

EFFECT OF COPPER OXYCHLORIDE, ELEMENTAL SULPHUR, AND RHIZOBIUM INOCULATION ON ROOT ROT DISEASE, NODULATION, AND GROWTH OF FABA BEAN PLANTS

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ABSTRACT

A pot experiment was conducted to evaluate the effect of applying copper oxychloride at a rate of 100 ppm and elemental sulphur at a rate of 100 kg/ feddan as a fungicides and/or *Rhizobium* inoculation for controlling faba bean root rot disease caused by *Rhizoctonia solani* and *Fusarium solani*. Nodulation, growth and N-percentage of faba bean shoots were also evaluated. The results were as follows:

In soil infected with *Rhizoctonia* application of the fungicides alone or in combination with *Rhizobium* inoculation increased seedling emergence compared to that infected with *Rhizoctonia* only. Infection of faba bean with *Fusarium* showed lower emergence percentage compared to soil infected with *Rhizoctonia*. Copper oxychloride or sulphur had negative effect on seedling emergence for controlling faba bean root rot caused by *Fusarium solani*. The most effective treatment in reducing infection % caused by *Rhizoctonia solani* was recorded in soil treated with copper oxychloride or elemental sulphur.

Rhizobium Inoculation in soil infected with *Rhizoctonia solani* or *Fusarium solani* decreased significantly infection percentage. Application of copper oxychloride or combination of *Rhizobium* and copper oxychloride had no effect on the infection % with *Fusarium solani*. In faba bean plants infected with *Rhizoctonia solani*, application of copper oxychloride or sulphur with or without *Rhizobium* inoculation had no effect on nodulation status.

Concerning to the effect of *Fusarium solani* on nodulation status, the results indicated that infection with *Fusarium* had an inhibitory effect on the nodulation of faba bean even in the presence of the fungicides. There was a significant increase in dry weight of shoots in soil infected with *Rhizoctonia solani* and treated with copper oxychloride compared to control plants. *Rhizobium* inoculation also increased significantly dry weight of faba bean shoots. Application of the fungicides had no effect on dry weight of shoots in soil infected with *Fusarium solani*.

Keywords: Copper oxychloride, Sulphur, Rhizobium, Faba bean, Root rot disease

INTRODUCTION

Root rot diseases are a major limiting factor in legume production. The disease depress seedling germination and cause emergence damping off, resulting in low yield. Root diseases of faba bean (*Vicia faba* L.) can rarely be ascribed to a single distinctive pathogen but is usually a complex pathological condition. Salt (1983) reported several soil-borne fungi as the causal organisms (*Rhizoctonia solani*, *Fusarium oxysporum*, *Fusarium solani*, *Pythium* spp, *Sclerotinia* spp.). Effective control strategies against root rot fungal pathogens have not been fully developed. Chemical seed treatment

before planting is a common practice to prevent seed and seedling rots, damping off and other fungal diseases. However, problems arise when the chemical seed treatments are to be used in conjunction with *Rhizobia* inoculants. In some cases, the applied seed fungicide may fail to protect against the intended pathogen or suppresses the effectiveness of the *Rhizobium* inoculants. Sulphur has been known and used as a pesticide since very early times, and has been registered for pesticide use in the United States since the 1920s (U.S.EPA, 1991). Currently, sulphur is registered for use as a fungicide and insecticide on several crops.

Copper-containing compounds have a long history of successful use as agricultural fungicides and are now being recognized as very hazardous pollutants. Copper salts are widely used to control bacterial and fungal diseases in agriculture (Mapper *et al.*, 1984). The beneficial effects of inoculating legumes with *Rhizobium* is well known and widely used in agriculture for crop improvement due to their ability to fix nitrogen (Roughley *et al.*, 1983). However, the recommended rates of the fungicides applied are often inhibitory to root nodule bacteria. For the last three decades, studies on the compatibility of *Rhizobium* strains with fungicides have been controversial. Application of Captan, Pentachloronitrobenzene (Curley and Burton, 1975) and Apron (Revellin *et al.*, 1993) on soybean (*Glycine max*) reduced the viability of *Bradyrhizobium japonicum* by 18, 75 and 61%, respectively, after one hour exposure.

Therefore, the objectives of this study were to investigate: (1) the influence of soil treatment with recommended rates of sulphur and copper oxychloride as a fungicides on the incidence of root rot disease of faba bean plants caused by *Rhizoctonia solani*, and *Fusarium solani*. (2) the effect of these fungicides on nodulation status and growth of faba bean plants and N-percentage in presence of the fungal pathogens.

MATERIALS AND METHODS

A Pot experiment was conducted at Agricultural Research Center, Giza, to study the effect of elemental S and Copper oxychloride as a fungicides on root rot disease, nodulation status and growth of faba bean plants.

Sources of seeds, fungicides and soil

- 1- Elemental sulphur (S) 100% and Copper oxychloride (Caprile 50%) were obtained from Plant Pathology Res. Inst., ARC, Giza, Egypt
- 2- *Rhizobium* inoculum (*Rhizobium leguminosarum*) was kindly obtained from BNF Unit, Microbiology Dept., Soils, Water, and Environment Res. Inst. ARC, Giza, Egypt.
- 3- Faba bean seeds (*Vicia faba*) Giza 3 cultivar were kindly provided by Field Crops Res. Inst., ARC, Giza, Egypt
- 4- Soil sample was collected from ARC farm, Giza. The mechanical and chemical analyses of the experimental soil are presented in Table (1).

Through this study two fungal isolates from diseased faba bean plants were used, one isolate of *Rhizoctonia* and one isolate of *Fusarium*. The isolates proved their pathogenic capability in the pathogenicity test.

Preparation of fungal inoculum

The fungal inocula were grown on sterilized barley grains medium for 12 days at $25 \pm 1^\circ\text{C}$. Each pathogen was added to the soil at a rate of 3% (w/w). The infested soil was watered daily for one week to enhance growth and distribution of the fungal inoculum.

The experiment divided into two groups: The first one infested with *Rhizoctonia solani* and included six treatments with three replicates and as follows: (1) Control (2) Soil treated with elemental sulphur at a rate of 100kg/feddan (3) Soil treated with copper oxychloride at a rate of 100 ppm (4) faba bean seeds inoculated with commercial *Rhizobium* inoculants (*Rhizobium leguminosarum*) (5) Seeds inoculated with *Rhizobium* and fertilized with starter dose of N fertilizer and the elemental S was mixed with the soil. (6) Seeds inoculated with *Rhizobium* and fertilized with starter dose of N fertilizer and the fungicide was mixed with the soil. The second included the same treatments but the soil was infested with *Fusarium solani*. *Rhizobium* inoculation was done before sowing. Seeds were coated with peat inocula using Arabic gum 16% as an adhesive agent. The pots were arranged in a complete randomized design. Four seeds were sown in each pot and plants were watered as needed.

Table (1): Mechanical and chemical properties of the soil

Properties	Value
Coarse sand (%)	7.59
Fine Sand (%)	23.00
Silt (%)	33.60
Clay (%)	35.81
Textural class	Clay loam
pH (1:5 soil suspension)	7.67
E.C. dSm^{-1} 1:5 (soil : water)	0.76
O.C. (%)	0.68
O.M. (%)	1.18
Total N (%)	0.42
Soluble cations (meq l^{-1})	
Ca ⁺⁺	0.77
Mg ⁺⁺	3.06
Na ⁺	0.79
K ⁺	0.79
Soluble anions (meq l^{-1})	
CO ₃ ⁻	--
HCO ₃ ⁻	2.60

Recommended dose of Calcium superphosphate (15.5% P₂O₅) was mixed with the soil at a rate of 100 kg/ fed. Ammonium nitrate (33.5% N) was applied as a recommended dose and a starter dose. Both calcium superphosphate and Ammonium nitrate were applied during soil preparation.

After 45 and 75 days from planting, the plants were uprooted and number and dry weight of nodules/plant were determined. Plant shoots were

dried at 70°C for 72 h., weighed, milled and analyzed for nitrogen percentage by semi- microkjeldahl procedure according to piper (1950).

Disease assessment:

Root rot was assessed on the basis of percent of seedling emergence and infection percentage. Percentage of seedling emergence was recorded after germination while infection percentage of infected faba bean roots with *Rhizoctonia solani* and *Fusarium solani* were recorded after 45 and 75 days from planting. Statistical analysis of collected data was done according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Effect of soil treated with copper oxychloride and sulphur on faba bean plants infected with *Rhizoctonia solani* and *Fusarium solani*

1- Seedling emergence

Data in Table (2) show that the soil infected with *Rhizoctonia solani*, scored the lowest seedling emergence among all treatments. Application of the fungicides alone or in combination with *Rhizobium* inoculation increased seedling emergence compared with control plants. On the other hand, infection of faba bean with *Fusarium* showed lower emergence compared to soil infested with *Rhizoctonia*. *Rhizobium* inoculation in soil infected with *Fusarium* had no effect on seedling emergence compared to control. Application of fungicides reduced seedling emergence compared to control treatment. These results indicated that using copper oxychloride or sulphur as fungicides for controlling faba bean root rot caused by *Fusarium solani* had negative effect on seedling emergence. However, *Rhizobium* inoculation combined with copper oxychloride showed higher emergence compared to those treated with copper oxychloride alone. These results are in agreement with those obtained by Muthomi *et al.* (2007). They stated that *Rhizoctonia* significantly reduced seedling emergence in legumes and application of copper oxychloride as a fungicide decreased significantly seedling mortality in *Rhizoctonia* treated seeds but the fungicide had little effect on *Fusarium*.

Table (2): Seedling emergence % of faba bean treated with fungicides and infected with the pathogens

Treatment	Soil infected with <i>Rhizoctonia</i>	Soil infected with <i>Fusarium</i>	Non-infected soil
Control	52.50	62.50	93.75
Sulphur(S)	87.50	31.25	100.0
Copper oxychloride(Cu)	93.70	50.00	100.0
<i>Rhizobium</i> inoculation (R)	75.00	62.50	93.75
S+R	81.25	50.00	87.50
Cu+R	81.25	62.50	93.75

2- Infection percentage

Data presented in Table (3) show that the soil infected with *Rhizoctonia solani* (control treatment) scored the highest percentage of infection after 45 and 75 days from planting compared to all other treatments. Inoculation with *Rhizobium* decreased significantly infection % in both periods. Soil treated

with copper oxychloride or sulphur also decreased significantly infection % compared to control. Combination of sulphur with *Rhizobium* inoculation showed less protection against *Rhizoctonia solani* compared to soil treated with sulphur only. The most effective treatment in reducing infection % caused by *Rhizoctonia solani* was recorded in soil treated with copper oxychloride or sulphur. These results are in agreement with those obtained by Muthomi *et al.* (2007).

Regarding to the effect of copper oxychloride and sulphur on faba bean plants infected with *Fusarium solani*, inoculation with *Rhizobium* decreased infection % in both periods. Soil treated with sulphur also decreased infection % compared to control. On the other hand, application of copper oxychloride or combination of *Rhizobium* and copper oxychloride had no effect on the infection % with *Fusarium solani* after 75 days from planting.

It could be concluded from these results that *Rhizobium* inoculation plays an important role in reducing the infection % of the fungal pathogens. These results are in agreement with those reported by Dileep Kumar *et al.* (2001). They found that some strains of *Rhizobium leguminosarum* bv.v *icea* produce siderophores and other compounds and may be involved in the mechanism of antagonism. Also, in a study of using *Rhizobium* species against various soil pathogens, Buonassisi *et al.* (1986) determined that some *Rhizobium* species isolated from bean inhibited *Fusarium* species. Chao (1990) and Ozkoc *et al.* (2001) demonstrated that *Rhizobium leguminosarum biovar phaseoli* was variably effective on the inhibition of *Fusarium*. Muthomi *et al.* (2007) investigate the effect of copper oxychloride as a seed dresser on legume root rot pathogens. They found that the fungicide seed dressing significantly increased seedling emergence and reduced seedling mortality. They found that *Rhizoctonia solani* and *Sclerotinia sclerotiorum* were more pathogenic and significantly increased seedling mortality and greatly reduced seedling emergence. Al-Kahal *et al.* (2003) found that inoculation of faba bean with *Rhizobium leguminosarum* and *Bradyrhizobium japonicum* as a plant growth promoting rhizpbacteria was significant for reducing faba bean root disease caused by *Fusarium oxysporum* under greenhouse conditions.

Table (3) Effect of copper oxychloride and sulphur on faba bean infection percentage

Treatment	Infected soil with <i>Rhizoctonia</i>		Infected soil with <i>Fusarium</i>	
	After 45 days	After 75 days	After 45 days	After 75 days
Control	62.5	62.5	62.8	57.0
Sulphur(S)	25.0	37.6	37.5	50.0
Copper oxychloride(Cu)	25.0	37.5	62.5	62.5
Rhizobium inoculation (R)	37.5	50.0	25.0	50.0
S+R	37.5	50.0	62.5	75.0
Cu+R	25.0	50.0	50.0	62.5
L.S.D. at 5%	10.00	12.54	23.65	9.34

3- Nodulation of faba bean

Results in Table (4) show that after 45 days from planting, there was a significant increase in number and dry weight of nodules in plants inoculated with *Rhizobium* compared to control ones (plants infected with *Rhizoctonia*

only). Muthomi *et al.* (2007) found that inoculation with *Rhizobium* improved nodulation in soil infected with *Rhizoctonia*. Combination of *Rhizobium* and the fungicides also increased significantly number of nodules, but there was a decrease in dry weight of nodules. After 75 days, there was a decrease in number of nodules in all treatments compared to control plants. However, application of the fungicides separately or in combination with *Rhizobium* increased dry weight of nodules. These results revealed that application of copper oxychloride or sulphur with or without *Rhizobium* inoculation had no effect on nodulation status in infected faba bean plants with *Rhizoctonia solani*. Concerning to the effect of *Fusarium solani* on nodulation status after 45 days, data in Table (4) showed that plants inoculated with *Rhizobium* scored the highest number and dry weight of nodules compared to all other treatments. There was a significant increase in number and dry weight of nodules in plants inoculated with *Rhizobium* compared with control ones (plants infected with *Rhizoctonia* only). Al-Kahal *et al.* (2003) found that inoculation of faba bean with *Rhizobium leguminosarum* and *Bradyrhizobium japonicum* as a plant growth promoting rhizpbacteria increased significantly number and dry weight of nodules and shoots dry weight of infested and non infested plants under greenhouse conditions. The same trend was also observed in plants infested with *Rhizoctonia solani* and inoculated with *Rhizobium*. Application of *Rhizobium* combined with the fungicides had no effect on nodulation. Application of the fungicide copper oxychloride or sulphur combined with *Rhizobium* had negative effect on nodulation status compared to control. In the second period, in spite of application of *Rhizobium* or combination of bacterial inoculum besides the fungicides increased number of nodules, but there was a reduction in dry weight of nodules. This result indicates that *Fusarium*, which cause root rot disease, had an inhibitory effect on the nodulation of faba bean even in the presence of the fungicides.

Table (4): Nodulation status of infected faba bean plants as affected by application of the fungicides and *Rhizobium* inoculation

Treatment	Soil Infected with <i>Rhizoctonia</i>				Soil Infected with <i>Fusarium</i>			
	After 45 days		After 75 days		After 45 days		After 75 days	
	No. nodules /plant	Dry wt. nodules /plant (mg)	No. nodules /plant	Dry wt. nodules/ plant (mg)	No. nodules /plant	Dry wt. nodules /plant (mg)	No. nodules /plant	Dry wt. nodules /plant(mg)
Control	12	0.047	62	0.023	19	0.023	43.5	0.09
Sulphur(S)	11	0.02	17.5	0.07	32.5	0.032	26.66	0.065
Copper oxychloride (Cu)	16.25	0.022	48	0.11	12.5	0.013	40	0.068
Rhizobium inoculation (R)	30.66	0.36	50.23	0.07	89.25	0.078	66.5	0.06
S+R	41.83	0.032	54.5	0.08	15.67	0.015	67.25	0.067
Cu+R	21.5	0.022	36.25	0.09	13	0.048	63.75	0.22
L.S.D.at 5%	13.67	0.042	24.53	0.06	23.88	0.026	13.65	0.19

4- Faba bean shoots dry weight

Data presented in Table (5) show that soil infected with *Rhizoctonia solani* (control) scored the lowest dry weight of shoots compared to all other treatments after 45 days from planting. There was no significant difference was recorded in soil treated with the fungicides and control treatment. *Rhizobium* inoculation scored the highest dry weight of shoots among all treatments. Muthomi *et al.* (2007) found that *Rhizobium* inoculation improved shoot dry weight of legumes in soil infected with *Rhizoctonia*. After 75 days from planting, there was a significant increase in dry weight of shoots in soil treated with Copper oxychloride compared to control plants. *Rhizobium* inoculation also increased significantly dry weight of faba bean shoots. It could be concluded from these results that *Rhizobium* inoculation had an inhibitory effect on the pathogen *Rhizoctonia solani* as indicated in both periods. Copper oxychloride or sulphur decreased the infection caused by the pathogen and in turn, increased shoots dry weight. Plants treated with copper oxychloride or sulphur scored the highest value of N-percentage.

Data in Table (5) reveal that plants infested with *Fusarium solani* scored the lowest dry weight of shoots and N-% among all treatments; however, there was a significant difference in dry weight of shoots after 45 days from planting in plants inoculated with *Rhizobium* only and control ones. Combination of *Rhizobium* inoculation and application of the fungicides gave an increase in dry weight of shoots but not significant compared to control. On the other hand, after 75 days, there was non-significant difference in all treatment. These results indicate that *Fusarium solani* was more pathogenic than *Rhizoctonia solani* in terms of its negative effect on dry weight of faba bean shoots.

Table (5) Dry weight of infected faba bean plants as affected by application of the fungicides and *Rhizobium* inoculation

Treatment	Infested soil with <i>Rhizoctonia</i>				Infested Soil with <i>Fusarium</i>			
	After 45 day		After 75 day		After 45 day		After 75 day	
	Dry wt. shoots /plant (g)	N-%	Dry wt. shoots /plant (g)	N-%	Dry wt. shoots /plant (g)	N-%	Dry wt. shoots /plant (g)	N-%
Control	0.50	4.8	1.850	2.7	1.01	6.2	4.30	6.1
Sulphur(S)	0.96	5.6	1.113	3.4	1.12	4.7	3.62	4.1
Copper oxychloride (Cu)	0.90	5.6	3.33	4.6	1.24	2.5	2.78	4.7
<i>Rhizobium</i> inoculation (R)	1.177	3.4	3.16	3.8	1.97	5.3	3.33	5.1
S+R	0.93	4.1	1.910	4.9	1.20	5.1	4.56	2.9
Cu+R	1.043	3.9	2.70	2.9	1.33	3.9	3.76	4.1
L.S.D.at 5%	0.49	--	0.90	--	0.338	--	0.93	--

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تأثير إضافة أكسي كلورو النحاس والكبريت المعدني والتلقيح بالريزوبيا علي
مرض أعفان الجذور وعلي العقد الجذرية ونمو نبات الفول البلدي
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أجريت تجربة أصص لتقييم استخدام أكسي كلورو النحاس بتركيز 100 جزء في
المليون والكبريت المعدني بمعدل 100 كيلوجرام للفدان كمبيدات فطرية كل علي حده أو مع
التلقيح بالريزوبيا علي مقاومة مرض تعفن الجذور في الفول البلدي المتسبب عن فطر الريزوكتونيا
وفطر الفيوزاريوم. وقد تم تقييم أيضا دراسة تأثير ذلك علي تكوين العقد الجذرية ونمو النبات ونسبة
النتروجين به. وكانت النتائج كالآتي:

في التربة المصابة بفطر الريزوكتونيا أدى استخدام المبيدات الفطرية سواء بمفردها أو
مع التلقيح بالريزوبيا إلي زيادة نسبة نمو البادرات مقارنة بالنباتات المصابة بفطر الريزوكتونيا
فقط. وكانت نسبة نمو البادرات المصابة بفطر الفيوزاريوم اقل من تلك المصابة بفطر الريزوكتونيا.
وقد أوضحت النتائج إن استخدام أكسي كلورو النحاس أو الكبريت كان له تأثير سلبي علي نسبة
نمو البادرات المصابة بفطر الفيوزاريوم ومن حيث نسبة الإصابة، كانت أكثر المعاملات فاعلية في
تقليل نسبة الإصابة بفطر الريزوكتونيا سولاني عند استخدام أكسي كلورو النحاس أو الكبريت
المعدني.

وقد أدى التلقيح بالريزوبيا في التربة المصابة بفطر الريزوكتونيا أو فطر الفيوزاريوم إلي
تقليل نسبة الإصابة معنويا. ولم يكن لاستخدام أكسي كلورو النحاس أو التلقيح بالريزوبيا مع أكسي
كلورو النحاس أي تأثير سلبي علي نسبة الإصابة بفطر الفيوزاريوم. وقد أوضحت النتائج إن
استخدام أكسي كلورو النحاس أو الكبريت سواء مع التلقيح بالريزوبيا أو بدونه لم يكن له أي تأثير
علي التعقيد الجذري لنباتات الفول البلدي المصابة بفطر الريزوكتونيا. وبالنسبة لتأثير فطر
الفيوزاريوم علي التعقيد الجذري فقد أكدت النتائج إن الإصابة بفطر الفيوزاريوم كان له تأثير مثبط
علي التعقيد الجذري لنباتات الفول البلدي حتي في وجود المبيدات الفطرية.

كانت هناك زيادة معنوية في الوزن الجاف للمجموع الخضري في التربة المصابة بفطر
الريزوكتونيا وذلك عند استخدام أكسي كلورو النحاس مقارنة بالكنترول. وقد أدى التلقيح بالريزوبيا
إلي زيادة معنوية في الوزن الجاف للمجموع الخضري. ولم يكن هناك أي تأثير علي الوزن الجاف
للمجموع الخضري في التربة المصابة بفطر الفيوزاريوم عند استخدام المبيدات الفطرية.