. The pots were arranged in a complete randomized design with four replications for each treatment and with a border untreated but inoculated with bradyrhizobial strains were served as controls.

At 45 and 75 days after planting, plant shoots were removed at soil level, dried at 70°C for 72hr., weighed, milled and analyzed for nitrogen concentrations by automated Nitrogen Analyzer, Model Carlo. Erba Instrument, (Batzli *et al.,* 1992). Phosphorus concentration in plant shoots was determined Spectrophotometrically in the acid solution of the digested samples using ammonium molybdate and stannus chloride reagents as described by Page *et al.* (1982).

The roots were uprooted from the soil and nodules were separated from the roots, counted, dried and weighed.

C. Determination of total chlorophyll :

The total chlorophyll of the soybean shoots was determined in plants grown under greenhouse conditions after 55 days from planting using Chlorophyll Meter (SPAD 501) as described by (Fergany, 1997).

D. Effect of the tested fungicides and *B. japonicum* on bacterial enzymes activity and total counts of microorganisms in soil:

The effect of fungicides on soil bacterial N_2-ase and dehydrogenase activities and total counts of fungi, bacteria and actinomycetes were determined from the greenhouse experiment. Soil samples were taken at 1, 3, 5, 7, 15, 30 and 45 days after sowing to determine these parameters.Dehydrogenase activity was assayed by using 2,3,5 triphenyl-tetrazolium chloride (TTC) method according to Page *et al.* (1982) while, nitrogenase activity (N_2-ase) was measured according to the procedure described by **Hardy *et al.* (1973)**.

RESULTS AND DISCUSSION

I. Effect of Four Copper Fungicides on the Growth of *B.japonicum* Strains *in vitro*:

The results in Table (1) reveal that the magnitude of fungicidal effect was increased by increasing its concentration. The most effective fungicide was copper oxychloride which completely inhibited the growth at 50 ppm followed by copper sulphate and copper hydroxide which showed efficacy of 83.8, 87.8, 83.2 and 89.4% for strains HH303 and 3407, respectively. While, the less effective fungicide against growth was coprous oxide. It had no lethal effect on growth of both strains of *B. japonicum* even at the highest tested concentration. Also, the growth of *Bradyrhizobium* strains was differed according to the tested

Table 1: Effect of different concentrations of copper fungicides on two *Bradyrhizobium japonicum* strains (cfu/plate) under laboratory conditions

Fungicides	Strains	Concentrations (ppm) 1		5		10		25		50		100	
		1	Efficiency %	5	Efficiency %	10	Efficiency %	25	Efficiency %	50	Efficiency %	100	Efficiency %
Coprous oxide	HH303	54.7	4.5	45.7	20.2	34.3	40.1	22.7	60.4	16.3	71.6	10.3	82.0
	3407	44.0	18.1	35.3	34.3	27.0	49.7	19.0	64.6	15.7	70.8	8.7	83.8
Copper hydroxide	HH303	19.3	66.3	15.0	73.8	12.0	79.1	9.0	84.3	7.0	87.8	0.0	0.0
	3407	17.7	67.0	14.0	73.9	10.7	80.1	8.0	85.1	5.7	89.4	0.0	0.0
Copper oxychloride	HH303	14.3	75.0	11.3	80.3	8.7	84.8	6.7	88.3	0.0	0.0	0.0	0.0
	3407	12.0	77.6	8.0	85.1	6.0	88.8	4.7	91.2	0.0	0.0	0.0	0.0
Copper sulphate	HH303	21.0	63.4	19.7	65.6	17.0	70.3	15.3	73.3	9.3	83.8	0.0	0.0
	3407	19.7	63.3	17.7	67.0	14.7	72.6	12.0	77.6	9.0	83.2	0.0	0.0
Control	HH303	57.3	-	57.3	-	57.3	-	57.3	-	57.3	-	57.3	-
(no fungicide)	3407	53.7	-	53.7	-	53.7	-	53.7	-	53.7	-	53.7	-

L.S.D at 0.05 for :

Fungicides (F) = 1.62
Strains (S) = 1.02
Concentrations (C) = 1.77
F x S = 4.44
F x C = 4.73
S x C = 3.82
F x S x C = n.s.

fungicide and the used strain. Data presented in Table (1) reveal that the used strains showed remarkable variations in their tolerant to the tested fungicides. Strain HH303 was the most tolerant to the chemical substances, while, strain 3407 was sensitive. These results are in agreement with those obtained by Tu (1980 and 1982) who mentioned that some pesticides have a bactericidal rather than bacteriostatic action on *Bradyrhizobium* strains. Similarly, Lal (1988) reported that fungicides have a direct action on survival of *Bradyrhizobium* and *Rhizobium* in soil and may directly affect the degree of infection and hence the amount of nodule formation. Results of the present study indicate that the tested fungicides had an adverse effect on survival of the examined *Bradyrhizobium japonicum* strains. Bradyrhizobial strain HH303 was more tolerant to fungicides than strain 3407.

II. Greenhouse Experiments:

A. Effect of the tested fungicides in the presence of two different strains of *Bradyrhizobium japonicum* on damping-off disease of soybean:

Data presented in Table 2 show that the soil artificially infested with the pathogenic fungi (control) gave the highest percentage of pre- and post-emergence damping-off, being 50.66 and 15.44%, respectively with *Bradyrhizobium* strain HH303 while, in case of strain 3407 gave 50.56 and 15.44%, respectively. No significant differences were found between effectiveness of the two strains of *Rhizobium* on infection percentage. Results of copper compounds indicated that all tested fungicides significantly decreased infection compared to control. The most effective fungicides were copper oxychloride and copper hydroxide followed by copper sulphate and copper oxide at 15days after sowing which they gave efficacy of 43.70%, 35.79%, 28.26% and 14.58%, respectively, with the strain HH303 of *Bradyrhizobium* and efficacy of 42.49%, 34.61%, 26.82% and 12.55%, respectively, with the *Bradyrhizobium* strain 3407. At 30 days after sowing, the most effective fungicides on infection with both of the bradyrhizobial strains were copper oxychloride and copper hydroxide which gave efficacy of 39.82% and 36.56%, respectively with the strain HH303 and 36.00 and 33.91%, respectively with the strain 3407.

Table 2: Effect of copper fungicides in the presence of two strains of *Bradyrhizobium japonicum* on controlling damping-off disease of soybean. under greenhouse conditions

Fungicides	Fungi	Strain HH303 Pre-emergence damping-off (15 days) % Infection	% Efficiency	Strain HH303 Post-emergence damping-off (30 days) % Infection	% Efficiency	Strain 3407 Pre-emergence damping-off (15 days) % Infection	% Efficiency	Strain 3407 Post-emergence damping-off (30 days) % Infection	% Efficiency
Coprous oxide	*M. phasiolina*	46.00	17.86	13.00	26.39	47.33	14.98	13.33	27.28
	F. oxysporum	42.67	18.97	13.67	14.56	43.67	16.55	14.33	6.52
	S. rolfsii	40.33	6.92	7.67	39.46	41.00	6.11	7.67	39.46
Mean (X̄)		43.00	14.58	11.45	26.80	44.00	12.55	11.78	24.42
Copper hydroxide	*M. phasiolina*	36.33	35.13	10.33	41.51	37.00	33.54	10.67	41.79
	F. oxysporum	33.67	36.10	12.67	20.81	34.33	34.40	13.00	15.20
	S. rolfsii	27.67	36.14	6.67	47.35	28.00	35.88	7.00	44.75
Mean (X̄)		32.56	35.79	9.89	36.56	33.11	34.61	10.22	33.91
Copper oxychloride	*M. phasiolina*	31.67	43.45	11.67	33.92	32.33	41.92	12.33	32.73
	F. oxysporum	30.00	43.03	12.00	25.00	30.33	42.04	12.67	17.35
	S. rolfsii	24.00	44.61	5.00	60.54	24.67	43.51	5.33	57.93
Mean (X̄)		28.56	43.70	9.56	39.82	29.11	42.49	10.11	36.00
Copper sulphate	*M. phasiolina*	40.33	27.98	13.00	26.39	41.00	26.35	13.66	25.48
	F. oxysporum	37.33	29.11	13.33	16.69	38.00	27.38	14.00	8.67
	S. rolfsii	31.33	27.69	8.00	36.86	32.00	26.72	8.67	31.57
Mean (X̄)		36.33	28.26	11.44	26.65	37.00	26.82	12.11	21.91
Control (no fungicide)	*M. phasiolina*	56.00	-	17.66	-	55.67	-	18.33	-
	F. oxysporum	52.66	-	16.00	-	52.33	-	15.33	-
	S. rolfsii	43.33	-	12.67	-	43.67	-	12.67	-
Mean (X̄)		50.66		15.44		50.56		15.44	

L.S.D at 0.05 for :

		Pre	Post
Strains	(S)	= 0.44	= n.s.
Fungicides	(F)	= 1.15	= 0.88
Fungi	(G)	= 0.89	= 0.69
S x F		= 1.63	= 1.25
S x G		= 1.27	= 0.97
F x G		= 2.00	= 1.53
S x F x G		= 2.83	= 2.17

On the other hand, as for pre-emergence damping-off, *M. phaseolina* was more tolerant to the copper fungicides followed by *F.oxysporum* and *S.rolfsii*, respectively. While, at post emergence damping-off, *F. oxysporum* was high virulent fungi followed by *M.phaseolina,* whereas, *S.rolfsii* was more sensitive to the used chemicals. These results are in agreement with those reported by Ibrahim *et al.* (1965) who stated that a protective control measure by fungicidal treatment would be the only feasible practices for controlling the disease. Meanwhile, Guy *et al.* (1989) found that Metalaxyl was effective in reducing lesion length caused by *Phytophthora megasperma* sp. *glycina* (the incitant of root and stem rot of soybean) in all cultivars of soybean. Also, Heweidy (1998) found that copper acrobat was effective in controlling chocolate spot disease of faba bean.

B. Effect of the tested fungicides on nodulation :

Results presented in Table (3) show that a significant reduction in both nodules number and nodules dry weight when copper fungicides were applied to the soil before inoculating with the two strains of *Bradyrhizobium* at 45 and 75 days after planting compared with the control ones. Also, data presented in Table (3) reveal that the magnitude of fungicidal effect was increased by increasing its concentration. At the mean time, these results referred to a high reduction in nodules number and nodules dry weight at the second period, 75 days after planting than the first one (45 days). The application of copper hydroxide and coprous oxide resulted highest number of nodules when inoculated with strain HH 303 either after 45 .and 75 days of planting or at both concentrations of the previous compounds. The results recorded 19.75, 15.50, 8.67 and 7.00 nodule/plant, respectively, compared with the control, being 32.75 and 32.67 nodule/plant. On the other hand, treatment with coprous oxide and copper hydroxide in the presence of strain 3407, caused 14.50, 14.00, 6.67 and 4.33 nodule/plant, respectively, compared with the control which gave 30.25 and 29.33 nodule/plant.

Dealing with dry weight (mg/plant) of nodules, the results in Table (3) show that application of coprous oxide with strain HH303 produced the highest number of nodules at both concentrations or periods after planting, being 230.25, 170.25, 31.00 and 24.33 mg/plant, respectively, compared with the control which reached

Table 3: Nodulation of soybean as affected by inoculation with *Bradyrhizobium japonicum* and application of some fungicides at different periods under greenhouse conditions.

Strains	Fungicides	Concentration (ppm)	After 45 days from planting Nodules/plant		After 75 days from planting Nodules/plant	
			Number	Dry weight (mg)	Number	Dry weight (mg)
HH 303	Coprous oxide	1000	18.75	230.25	8.67	31.00
		2000	13.25	170.25	7.00	24.33
	Copper hydroxide	1000	19.75	171.50	6.67	17.33
		2000	15.50	149.50	4.67	10.00
	Copper oxychloride	1000	10.00	158.25	5.33	15.33
		2000	10.00	136.25	2.67	7.67
	Copper sulphate	1000	16.50	129.50	6.33	11.00
		2000	13.00	124.50	5.33	9.00
	Control		32.75	344.75	32.67	239.33
3407	Coprous oxide	1000	14.50	178.75	5.00	18.33
		2000	14.00	100.00	4.67	17.00
	Copper hydroxide	1000	12.25	105.50	6.67	17.00
		2000	10.00	74.75	4.33	15.33
	Copper oxychloride	1000	6.75	77.00	4.33	9.00
		2000	4.75	61.25	2.00	5.67
	Copper sulphate	1000	8.25	86.25	6.33	9.33
		2000	7.25	80.00	3.67	6.33
	Control		30.25	295.00	29.33	135.00

L.S.D at 0.05 for :

Strains (S)	=	3.7	3.66	n.s.	0.60
ungicides (F)	=	2.2	2.46	1.5	0.74
Concentrations (C)	=	1.9	2.13	2.1	0.64
S x F	=	3.1	3.47	1.3	1.10
S x C	=	2.7	3.01	1.9	0.91
F x C	=	2.8	4.25	2.6	1.28
S x F x C	=	5.4	6.01	3.7	1.82

344.75 and 239.33 mg/plant. The same trend was found when soybean was inoculated with strain 3407.

The obtained results are in accordance with those of Saleh *et al.* (2001) who found that fungicides-resistant bradyrhizobia strains were more efficient than the original ones. Also, Lal (1988) reported that fungicides had a direct action on the survival of *Bradyrhizobium* and *Rhizobium* in soil and may indirectly affect the degree of infection and hence the amount of nodule formation.

Although the concept "Integrated Pest Management, IPM" recently occupies a big area of scientists deal with pests control to restrict the over use of pesticides, a large number of field crops still relying on pesticide application. Soybean is among such crops where a variety of fungicides are recommended as antifungal agents.

C. Effect of fungicides and *Bradyrhizobium japonicum* on shoots dry weight and contents of nitrogen, phosphorus and chlorophyll:

Data presented in Table (4) demonstrate that a significant reduction in plant shoot dry weight and plant nitrogen content resulted when fungicides were applied to the soil either at 1000 and 2000 ppm or at different periods of planting. When soybean seeds were inoculated with fungicides tolerant strain HH303 after 45 days from planting, copper oxychloride gave the highest shoot dry weight at 1000 ppm (10.92 g/plant) and 279.75 mg/plant N-content at 2000 ppm while, the control recorded 7.57 g/plant dry weight and 205.50 mg/plant N-content, respectively. At 75 days after planting, the control ones should enhanced plant growth and increased plant nitrogen content over those planted in soil treated with fungicides. All fungicides significantly reduced mass and plant nitrogen content. The degree of reduction varied depending on the used fungicide. Inoculation of soybean seeds with the sensitive strain 3407 (as a control) significantly enhanced plant growth and nitrogen content at 45 days or 75 days after sowing, being 7.64, 11.91/plant shoot dry weight and 188.75, 332.33 mg/plant nitrogen content. A significant reduction in both of shoots dry weight and nitrogen content was recorded when the fungicides were applied to the soil giving various reduction levels depending on the tested fungicide and on the tested concentration.

Table 4: Effect of some copper fungicides on dry weight (g/plant), N-content (mg/plant), phosphorus content (mg/plant) and chlorophyll content (mg/g fresh weight) of soybean shoots as affected by inoculation with *Bradyrhizobium japonicum* at different periods under greenhouse conditions

Strains	Fungicides	Concentration (ppm)	At 45 days after planting			At 75 days after planting			Chlorophyll content (mg/g fresh weight) (after 55 days of planting)
			Dry weight (g/plant)	N-content (mg/plant)	P-content (mg/plant)	Dry weight (g/plant)	N-content (mg/plant)	P-content (mg/plant)	
HH 303	Coprous oxide	1000	9.68	201.00	35.25	11.10	294.00	25.00	30.80
		2000	6.10	218.75	47.75	10.57	327.67	47.67	31.85
	Copper hydroxide	1000	9.62	155.25	36.50	8.50	255.00	18.00	32.47
		2000	6.29	260.75	55.75	10.79	281.00	23.00	30.45
	Copper oxychloride	1000	10.92	267.25	68.50	11.37	351.00	73.33	35.70
		2000	8.88	279.75	71.00	11.07	316.67	80.67	30.50
	Copper sulphate	1000	6.95	171.75	23.00	12.51	320.00	26.00	32.45
		2000	5.87	141.00	23.30	10.30	250.61	21.66	32.72
	Control		7.57	205.50	30.50	12.83	375.33	49.00	30.62
3407	Coprous oxide	1000	6.71	151.50	18.50	10.80	261.00	20.66	31.40
		2000	5.98	159.00	25.75	9.43	256.00	27.00	34.92
	Copper hydroxide	1000	5.81	139.50	30.00	11.49	191.67	15.33	34.50
		2000	6.60	166.75	30.25	6.77	277.00	24.00	33.17
	Copper oxychloride	1000	5.89	159.50	38.00	11.50	277.33	58.67	33.62
		2000	6.59	245.50	49.75	12.44	337.00	86.00	30.30
	Copper sulphate	1000	6.61	149.25	21.25	9.67	276.66	20.00	32.25
		2000	6.18	130.25	44.00	9.32	212.33	20.25	32.45
	Control		7.64	188.75	32.00	11.91	332.33	50.00	34.10
L.S.D at 0.05 for :									
	Strains (S)	=	n.s.	2.22	1.19	n.s.	5.02	1.66	0.94
	Fungicides (F)	=	0.60	2.15	1.10	1.10	2.56	1.45	0.47
	Concentrations (C)	=	0.50	1.86	0.59	0.90	1.81	1.26	0.56
	S x F	=	0.90	3.04	1.56	1.50	2.95	2.05	0.40
	S x C	=	0.70	2.63	1.35	1.30	2.56	1.78	0.56
	F x C	=	1.04	3.72	1.91	1.90	3.62	2.51	0.79
	S x F x C	=	1.50	5.26	2.70	2.60	5.11	3.55	1.19

Dealing with phosphorus and chlorophyll contents, results in Table (4) reveal that inoculation of soybean seeds with both strains either resistant or sensitive to fungicides appeared significant increase of P-content at 75 days after sowing than at 45 days, being 49.00, 50.00,30.50 and 32.00 mg/ plant P-content. While application of fungicides in addition to soil inoculation with the two different strains of bradyrhizobia showed that copper oxychloride treatments gave the highest values of P-content either at 45 or 75 days after sowing or at both tested concentrations, being 68.5, 73.33, 71.00 and 80.67 mg/plant P-content with strain HH303, while strain 3407 recorded 38.00, 58.67, 49.75 and 86.00 mg/plant P-content.

On the other hand, the total chlorophyll content was significantly affected with both *Rhizobium* strains, using all tested treatments, being 35.70-30.80 at 1000 ppm with strain HH303 and 32.72- 30.45 at 2000 ppm. While, it was 34.50- 31.40 at 1000 ppm with strain 3407 and 34.92-30.30 mg/g fresh weight at 2000 ppm compared with 30.62 and 34.10 mg/g fresh weight in control. The obtained results are in agreement with those obtained by Chahal and Sidhu (1992), who reported that thiram may have adverse effects on *Bradyrhizobium* spp. symbiosis with groundnut. Their results also indicated that thiram resistant strain of *Bradyrhizobium* spp. Increased dry weight and N-content of shoots compared to plant nodulated by parental strains.

In contrast to the obtained data, Chambe and Montes (1982) reported that fungicides either had no effect or had a stimulatory effect on nodulation, shoot dry weight and nitrogen content, and this may be due to the low concentration of fungicides used or to the methods of inoculant application. Also, little information has been obtained with copper fungicides effect on soil borne diseases, and most of studies were on foliage diseases of soybean plants.

D. Effect of fungicides on enzymes activity and total counts of microorganisms in soil:

Nitrogenase and dehydrogenase activities of soil of soybean plants are presented in Table (5). Data show that, in the absence of fungicides the highest values of N_2-ase activity were expressed at

Table 5: Nitrogenase and dehydrogenase activities in soil of soybean as affected by application of some copper fungicides at different periods after sowing under greenhouse conditions

Fungicides	Periods/ days after sowing	Enzyme activity			
		N_2-ase activity (nmol C_2H_4/100 g soil/h.)		Dehydrogenase activity (mg TPF/100 g dry soil)	
		1000 ppm	2000 ppm	1000 ppm	2000 ppm
Coprous oxide	1	6	4	53	48
	3	4	3	50	33
	7	4	0	74	47
	15	3	0	61	47
	30	0	0	64	40
	45	4	2	64	36
Mean (X̄)		3.5	1.5	61.0	41.8
Copper hydroxide	1	8	3	58	42
	3	5	0	50	39
	7	0	0	37	3
	15	3	0	54	32
	30	0	0	52	48
	45	9	2	61	53
Mean (X̄)		4.2	0.8	52.0	41.0
Copper oxychloride	1	9	6	67	53
	3	6	5	64	54
	7	4	0	47	54
	15	0	0	54	43
	30	0	0	69	39
	45	4	0	70	61
Mean (X̄)		3.8	1.8	61.8	47.3
Copper sulphate	1	4	4	53	42
	3	2	0	48	43
	7	3	0	43	35
	15	0	0	56	48
	30	2	0	59	45
	45	2	0	56	42
Mean (X̄)		2.2	0.6	52.5	42.5
Control	1	48		72	
	3	220		92	
	7	232		112	
	15	187		111	
	30	323		68	
	45	617		49	
Mean (X̄)		271.2		84.0	

all of the experimental periods, which were 617 and 48 nm C_2H_4/100 g dry soil/h after 45 and 1 day, respectively. Application of fungicides led to the greatest reduction in N_2-ase activity and reached the level of no activity at all in most treatments at 2000 ppm. While, at 1000 ppm the highest reduction was found when copper sulphate was used, here in, N_2-ase activity was 2.2 and the lowest within copper hydroxide, being 4.2 compared with the control 271.2 nmole C_2H_4/100 g dry soil/h. Dehydrogenase activity as well as in the absence of the fungicides recorded the highest values of activity within 3, 15 and 7 days after sowing were 92, 111 and 112 mg TPF/100 g dry soil and the lowest value was 49 mg TPF/100 g dry soil with the last period of the experiment. When the fungicides were applied, data in Table (5) reveal that the magnitude of fungicidal effect was increased by increasing its concentration. It was found that dehydrogenase activity was high within copper oxycholride, being 61.8 and 47.3 at 1000 and 2000 ppm, respectively, while it was low when copper hydroxide was used, being 52.0 and 41.0 mg TPF/100 g dry soil, respectively, compared with the mean value obtained of the control one (84.0 mg TPF/100 g dry soil).

On the other hand, results in Table (6) indicate that when the four copper fungicides were applied, this led to a great reduction of total counts of fungi, bacteria and actinomycetes in soybean rhizosphere, especially the total count of bacteria. Also, there were a magnitude fungicidal effect on reduction of microorganisms total counts by increasing the fungicides concentrations. In case of fungi total count, copper oxychloride gave the lowest total counts, being 4.3 x 10^3 and 2.9 x 10^3/g dry soil at 1000 and 2000 ppm, respectively, while copper hydroxide gave the highest number of fungi (7.1 x 10^3/ g dry soil) at 1000 ppm compared with the control which recorded 21.5 x 10^3/g dry soil. Dealing with total count of bacteria, it was found that when copper sulphate was applied, more effect was noticed and lowest number of bacteria was resulted at both concentrations, which recorded 2.6 x 10^6 and 0.8 x 10^6/ g dry soil at 1000 and 2000 ppm,respectively,while the coprous oxide recorded the highest total counts which were 3583.3 x 10^6 and 478.3 x 10^6/g dry soil at

Table 6: Total counts of fungi, bacteria and actinomycetes in soil of soybean as affected by application of some copper fungicides at different periods after sowing under greenhouse conditions

Fungicides	Periods/ days after sowing	Total counts of Fungi (10^3)/g dry soil 1000 ppm	Fungi 2000 ppm	Bacteria (10^6)/g dry soil 1000 ppm	Bacteria 2000 ppm	Actinomycetes (10^4)/g dry soil 1000 ppm	Actinomycetes 2000 ppm
Coprous oxide	1	9.5	8.2	2700	1300	51	11.0
	3	9.2	6.1	2200	610	51	9.8
	7	8.2	6.1	1100	590	35	4.3
	15	7.8	5.3	3900	130	34	4.5
	30	2.2	1.1	5800	120	15	5.9
	45	1.1	0.17	5800	120	16	4.9
Mean (X')		6.3	4.5	35.83	478.3	33.7	6.7
Copper hydroxide	1	13.0	13.0	34	8.1	43	0.25
	3	12.0	6.9	12	7.6	35	0.068
	7	3.8	1.0	16	3.0	39	0.025
	15	3.6	0.12	21	1.5	14	0.023
	30	4.8	0.12	23	1.2	11	0.009
	45	5.2	0.12	28	1.2	2.3	0.009
Mean (X')		7.1	3.5	22.3	3.8	24.1	0.06
Copper oxychloride	1	9.6	8.2	37.0	3.2	20.0	5.20
	3	7.5	6.0	8.8	2.8	6.3	0.37
	7	5.2	2.0	2.9	2.1	0.22	0.12
	15	2.4	0.9	1.1	0.12	0.20	0.11
	30	1.2	0.12	20.0	6.1	1.10	0.20
	45	0.22	0.15	37.0	7.3	2.10	0.061
Mean (X')		4.3	2.9	17.8	3.6	4.9	1.00
Copper sulphate	1	14.0	13.0	6.1	1.1	12.0	3.80
	3	2.5	6.3	3.8	0.92	11.0	0.76
	7	3.8	3.8	3.8	0.88	2.20	0.51
	15	11.0	4.9	1.1	0.85	0.24	0.12
	30	6.1	4.4	0.61	0.62	0.21	0.037
	45	1.2	1.2	0.49	0.24	0.37	0.033
Mean (X')		6.4	5.6	2.6	0.8	4.3	0.90
Control	1	15		5900		56	
	3	19		6100		55	
	7	20		6800		51	
	15	25		7600		41	
	30	30		7700		29	
	45	20		7100		23	
Mean (X')		21.5		6866.7		42.5	

1000 and 2000 ppm, respectively, in comparison with the control mean which gave 6866.7×10^6/g dry soil. Concerning the effect of copper fungicides on total count of actinomycetes, Table (6) show a similar trend as much as bacteria. The obtained results of this study introduced; A) a reduction of soil born diseases, B) a stimulation of plant self resistance and C) N_2-fixation activity even if it was partly reduced which confirm those obtained by Azad *et al.* (1988) who observed that size and number of nodules in fungicides received treatments were lower than those of untreated plants which might be attributed to the inhibitory effect of the fungicides on nitrogenase activity. Also, Angle *et al.* (1981) reported that the fungicides decreased the number of harmful organisms, suggesting that they have no effect on the decline of rhizobia in the rhizosphere soil in soybean. In contrast, Chahal and Sidhu (1992) found that inoculation with fungicides-resistant strains of *Bradyrhizobium* spp. increased the number of nodules and nitrogenase activity as well as nodules dry weight and N-content of plant shoots compared to plants nodulated by parent strains. They suggested that development of fungicides resistance in *Bradyrhizobium* spp. may lead to an increase in crop yield if used in conjunction with the fungicides.

In conclusion, development of fungicides against soybean soil borne diseases must be chosen as fungicide-resistant strains of *Bradyrhizobium* as inoculants for soybean became an important approach in Egyptian agriculture to overcome the inhibitory effects of fungicides commonly applied to increase the crop production. This is in harmony with the conclusions of Tesfai and Mallik (1986). It is of rather interest to clarify that the copper is heavy metal which can result phytotoxicity and increasing the movement of copper ions into the plant tissues which is in accordance with the fact that most heavy metal ions have strong affinity to make interruption for protein chains in microbial cells, so why copper compounds has been of a negative effects on biological activity in the soil. In the meanwhile, the rhizobial growth has a great effect in plant resistance through rhizobial phytohormons production which may be contributed to exclusion of some signals for the plant to start up inducing self resistance.

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فاعلية سلالتين من بكتيريا العقد الجذرية ومعاملة التربة ببعض المبيدات النحاسية فى مقاومة مرض موت البادرات والنشاط

الحيوى النباتى فى فول الصويا

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أجريت بعض التجارب لتقييم فاعلية أربعة مركبات نحاسية هى أكسيد النحاس ، هيدروكسيد النحاس ، أوكسى كلوريد النحاس وسلفات النحاس فى وجود سلالتين مختلفتين من بكتيريا العقد الجذرية لنبات فول الصويا هما HH-303 (متحملة لتأثير المبيدات) والأخرى 3407 (حساسة للمبيدات).

تم دراسة تأثير هذه المركبات النحاسية معملياً على (نمو السلالتين فى المزارع السائلة) وفى الصوبة على تكوين العقد البكتيرية وتثبيت النيتروجين. كذلك تم تقدير النسبة المئوية لمرحلتى ما قبل ظهور البادرات فوق سطح التربة ولمرحلة ما بعد ظهور البادرات فوق سطح التربة (مرحلتى مرض موت البادرات) ، وكذلك محتوى الفوسفور والكلوروفيل والنشاط الأنزيمى والعدد الكلى للكائنات الدقيقة فى التربة المستعملة.

وقد أظهرت الدراسة أن أوكسى كلوريد النحاس يعتبر من أكثر المركبات النحاسية تثبيطاً لنمو كلا من هاتين السلالتين عند تركيز ٥٠ جزء فى المليون. وفى نفس الوقت أدى الى حدوث نقص معنوى فى النسبة المئوية للإصابة بالفطريات مثل ماكروفومينا فاسيولينا ، فيوزاريوم أوكسيسبورم و سكليروشيم رولفزياى مقارنة بمعاملة الكنترول أو بأحد المبيدات الأخرى المستخدمة مع السلالتين من البكتيريا المكونة للعقد البكتيرية. كذلك أظهرت الدراسة أن المركبات النحاسية تقلل من تكوين العقد البكتيرية والوزن الجاف للمجموع الخضرى والمحتوى النيتروجينى. أيضاً أتضح أن هناك علاقة سالبة بين استخدام هذه المبيدات وكلا من النشاط الأنزيمى والعدد الكلى للكائنات الدقيقة فى منطقة الريزوسفير لنباتات فول الصويا.

لوحظ أيضاً أن هناك نقص واضح فى نشاط كل من أنزيمى النيتروجينيز والديهيدروجينيز والعدد الكلى لكل من الفطريات والبكتيريا والأكتينوميستات. كذلك أوضحت النتائج أن المبيدات النحاسية المستخدمة لها تأثير معاكس على النشاط الحيوى الكلى لكل من النبات والكائنات الدقيقة بالريزوسفير.