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Efficacy of Sulfur, Copper and *Rhizobium leguminosarum* against Faba bean damping-off caused by *Fusarium solani*

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ABSTRACT
The efficacy of the organic fungicides elemental sulfur (S) and copper oxychloride (Cu) as well as a soil treatment *Rhizobium leguminosarum* were evaluated under laboratory, plastic houses and the field conditions, to control faba bean damping-off disease caused by *Fusarium solani*. Under laboratory conditions, the fungicides produced reduced mycelial growth of F. solani compared to *R. leguminosarum* as their effect was weak in reducing its growth. Under plastic houses and/or field conditions during (2015/2016 and 2016/2017) growing seasons, the faba bean plant was treated with the organic fungicides elemental sulfur at 500 ppm, copper oxychloride at 500 ppm (as a soil treatment) and / or seed packaging with R. leguminosarum, individually or combined. All treatments reduced the pre- and post-emergence damping-off, as well as increased the remaining plants, fresh & dry weight shoots and Nodulation of faba bean compared to the comparison treatment. In addition, under field condition, it caused a significant increase in the yield components for example (the number of pods per plant and total yield per Fed of both seasons. The combination of *R. leguminosarum* and the fungicide elemental sulfur was the best of these treatments compared to the other treatments.
Keywords: Elemental sulfur, Copper oxychloride, *Rhizobium leguminosarum*, Faba bean, Seedlings damping-off.

INTRODUCTION
Faba beans (*Vicia faba* L.) are considered one of the most important sources of nutrition in many countries of the world, developing and industrial ones, as it is used in human and animal nutrition as well as birds, and due to its high protein content estimated at 20-41%, it is used as an alternative to meat (Ahmed, 2013; Albdaiwi *et al.,* 2019). Faba bean plant is exposed to many diseases that cause a significant decrease in the amount of yield, the most important of these diseases are damping-off and root- rot (Abd-El-Kader *et al.,* 2015; Habtegebriel and Boydom, 2016). Faba Bean root disease is rarely attributed to a distinct pathogen but is usually a complex disease condition. (Salt, 1983, Elwakil *et al.*, 2009; Golpayegani *et al.*, 2010; Rashad *et al.*, 2012; Chang, *et al.*, 2014; Noher *et al.,* 2018) determined that: There are many soil-borne fungi (*Rhizoctonia solani*, *Fusarium oxysporum*, *Fusarium solani*, *Pythium* spp., and *Sclerotinia* spp.). It is the main cause of these diseases. So far, no modern methods have emerged to combat these diseases, as chemical pesticides are used in treating the seeds before planting them, as they are considered the usual method for controlling the fungi that cause disease of damping-off (Noher, *et al.,* 2018). However, problems do arise when chemical seed treatments are used in conjunction with *Rhizobium inoculum*. Often, fungicides may not give satisfactory results in combating these diseases or interfere with inoculation with *rhizobia* (Revellin, *et al.,* 1993). The organic fungicide elemental sulfur has been known for a long time for its use as a pesticide, as it was approved in 1929 as a pesticide in the United States.

At present, sulfur has been approved as one of the pesticides used in controlling fungi for many crops. Chemical compounds containing copper salts have been known as fungicides for a long time since they are widely used in combating bacterial and fungal diseases in agriculture (Mapper *et al.*, 1984, Hussein *et al.*, 1991, El-Fiki 1994, Zaghloul, 1997 and Noher, *et al.,* 2018). The inoculation of legumes with *rhizobia* and the effect of this on the growth of these crops have been known for a long time since it has been used to a large extent in agriculture to improve crops due to its ability to fix nitrogen (Chen *et al.,* 2018). *Rhizobium* symbiosis with legumes produces 50% of 175 million tons of total biological N_2 fixation annually worldwide (Roughley, *et al.*, 1983; Shaban and Bramawy, 2011; Farfour and Al-Saman, 2014, Yadav and Verma, 2014; Ouma *et al.,* 2016; Chen *et al.,* 2018). *Rhizobia* can also serve as biological control agents of some plant pathogens in legume and non-legume plants (Antoun and Prévost, 2005; Kumawat *et al.,* 2021). In fields infested with *Pythium spp*., inoculation with *R. leguminosarum* significantly increased seedling emergence in pea and sugar beets (Bardin *et al.,* 2004) and reduced the incidence of a disease induced by soil-borne pathogens of pea and lentil (Huang and Erickson, 2007). Treatment of faba bean seeds with *R. leguminosarum* under soil infestation conditions by *R. solani* caused a significant reduction in damping-off as compared to the untreated control (Shaban and El-Bramawy, 2011). However, using fungicides at the recommended rates often inhibits the action of root nodule bacteria. Therefore, in the past three decades, researchers have been interested in studying the compatibility of *rhizobia* strains with fungicides, as these studies have been controversial. Curley and Burton (1975); Apron Revellin *et al.* (1993) indicated that when using the fungicide captan and pentachloronitrobenze on soybeans (*Glycine max*) inoculated with *Rhizobium*, this reduced the ability of *Bradyrhizobium japonicum* to fix nitrogen by 18,75 and 61%, respectively, after exposure to the pesticide for 1 hour.

Therefore, the objectives of this study were to verify: 1) The effect of soil treatment with the organic fungicides elemental sulfur and copper oxychloride on the incidence of pre- and post-emergence damping- off of faba bean plants caused by *F. solani*(2) The effect of those organic fungicides on the condition of the node and growth of faba bean plants in the presence of the pathogen. (3) To study the possibility of *R. leguminosarum* bacteria coexisting with these organic fungicides when used in controlling root- rot disease under field conditions. (4) Determine the effect of *R. leguminosarum* alone or in combination with those organic fungicides on stimulating the growth of the faba bean plant and crop components under field conditions.

MATERIALS AND METHODS

Source of the causal fungi and *Rhizobium*:

The pure pathogenic isolates of *Fusarium solani* were taken from Legeme Dis. Res. Dept., Plant Pathol. Res. Inst., ARC, Egypt. Moreover, the *Rhizobium leguminosarum* inoculum was obtained from the BNF Unit, Microbiology Dept., Soil, Water, and Environment Res. Inst. ARC, Giza, Egypt. The inoculum of *F. solani* was maintained on PDA slants whereas, inoculum of *R. leguminosarum* was maintained on Mannitol Yeast Extract Agar Medium (MYA) slants. Both fungal and bacterial cultures were Kept at 4°C until their use.

Source of soil sample and seeds:

The soil sample used in the study was obtained from the Agricultural Research Center farm in Giza, Egypt. Meanwhile, the Giza 3 Faba bean variety used in the experiments for this investigation was taken from the Legume Crop Research Department, Field Crop Res. Inst. Agric. Res. Center, Ministry of Agriculture, Egypt.

Laboratory tests:

Effect of the two organic fungicides elemental sulfur and copper oxychloride on the linear growth of the pathogen *Fusarium solani as well as growth of Rhizobium leguminosarum*:

The two organic fungicides elemental sulfur (S) 100% and copper oxychloride (Cu 50%) were tested under laboratory conditions to study the inhibitory efficacy of both on the growth of *F. solani* using the toxic food method described by Schmitz, (1930). Multiple concentrations of both organic fungicides were prepared, for example, 0.0, 50, 100, 200, 300, 400, and 500 ppm. Each concentration was added separately to a sterile PDA medium, immediately before solidification and poured into sterile 9 cm Petri dishes. The plates were inoculated with 5 mm discs of *F. solani* obtained from 7-day-old cultures. After inoculation, the plates were incubated at 27 ± 1 ° C, where five dishes were used for each concentration. When the growth of fungus reached the edge of a plate that was used as a comparison treatment, the average growth of fungus for each treatment was calculated according to Topps and Wain, (1957). On the other hand, the efficacy of these organic fungicides on the growth of *R. leguminosarum*, which was grown on a sterile (MYA) medium, was studied using the method for measuring the diameters of the colonies formed, as described by Bollich, *et al.,* (1985) The concentrations of each fungicide were prepared as previously mentioned, and each concentration was added individually to MYA medium before solidification and poured into a sterile Petri dishes (9 cm), five plates were used for each treatment. 2 ml of *Rhizobium* suspension was taken from a 3-day-old culture, inoculated with cold medium (MYA) using a sterile pipette and then dispersed evenly on the growth medium using a sterile glass rod. The dishes were incubated inverted after inoculation at a temperature of 27 ± 1 °C. The diameters of the colonies formed after 3 days of incubation were measured.

Effect of soil treated with the two organic fungicides elemental sulfur and copper oxychloride against *Fusarium solani* on faba bean and their effect on fresh and dry weight, as well as, nodulation*:*

1-Under greenhouse conditions:

The potted experiment was designed under greenhouse conditions in the Agricultural Research Center, Giza, Egypt. Aiming study the efficacy of the two organic fungicides elemental sulfur (S) and copper oxychloride (Cu) (as a soil treatment) alone or combined with the root knot bacterium *R. leguminosarum* (R) (as inoculated seed) in reducing pre- and post-emergence damping-off of faba bean plants, besides its effect on fresh and dry weight, as well as, nodulation. In this experiment, sterile plastic pots of 25 cm in diameter were used that contained sterile soil (2.4 kg /pot) consisting of a mixture of clay and sandy soils in a ratio of (4: 1 w / w), respectively. The pathogen *F. solani* were grown on sterile barley grain medium, for 12 days at 25 ± 1 ° C. The pathogen was added to the soil at a rate of 3% (w / w). The inoculated soil was watered daily for seven days to stimulate growth and distribution of the inoculum pathogen. Faba bean seeds Giza 3 were inoculated with *R. leguminosarum* at the rate of 10 g / kg $^{-1}$ seed before planting by immersing them in a sugar solution at a concentration of 20% where it was used as an adhesive substance. The treated seeds of peat pollen were coated with 16% gum arabic as an adhesive agent and left for 30 minutes in the shade Until completely dry according to Farfour and Al-Saman, (2014). Six faba bean seeds were planted in each pot, and five pots were identified as duplicates for each transaction in a completely random template design. The pots were watered as needed.

The experiment included the following treatments:

(1) Comparative treatment (Soil inoculated with *F. solani* only).
(2) Soil treated with the fungicide elemental sulfur (S) at a rate of 500 ppm.
(3) Soil treated with the fungicide copper oxychloride (Cu) at a rate of 500 ppm.
(4) Seeds inoculated with the commercial *R. leguminosarum* inoculant).
(5) Seeds inoculated with *R. leguminosarum* and fertilized with a starting dose of nitrogen fertilizer and the fungicide elemental sulfur mixed with soil
(6) Seeds inoculated with *R. leguminosarum* and fertilized with a starting dose of nitrogen fertilizer and the fungicide copper oxychloride mixed with the soil.

Pots were fertilized with the recommended dose of Calcium Superphosphate (15.5% P_2O_5), Ammonium Nitrate (33.5% N as per the Egyptian Ministry of Agriculture guidelines, that were mixed with the soil.

Disease assessment:

The incidence of pre -and post -emergence damping–off was calculated 20 days after sowing. Forty-five days after sowing, the remaining plants were counted and recorded. Also, fresh and dry weight (g/plant), as well as, number and dry weight of nodules (mg/plant), of faba bean plants, were calculated for the remaining plants 60 days after planting.

2-Under field conditions:

A field experiment was conducted in Menouf Center in Menoufia Governorate, Egypt, under conditions of natural infection with pathogen *F. solani* during the growing seasons 2015-2016 and 2016-2017 to study the effectiveness of tested organic fungicides elemental sulfur (S) and copper oxychloride (Cu) (as a soil treatment) alone or combined with *R. leguminosarum* (R) (as inoculated seed) in reducing pre- and post-emergence damping-off of faba bean plants , besides its effect on fresh and dry weight, nodulation, as well as, on yield components. The experiment was designed as a complete randomized block that contained three replicates. The experimental unit was calculated as 10.5 m2 (3.5 x 3 m). Each unit contained five rows, each row 2.5 meters long with 60 cm spacing. Faba bean seeds (Giza 3) were treated with R. leguminosarum as described above. The treated seeds were sown in hills of 25 cm on both sides with a length of 6 cm, with two seeds per mound in both seasons. The faba bean seed was grown free of any treatment and was used as a comparison treatment. All tested units were fertilized and all agricultural processes used in growing faba bean were followed according to the Egyptian Ministry of Agriculture guidelines. The incidence of the pre- and post-emergence damping-off of the faba bean was estimated as previously mentioned. Also, at the end of the experiment, some plants were uprooted to estimate the faba bean fresh and dry weight (g/plant), as well as, number and dry weight of nodules (mg/plant). When faba bean yield was harvested, number of pods / plant and total yield (kg /Fedn) were measured.

Statistical analysis:

The obtained data were subjected to the proper statistical analysis using the MSTAT statistician software (MSTAT-C, 1991), and the comparison was done using, L.S.D (P<0.05).

RESULTS

Laboratory tests:

Effect of the two organic fungicides elemental sulfur and copper oxychloride on the linear growth of the pathogen *Fusarium solani* as well as growth of *Rhizobium leguminosarum*:

The results in **Table (1)** show that when using both the organic fungicides elemental sulfur (S) and copper oxychloride (Cu) to study their effect on the linear growth of the pathogen *F. solani* as well as the *R. leguminosarum* nodulating bacteria. These fungicides caused a significant decrease in the linear growth of F. solani and R. leguminosarum compared to the comparison treatment. This decrease in linear growth was related to an increase in the concentration of both organic fungicides. It was noted from the results, that the fungicide elemental sulfur had a higher effect than the fungicide copper oxychloride in inhibiting the linear growth of the pathogen, as it was recorded 5.82, 4.33, 3.82, 2.55 and 1.40 cm at 50, 100, 200, 300 and 500 ppm, respectively, while, the fungicide copper oxychloride had a low effect in reducing the linear growth of *F. solani* as it was recorded, 6.33, 5.42, 4.53, 3.66 and 2.82 cm at 50, 100, 200, 300 and 500 ppm, respectively. On the other hand, it was also noticed from the results that both organic fungicides recorded the least effect of growth of *R. leguminosarum* bacteria as it was recorded 8.72, 7.99, 6.33, 5.82, 5.12 9.00, 9.00, 8.66, 8.22 and 7.99 cm at 50, 100, 200, 300 and 500 ppm, respectively.

Table 1: Effectiveness of multiple concentrations of the two organic fungicides elemental sulfur (s) and copper oxychloride (cu) on inhibition of linear growth of *Fusarium solani* as well as growth of *Rhizobium leguminosarum*.

Isolates	Linear growth (cm) on each concentration (ppm) tested											
	Elemental sulphur						Copper oxychloride					
	0	50	100	200	300	500	0	50	100	200	300	500
Fusarium solani	9.00	5.82	4.33	3.82	2.55	1.40	9.00	6.33	5.42	4.53	3.66	2.82
R. leguminosarum	9.00	8.72	7.99	6.33	5.82	5.12	9.00	9.00	9.00	8.66	8.22	7.99

L.S.D. at 0.05 % for: Isolates (I) = 1.70, Fungicides (F) = 2.10, Concentration(C) = 2.40, IxF= 3.90, IxC= 3.80, FxC= 4.00 and IxFxC= 5.20.

Effect of soil treated with the two organic fungicides elemental sulfur and copper oxychloride against *Fusarium solani* on faba bean and their effect on fresh and dry weight, as well as, nodulation:

1-Under greenhouse conditions:

1-a. Estimating the percentage of infection with the disease and the percentage of remaining plants:

The results in **Table (2)** showed that all treatments resulted in a significant decrease in the incidence of pre- and post-emergence damping-off, as well as an increase in the percentage of the remaining plants of the faba bean plant that were artificially infected by pathogen *F. solani* compared to the comparison treatment. Also, it was noticed from the obtained results that when the faba bean seeds were inoculated with *R. leguminosarum* (R) and the combination of those bacteria with both the organic fungicides elemental sulfur (s+R) and copper oxychloride (Cu+R) (as a soil treatment) had caused a significant decrease in the incidence of pre- and post-emergence damping-off, as well as an increase the percentage of the remaining plants of faba bean, overcome some treatments alone. In addition, the results indicated that when the soil was treated with both organic fungicides, they had a significant effect in reducing the infection rate and increasing the remaining plants more than the use of *R. leguminosarum* when applying these treatments alone. On the other hand, when the fungicide elemental sulfur was combined with *R. leguminosarum*, it caused a significant increase in the percentage of the remaining plants more than the combination of the fungicide copper oxychloride with *R. leguminosarum* it was recorded 93.34%, and 86.27% respectively. Conversely, the seeds treated with *R. leguminosarum* had the lowest incidence of infestation and an increase in the percentage of remaining plants it was recorded 60.01%. In addition, when using each of the two organic fungicides alone to combat faba bean damping-off disease, it was noted from the results that the fungicide elemental sulfur had a high effect in reducing the incidence of pre- and post-emergence damping-off, as well as, increasing the percentage of the remaining plants more than using the fungicide copper oxychloride scoring 80.01% and 70.00 %remaining plants, respectively.

Table 2. Efficiency of using the two organic fungicides elemental sulfur and copper oxychloride (as a soil treatment) as well as *Rhizobium leguminosarum* (as inoculated seed) alone or combined to compact damping-off disease of faba bean plant artificially infected with *Fusarium solani*, as well as, the remaining plants under greenhouse conditions.

Treatments	Pre- emergence damping-off (%)	Post- emergence damping off (%)	Remaining Plants (%)
Control	40.00	16.66	43.34
Elemental sulfur (S)	13.33	6.66	80.01
Copper oxychloride (Cu)	20.00	10.00	70.00
***R. leguminosarum* (R)**	23.33	16.66	60.01
S + R	6.66	0.00	93.34
Cu + R	10.00	3.33	86.27
L. S. D. at 0.05% for	2.22	1.09	4.09

1-b. Estimate the faba bean fresh and dry weight (g/plant):

The results in **Table (3)** show that when treating faba bean witch artificially infected with *F. solani* with *R. leguminosarum* (R) (as inoculated seed) and with the two organic fungicides elemental sulfur (S) and copper oxychloride (Cu) (as a soil treatment), alone or combined, increase the fresh and dry weight of faba bean plants (g/plant), compared with comparison treatment. In addition, when applying these treatments separately, it was observed from the results that both of the organic fungicides gave an increase in the fresh and dry weight were greater than using *R. leguminosarum* alone. It was also noted from the results that the fungicide elemental sulfur was more effective in increasing the fresh and dry weight when the soil was infected with the pathogen than the fungicide copper oxychloride, where it was recorded 19.06 and 3.96 (g/plant), respectively, while the fungicide copper oxychloride gave the following results 17.53 and 3.16 (g/plant), respectively. On the other hand, when *R. leguminosarum* and the fungicide elemental sulfur combined, this treatment gave an increase in the fresh and dry weight, where it was recorded 21.16 and 4.72 (g/plant), respectively, more than one union those bacteria with the fungicide copper oxychloride where it was recorded 20.03 and 4.15 (g/plant), respectively. It was also observed from the results that the least increase in the fresh and dry weight of the plants was recorded when the faba bean were treated with *R. leguminosarum* where it was recorded 14.23 and 2.69 (g/plant), respectively.

Table (3): Efficiency of using the two organic fungicides elemental sulfur and copper oxychloride (as soil treatment) as well as *Rhizobium leguminosarum* (as inoculated seed)on the fresh and dry weight (g/plant) of faba bean plants artificially infected with *Fusarium solani* under green house conditions.

Treatments	Fresh weight (g/plant)	Dry weight (g/plant)
Control	8.08	1.90
Elemental sulfur (S)	19.06	3.96
Copper oxychloride(Cu)	17.53	3.16
***R. leguminosarum* (R)**	14.23	2.69
S + R	21.16	4.72
Cu+R	20.03	4.15
L. S. D. at 0.05% for	1.75	0.86

1-c. To estimate the root nodule number/plant, root nodule dry weight/plant (mg):

The nodulation results obtained in **Table (4)** indicated that the two organic fungicides (elemental sulfur (S) and copper oxychloride (Cu) (as a soil treatment) and *R. leguminosarum* (R) (as inoculated seed) may be used separately or together. These treatments resulted in a significant increase in the number and dry weight of the root nodules /plant (mg) of the faba bean plant witch artificially infected with *F. solani* compared to the comparison treatment. It was observed from the results that the *R. leguminosarum* combined with both organic fungicides resulted in a significant increase in both the number and the dry weight of the root nodules, superior to use any of them alone. Moreover, when the *R. leguminosarum was* used alone, scored better in increasing the number and dry weight of root nodules than using both organic fungicides *alone*. It was also noted from the results that the fungicide elemental sulfur was more effective in increasing the number and dry weight of root nodules when the soil was infected with pathogen than the fungicide copper oxychloride, where it was recorded 43.50 /plant and 0.07 /plant (mg) respectively, while the fungicide copper oxychloride gave the following results 40.00 /plant and 0.06 /plant (mg), respectively. In general, the S + R combination scored the highest number and dry weight of root nodules where it was recorded 62.50 /plant and 0.20 /plant (mg) respectively, followed by Cu + R where it was recorded 54.50 /plant and 0.14 /plant (mg) respectively. On the other hand, the fungicide copper oxychloride scored the lowest number and dry weight of root nodules in this study.

Table (4): Efficiency of using the two organic fungicides (elemental sulfur and copper oxychloride) (as a soil treatment) as well as *Rhizobium leguminosarum* (as inoculated seed) alone or combined together on the number /plant and dry weight of root nodules /plant (mg) of faba bean plants artificially infected with *Fusarium solani* under greenhouse conditions.

Treatments	No.root nodules/plant	Dry weight root nodules (mg/plant)
Control	32.50	0.05
Elemental sulfur (S)	43.50	0.07
Copper oxychloride (Cu)	40.00	0.06
***R. leguminosarum* (R)**	50.23	0.08
S+R	62.50	0.20
Cu+R	54.50	0.14
L. S. D. at 0.05%	1.65	0.03

2- Under field conditions:

2-a. Estimating the percentage of infection with the disease and the percentage of remaining plants:

Data in (**Table 5**) demonstrate that all treatments significantly reduced the incidence of pre- and post-emergence damping-off, as well as, an increase in the percentage of the remaining plants of faba bean plants, witch naturally infected with *F. solani* under field condition during the two seasons 2015-2016 and 2016-2017 compared with the comparison treatment. Faba bean seed inoculated with *R. leguminosarum* and sown in soil previously treated with organic fungicide were more effective than using either treatment alone. In addition, the obtained data show that soil treated with any of organic fungicides (elemental sulfur or copper oxychloride) was more effective to reduce pre- and post-emergence damping-off, as well as, an increase in the percentage of the remaining plants than *R. leguminosarum*. Elemental sulfur (S) + *R. leguminosarum* (R) recorded the highest reduction of pre- and post-emergence damping-off, as well as, an increase in the percentage of the remaining plants in both seasons, minor increase in the second season. Its recorded 94.27%, 90.28%, 92.27% and 86.00% remaining plants compared with 50.01% and 43.27% in comparative treatment in 2015/2016 and 2016/2017, respectively. On the contrary, faba bean seeds coated in *R. leguminosarum* recorded the lowest values in both seasons (73.34% remaining plants in first seasons and 68.00% in the second season, respectively).

Table(5): Efficiency of using the two organic fungicides (elemental sulfur and copper oxychloride) (as soil treatment) as well as *Rhizobium leguminosarum* (as inoculated seed)individually or in combination in controlling faba bean damping-off disease caused by *Fusarium solani* , and remaining plants under field conditions during growing seasons 2015-2016 / 2016-2017.

Treatments	Season 2015-2016			Season 2016-2017		
	Pre-emergence damping-off (%)	Post-emergence damping off (%)	Remaining plants (%)	Pre-emergence damping-off (%)	Post-emergence damping off (%)	Remaining plants (%)
Control	33.33	16.66	50.01	36.33	20.00	43.27
Elemental sulfur (S)	12.00	6.66	81.34	13.33	8.00	78.27
Copper oxychloride(Cu)	13.33	8.66	78.01	16.66	10.00	73.34
***R.leguminosarum* (R)**	16.66	10.00	73.34	20.00	12.00	68.00
S + R	4.00	1.33	94.27	6.66	2.66	90.28
Cu + R	5.33	2.00	92.27	10.00	4.00	86.00

L.S.D. at 0.05%for: Treatments (T)=0.09, Seasons (S)= 1.00, TxS= 2.21

2-b To estimate the root nodule number/plant, root nodule dry weight/plant (mg), fresh and dry weight shoots/plant (g) and yield components of faba bean:

The results in **Table (6)** showed that when the two organic fungicides were combined with *R. leguminosarum,* witch faba bean plants naturally infected with *F. solani* under field condition, this led to an increase in the number of root nodules, root nodules dry weight, fresh and dry weight of faba bean plants as well as an increase in the yield components more than using them alone. In addition, the soil treated with any of the investigated organic fungicides was highly effective in this regard than using *R. leguminosarum*. Synthesis between *R. leguminosarum* and elemental sulfur was the most effective as it recorded the highest components of the faba bean yield eg number of pods per plant (24.30 and 24.90 pods / plant) as well as total faba bean yield (1851.0 and 1986.0 kg /Fedn) in both growing seasons respectively.

Moreover, the results obtained showed that all the treatments led to a significant increase in the number of root nodules/plant, as well as, the dry weight of the root nodules /plant (mg), and fresh and dry weight shoots (g/plant), in both growing seasons compared to the comparison treatment.

DISCUSSION

In leguminous crops, nitrogen is fixed to a specific root or stem organ called nodule. To obtain good plant growth, this requires compatibility between leguminous crops and *Rhizobia*, as this leads to efficient nitrogen fixation. Inoculation of legumes with *Rhizobia* increases growth when root nodes are not present or when root nodes are not effective (Chen, *et al.,* 2018). There are some organic fungicides that farmers can use to control plant diseases, such as sulfur and copper. Where the use of these organic fungicides in the control of diseases of leguminous crops leads to obtaining a product of high marketing and pricing value. Given that the use of organic fungicides in controlling plant diseases is more frequent than chemical fungicides, this study aims at the possibility of coexistence of *Rhizobia* with the repeated use of those organic fungicides to combat the faba bean damping-off disease caused by the fungus *Fusarium solani* under laboratory, greenhouse and field *conditions*. under laboratory conditions, the obtained results showed that both the organic fungicides elemental sulfur and copper oxychloride had a high inhibition of the linear growth of the pathogen *Fusarium solani*, and this inhibition was increased with increasing concentrations of both organic fungicides. On the other hand, these organic fungicides did not significantly affect the growth of *Rhizobium leguminosarum*. These results have been interpreted by Muthomi, *et al.* (2007) and Noher *et al.,* (2018) where he explained that the inhibitory effect of tested organic fungicides on pathogen may be due to their toxic effect of those organic fungicides at high concentration. Moreover, this effect was more pronounced on *F. solani* than on *R. leguminosarum*, possibly since organic fungicides such as sulfur is specialized in killing fungi only, while, copper is specialized in killing fungi and spors (Barbara and Bradley 1992). This reduced effect on *R. leguminosarum* encouraged this bacterium to stop the growth of pathogens due to its antifungal effect (Antoun and Prévost, 2005).

Under greenhouse and/or field conditions during (2015/2016 and 2016/2017) growing seasons, the use of both the organic fungicides elemental sulfur and copper oxychloride, as well as, *Rhizobium leguminosarum* alone or combined together, reduced the incidence of pre- and post-emergence damping-off, as well as, increased the number of remaining plants, fresh and dry weight shoots and number and dry weight root nodule of the faba bean plant. Especially when the fungicide elemental sulfur was combined with *R. leguminosarum*. This treatment gave the best results obtained. These results are in agreement with that reported by Muthomi *et al.* (2007), they explained that when pollinating the soil with *Rhizoctonia solani* and treating that soil with the fungicide copper oxychloride, this treatment led to a reduction in faba bean damping-off and root-rot diseases, as well as an increase in the number of plants that appeared above the soil surface. Also, Abdel-Monaim, (2013) and Noher, *et al.,* (2018) showed that inoculating the soil with *Fusarium solani* significantly decreased seedlings appearing above the soil surface in legumes. On the other hand, it can be concluded from the results obtained that, inoculation with *R. leguminosarum* plays an important role in reducing the incidence of fungal pathogens. These results are in line with Kumar, *et al.* (2001) and Shaban and El-Bramawy, (2011) where they found that treatment of faba bean seeds with *R. leguminosarum* under soil infestation conditions by *R. solani* caused significant reduction in damping-off as compared to the untreated control. This effect is due to some strains of *Rhizobium leguminosarum pv.vicea* produce siderophores and other compounds such as flavonoids and betaine, these compounds play an important role in inhibiting the growth of pathogens and preventing their progression (Demir, 2005; Wehner, *et al.,* 2010; Abd-Alla, 2011; Abohatem, *et al.,* 2011). Also, in the study by Buonassisi, *et al.* (1986), in which the *Rhizobium* species were used against various soil pathogens, it was determined that, there are some *Rhizobium* species isolated from Bean that prevent infection with *Fusarium*. Chao, (1990), Özkoç, *et al.* (2001) and Bardin, *et al.,* (2004), are also mentioned that, *Rhizobium leguminosarum biovar phaseoli* was moderately effective in inhibition of *Fusarium and Pythium spp*. Al-Kahal, *et al.* (2003) and Huang and Erickson, (2007), also reported that inoculating faba bean, pea and lentil with *Rhizobium leguminosarum* and *Bradyhizobium japonicum* as a vegetative growth promoting resulted in a significant reduction of the incidence of faba bean root-rot disease caused by *Fusarium oxysporum* under greenhouse conditions and reduced the incidence of a disease induced by *Pythium spp* of pea and lentil under fields conditions. In adition, AL-Kahal *et al.,* (2003), and Ouma, *et al.,* (2016), where they found from the results obtained and conducted under greenhouse conditions that inoculation of faba bean, common bean and soybean by *Rhizobium leguminosarum* and *Bradyrhizobium japonicum* as plant growth promoting resulted in a significant increase in the number and weight of dry root nodules, this increase was present in the infected and healthy plants. Also, Muthomi, *et al.* (2007), Müller, *et al.,* (2016) and Hussain, *et al.,* (2018) observed that The pollination of leguminous crops by R. leguminosarum plays an important role in plant growth and development. Also, it has an important role in protecting plants from the invasion of various pathogens and abiotic pressures. They also mentioned, inoculation by *R. leguminosarum* leads to improvement of the node and dry weight of legumes in *Rhizoctonia*-infected soils under field conditions. Under field conditions, it was observed from the obtained results an increase in both the number of pods of the faba bean plant and an increase in the quantity of the yield in both growing seasons as a result of treating the faba bean plant with the same treatments mentioned above. These results are consistent with those obtained by Hussein, *et al.* (1991), El-Fiki, (1994), Zaghloul, (1997), Trabelsi, *et al.* (2012), and Denton, *et al.,* (2013), where they found, root pollination (with *R. leguminosarum*) enhanced phosphate solubility, as well as P and N absorption and iron content in the faba bean. Therefore, the root pollination increased the growth of branches, number of pods, and grain yield of faba bean.

Table 6: Efficiency of using the two organic fungicides elemental sulfur and copper oxychloride (as soil treatment) as well as R*hizobium leguminosarum* (as inoculated seed) alone or combined under naturally infected with *Fusarium solani* on the fresh and dry weight of faba bean shoots/plant (g), number of root nodules/plant, root nodules dry weight /plant (mg) and yield under field conditions during the growing seasons 2015-2016 And 2016-2017.

Treatment	Season 2015-2016						Season 2016-2017					
	No.root nodules/ plant	Dry wt.root nodules/ plant (mg)	Fresh wt. shoots / plant(g)	Dry wt. shoots / plant(g)	No. of pods/ plant	Total yield/ Fed	No.root nodules/ plant	Dry wt.root nodules/plant (mg)	Fresh wt.shoots/plant(g)	Dry wt. shoots/plant(g)	No. of pods /plant	Total yield/ Fed
Control	11.25	0.02	15.99	1.70	12.50	1060.0	12.66	0.03	16.12	1.90	13.50	1102.8
Elemental sulfur (S)	15.00	0.06	20.97	4.40	18.40	1562.5	16.50	0.07	23.11	5.80	19.60	1729.0
Copper oxychloride (Cu)	16.25	0.04	18.20	3.90	17.30	1444.5	17.00	0.05	20.33	4.40	18.40	1633.0
***R. leguminosarum* (R)**	21.50	0.07	17.04	2.60	16.60	1229.0	23.75	0.09	19.42	3.30	16.80	1458.7
S+R	41.83	0.36	27.34	8.90	24.30	1851.0	47.25	0.42	28.44	9.90	24.90	1986.0
Cu+R	30.66	0.25	24.82	6.80	22.00	1733.0	36.50	0.39	26.97	8.60	22.40	1833.0

L.S.D. at 0.05%for: Treatments (T)=1.23 Seasons(S)= 2.55 TxS= 3.74

CONCLUSION

It was concluded that, the combination of the organic fungicide elemental sulfur with the nitrogen nodulating fixing bacteria *Rhizobium leguminosarum* had a good effect on controlling the faba bean damping-off disease caused by *Fusarium solani*, as the organic fungicides had no effect on growth of *R. leguminosarum* and could coexist in the presence of the organic fungicides.

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كفاءة الكبريت و النحاس ريزوبيوم ليجومينوساروم ضد مرض تعفن جذور الفول المتسبب عن الفيوزاريوم سولاني

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الملخص العربى

تم تقييم فعالية مبيدات الفطريات العضوية (الكبريت المعدني وأوكسي كلوريد النحاس) (كعلاج للتربة) تحت الظروف المعملية, البيوت البلاستيكية والحقلية للسيطرة على مرض تعفن جذور الفول الناجم عن الفيوزاريوم سولاني وكذلك على نمو ريزوبيوم. في ظل الظروف المعملية ، أدت المبيدات الفطرية المنتجة إلى تقليل النمو الميسليومي للفطر *F. solani*مقارنة بـ *R. leguminosarum* حيث كان تأثيرها ضعيفًا في الحد من نموها.

تحت ظروف البيوت البلاستيكية و/أو الحقلية خلال موسمي النمو (2016/2015 و 2017/2016)، تمت معالجة نبات الفول بمبيدات الفطريات العضوية كبريت معدني عند 500 جزء في المليون ، أوكسي كلوريد النحاس عند 500 جزء في المليون (كمعاملة للتربة) و / أو تغليف البذور باستخدام *R. leguminosarum* ، منفردة أو مجتمعة. أدت جميع المعالجات إلى تقليل التعفن قبل وبعد ظهور نباتات الفول ، بالإضافة إلى زيادة كل من النباتات المتبقية, الوزن الطازج والجاف ، العقد الجذرية لنبات الفول مقارنةً بمعاملة المقارنة. بالإضافة إلى ذلك ، في ظل الظروف الحقلية ، أدى ذلك إلى زيادة معنوية في مكونات المحصول على سبيل المثال (عدد القرون لكل نبات وإجمالي المحصول لكل فدان في كلا موسمي النمو، كان الجمع بين *R. leguminosarum* ومبيد الفطريات الكبريت المعدني هو أفضل هذه المعاملات مقارنة بالمعاملات الأخرى.

الكلمات المفتاحية: عنصر الكبريت ، أوكسي كلوريد النحاس ، ريزوبيوم ليجومينوساروم ، الفول ، موت البادرات