

## BIOTOXIC EFFECTS OF SOME FUNGICIDES, MICRO-ELEMENTS AND THEIR MIXTURES AGAINST SOME SOIL BORNE FUNGI WHICH CAUSE ROOT-ROT AND DAMPING OFF TO SOYBEAN SEEDLINGS

BY

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### ABSTRACT

Soybean seedlings are exposed to a lot of pathogenic fungi, i.e. *R.solani*, *S.rolfsii*, *M.phaseolina* and *P.debaryanum*, causing damping-off and root-rot diseases. Studies were conducted to evaluate the toxicity of some fungicides, i.e. Rizolex T/thiram 50%, Vitavax 200 75%, Topsin -M 70%, Monceren 25% and Monceren / thiram 47%, and the efficiency of some important elements i.e.  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  and  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ . Pathogenicity test revealed that *S.rolfsii* was highly virulent, it caused root-rot and damping-off disease to soybean plants and decreased percentage of survival plants. Rizolex T /thiram gave the most toxic effect against *R.solani* and *P.debaryanum*, followed by Vitavax 200. Rizolex T /thiram was the most toxic fungicide against *M.phaseolina*, followed by Topsin -M fungicide; while Vitavax 200 and Monceren /thiram were the most toxic ones against *S.rolfsii* followed by Rizolex T /thiram. Cu was the most toxic element against *R.solani*, *M.phaseolina* and *P.debaryanum*, followed by Fe element, which gave the most toxic effect against *S.rolfsii*, followed by Cu. The joint toxic effect was studied between Vitavax 200 and other compounds. The synergistic effects were observed in the case of *R.solani*, when the Vitavax 200 fungicide was combined with elements Fe, Mn and Zn

### INTRODUCTION

Egyptian soil is infested with many soil-borne pathogenic fungi, which attacked many crops i.e. *Rhizoctonia solani* (Kuhn), *Sclerotium rolfsii* (Sacc), *Macrophomina phaseolina* (Tasii) and *Pythium debaryanum*. The latter attacked seedlings and roots of some crops such as soybean causing damping-off and root - rot diseases (Khalifa, 1987).

Soybean (*Glycine max*(L.) Merrill) is one of the most important commercial crops playing a key economic and social affairs in Egypt, it is

an important food and feed crop in many regions of the world. It is one of the world's important sources of oil and protein. It represent nearly 50% of global oil seed production ,28% global vegetable and marine oil supply and 58% of global protein meal supply ( Rosier,1989). It is affected by various diseases. Damping-off and root-rot diseases are the most important diseases that affect plant stand causing great losses in soybean yield, total nitrogen and protein contents in soybean seeds.

Continuous use of synthetic pesticides for disease control has resulted in several environmental problems such as, long persistence period (Beye,1978), pollutive effects (Dubey and Mall, 1972), phytotoxicity (Fawcett and Spencer, 1970), teratogenicity (Javoraska, 1978) and carcinogenicity (Epstein et al., 1967). These factors emphasize the need for new methods to control disease (Wilson et al., 1987).Micro- elements were effective, hence it inhibited radial growth of pathogens, also, seed treatment with some micro elements reduced percentage of infected plants of fungi (Abdel Moneem, 1996 and Savita-Pareek and Pareek, 1999).

The present investigation aims to study the efficacy of some fungicides and micro-elements, against four pathogenic fungi and joint toxic effects of carboxin /thiram with the tested compounds against the tested pathogenic fungi in vitro.

## MATERIALS AND METHODS

**1- Soybean *Glycine max* (L.) (Merrill) seeds, cultivar (Giza 82) :**A sample was Kindly supplied by the legumes Department, El-Gemmeiza Agricultural Research Station, Agricultural Research Center.

### **11- Tested fungi:**

Four pathogenic fungi were used in these experiments namely:

*Rhizoctonia solani* , *Sclerotium rolfsii*, *Macrophomina phaseolina* and *Pythium debaryanum*.

### **111- Tested fungicides and elements :**

#### **A- Tested fungicides**

- 1- Vitavax-200 Consists of 37.5% thiram (Bis (Dimethyl thio -Carbamyl) disulphide + 37.5% Vitavax (5,6 dihydro - 2 methyl - N- phenyl - 1.4 oxathion -3-Carboxamide.) :
- 2- Rizolex - T :consists of 20% Rizolex (O,O-dimethyl-O- (2.6 dichloro - 4 methyl phenyl Phosphoro - thioate) + 30% Thiram (Bis (Dimethyl thio - Carbamyl) disulphide)

3.- Topsin - M (70% W.P):

Dimethyl (1,2 - Phenyl bis (imino - carbonothioyl) bis Carbamate).

4 -Monceren (25% W.P):

1- (4- chloro - benzayl) - 1-1 cyclo-pentyl - 3 - phenyl urea.

5 -Monceren - T (47% W.P)

Consists of (15% Monceren + 32% Thiram).

### B- Tested elements:

Four micro - elements were used namely :

1- Copper	as	Cu SO <sub>4</sub> . 5H <sub>2</sub> O
2- Manganese	as	Mn SO <sub>4</sub> . H <sub>2</sub> O
3- Zinc	as	Zn SO <sub>4</sub> . 7 H <sub>2</sub> O
4- Iron	as	FeSO <sub>4</sub> . 7 H <sub>2</sub> O

### IV- Biological tests

#### 1- Evaluation of the fungicidal activity of tested compounds:

A laboratory study was performed to examine the sensitivity of the fungi to the tested fungicides, and micro - elements. Five concentrations of each fungicide, i.e. 0.01,0.1,1.0,10 and 100 ppm, and four concentrations of micro - elements were used i.e. 0.01,0.1,1.0 and 10 ppm, were used. The required concentrations were obtained by adding the appropriate amount of stock solution used to 100 ml portions of autoclaved PDA cooled to about 45°C. Four petri-dishes, 9 cm diameter, were used as replicates for each concentration . After solidification of the medium, each dish was inoculated centrally with a mycelial disk (0.5 cm in diameter) taken from the cultures of each fungus (7 days - old). Dishes were incubated at 28 ±1oc for 4 days and colony diameters were measured till the untreated dish reached the maximum growth. Inhibition percent ( I % ) was calculated according to the following equation ( Topps and Wain ,1957 ):

$$I\% = \frac{A-B}{A} \times 100$$

Where :

I% = Percent of inhibition.

A = Mean diameter growth in the control .

B = Mean diameter growth in a given treatment.

## 2- The joint toxic action :

IC<sub>25</sub> of carboxin/thiram (Vitavax-200) as a traditional fungicide which exhibited the highest or the lowest effect on the pathogenic fungi, was mixed with IC<sub>25</sub> from every compound alone against the tested pathogenic fungi.

The Joint toxic action of the different combinations was evaluated according to (Mansour et al 1966 ).Results were statistically analyzed according to Finney (1952), then regression lines were drawn on probit-log paper and the median inhibition concentration (IC<sub>50</sub>) was detected . Analysis of variance was made. L.S.D (Least significant difference) and used if differences seemed to be found (Snedecor (1965)). Correction for the control mortality was made by using Abbott's formula (1925).

## RESULTS AND DISCUSSION

### 1- Pathogenicity test :

Data in Table (1) showed that all tested fungi are pathogenic and caused root-rot and damping-off in "Giza 82" soybean cultivar. They varied in their virulence in pre-and post- emergence stages. The tested fungi apparently resulted in significant parameters compared with the noninfested control. *S. rolfsii* was highly virulent to "Giza 82" soybean cultivar. *R. solani* and *P. debaryanum* were moderately virulent. The results of pathogenicity revealed that *S. rolfsii* and *R. solani* are the most important pathogens at the early growth stages of soybean. On the other hand *S. rolfsii* was the most pathogenic fungi, it caused decreasing of morphological characters i.e plant height, fresh and dry weights of shoots and roots, and chlorophyll content, followed by *R. solani* and *P. debaryanum*. However *M. phaseolina* was the least fungus affected the morphological characters. These results were confirmed by the findings of Smartt (1960) who found that soybean was susceptible to infection by *S. rolfsii* at seedlings stage. Khalifa (1987) also found that *S. rolfsii* was the most pathogenic fungi to soybean plants followed by *R. solani*, while *M. phaseolina* was the least pathogenic fungi to soybean plants. Yang et. al., (1990) and lui and Sinclair (1991) reported that *R. solani* was highly pathogenic to soybean.

**Table ( 1 ) : Pathogenicity of tested fungi and their effect on the morphogenesis of soyabean plants cv.Giza "82".**

Parameters fungi	Pre emergence damping off%	Post-emergence damping off%	Survival plants %	Plant height "cm"	Shoot "g"		Root "g"		Chlorophyll content		
					fresh	Dry	fresh	Dry	Chl a	Chl b	Chl a+b
R.solani	64.58	12.50	22.92	28.97	6.573	1.739	0.714	0.145	2.192	2.034	4.226
S.rolfsii	77.15	8.33	14.52	24.83	6.1	1.285	0.378	0.118	3.258	1.399	4.657
M.phaseolina	54.28	9.72	36.00	27.77	10.393	2.103	0.906	0.259	2.8	1.411	4.211
P.debaryanum	68.75	6.25	25.00	28.07	7.678	1.301	0.445	0.238	3.348	1.874	6.222
Control*	4.17	0.00	95.83	29.15	15.38	2.88	1.933	0.705	4.833	1.392	6.225
L.S.D. at 0.05	4.133	2.277	2.718	1.203	1.385	0.329	0.193	0.006	0.311	0.119	0.362
0.01	6.012	3.313	3.954	1.751	0.479	0.281	0.281	0.009	0.453	0.174	0.526

\* untreated seeds sown were in sterilized soil.

## 2- In vitro evaluation of biological activity of tested compounds on tested fungi:

The biological activity of the tested compounds was evaluated against all tested pathogenic fungi :

### a- Evaluation of tested fungicides:

Results in Table (2) showed that Rizolex -T/thiram was the most effective fungicide against *R.solani*, followed by Vitavax 200 and Topsin -M, while Monceren /thiram and Monceren were the least effective compounds on the same fungus. In case of *S.rolfsii*, the data clearly indicated that Vitavax 200 and Monceren /thiram were the most toxic compounds, followed by Rizolex -T /thiram and Topsin -M. Monceren fungicide was the least toxic compound on the same fungus. The results also revealed that *M. phaseolina* fungus was sensitive to Rizolex -T/thiram, Topsin -M, Vitavax 200 and Monceren / thiran, while Monceren had a poor fungicidal action even at its higher concentration

Concerning the tested fungus *P. debaryxanum*, the data clearly revealed that Rizolex T/thiram was the most toxic compound, followed by Vitavax 200 and Topsin -M. Monceren/thiram gave moderate effect, while Monceren was the lowest toxic fungicide against *P.debaryanum*. Rizolex T/thiram had the strongest activity against all tested pathogens. The tested fungi were also strongly inhibited by Vitavax 200 , Topsin -M and Moceren/thiram. On the other hand, pencycuron was not effective against *S.rolfsii*, *M.phaseolina* and *P.debaryanum*, but showed strong activity against *R.solani*. These results are in full agreement with these of Kataria

and Verma (1990), Smiley et al., (1990), Zein et al., (1990) and Kataria et al., (1991).

They reported that Rizolex T/thiram was inhibitory in vitro against *R.solani*. Henriquez and Montealeger (1992), Rahman et al., (1994) and Rondon et al., (1995) reported that carboxin / thiram had a high antifungal activity against the mycelial growth of *S.rolfsii*. Abdel-Aziz et al., (1996) found that Rizolex T /thiram caused strong inhibition against mycelial growth of *R.solani*, *S.rolfsii* and *M. phaseolina*, while pencycuron was non effective against *M. phaseolina* and *S. rolfsii*, but showed strong activity against *R.solani*.

#### **b- Evaluation of the tested elements:**

Results in Table (2) showed that most of the tested elements had a toxic effect on growth of the tested pathogenic fungi in vitro. Toxicity of such elements to the tested pathogenic fungi increased by increasing the concentrations. Cu gave the most toxic effect on *R.solani* , followed by Fe and Zn . Mn gave the least toxic effect on *R.solani* . Also *S.rolfsii* was highly sensitive to Fe, at the same time it was moderately sensitive to Cu and Mn. Zn was the potent micro-element on *S.rolfsii*. Cu was very toxic against *M. phaseolina*, followed by Fe , while Mn was moderately toxic against the same fungus. Zn gave the lowest toxic effect against *M.phaseolina* . In case of *P. debaryanum*, Cu was the most toxic element , followed by Fe and Zn. Moreover, *P. debaryanum* was the least sensitive fungus to Mn .

Based on  $ic_{50}$  values presented in Table (2) it is quite clear that Cu was the most toxic element against all tested pathogenic fungi, followed by Fe and Zn. Mn was the least toxic one against all tested pathogenic fungi. These findings may be attributed to the effect of such metals in inhibiting fungal enzymes (Byrde et al., 1960); or may be due to the acting of some metals at the cell membrane of fungi causing changes in permeability (Passow et al., 1961); or may be due to the interacting of copper with the genetic apparatus of fungi (Antoine, 1965). These results are agreed with these obtained by Abdel-Moneem(1996). He found that cobalt, Cu, Ni and Zn elements gave toxic effect on *F.oxysporum* f.sp.sesami and *Sclerotium battaticola* (*S.rolfsii*) in vitro. Copper was the most effective one. Savita-pareek and Pareek (1999) mentioned that micro-elements Zn, copper, iron, manganese, magnesium and boron were effective in reducing the charcoal. -rot disease induced by *M.phaseolina*.

**Table (2) : Effects of fungicides and micro-elements on the linear growth of tested pathogenic fungi on PDA medium in vitro ( four days old )**

Fungicides and micro-elements	Pathogenic fungi	I C <sub>50</sub> ppm	Confidence limits		slope
			Lower	Higher	
Vitavax 200	<i>R. solani</i>	0.036	0.015	0.072	0.64
	<i>S. rolfsii</i>	0.0082	0.008	0.03	0.37
	<i>M phaseolina</i>	0.43	0.27	0.67	0.32
	<i>P. debaryanum</i>	0.37	0.23	0.72	4.7
Rizolex-T/thiram	<i>R. solani</i>	0.011	0.004	0.026	0.60
	<i>S. rolfsii</i>	0.076	0.04	0.14	0.7
	<i>M phaseolina</i>	0.0112	0.0009	0.046	0.32
	<i>P. debaryanum</i>	0.18	0.09	0.33	5.6
Topsin -M	<i>R. solani</i>	0.12	0.03	0.32	0.38
	<i>S. rolfsii</i>	1.73	0.99	3.08	0.7
	<i>M phaseolina</i>	0.25	0.001	4.02	0.51
	<i>P. debaryanum</i>	0.45	0.069	2.01	0.23
Monceren	<i>R. solani</i>	0.15	0.017	0.58	0.28
	<i>S. rolfsii</i>	43.34	11.33	451.66	0.31
	<i>M phaseolina</i>	1439.48	146.41	3.09 x10 <sup>5</sup>	0.3
	<i>P. debaryanum</i>	24.06	7.45	154.62	0.33
Monceren/thiram	<i>R. solani</i>	0.12	0.05	0.25	0.54
	<i>S. rolfsii</i>	0.0068	0.009	0.02	0.49
	<i>M phaseolina</i>	1.84	1.16	2.96	0.91
	<i>P. debaryanum</i>	2.39	1.19	5.06	0.52
Cu	<i>R. solani</i>	0.071	0.05	0.1	1.56
	<i>S. rolfsii</i>	0.7	0.49	0.99	1.89
	<i>M phaseolina</i>	0.014	0.0068	0.023	1.08
	<i>P. debaryanum</i>	0.063	0.044	0.089	1.86
Fe	<i>R. solani</i>	0.1	0.07	0.14	1.58
	<i>S. rolfsii</i>	0.22	0.025	3.15	1.3
	<i>M phaseolina</i>	0.040	0.027	0.22	0.96
	<i>P. debaryanum</i>	0.21	0.13	0.29	1.32
Mn	<i>R. solani</i>	0.49	0.3	0.61	1.61
	<i>S. rolfsii</i>	1.11	0.75	1.67	1.27
	<i>M phaseolina</i>	0.44	0.26	0.77	0.79
	<i>P. debaryanum</i>	1.3	0.095	514.6	0.97
Zn	<i>R. solani</i>	0.14	0.093	0.2	1.38
	<i>S. rolfsii</i>	1.22	0.81	1.88	1.2
	<i>M phaseolina</i>	0.37	0.0025	71.56	1.25
	<i>P. debaryanum</i>	0.85	0.64	1.14	2.47

**3- Joint toxic action of the tested compounds against the tested pathogenic fungi under laboratory conditions:**

The fungitoxic effects of the combinations of carboxin/ thiram with both of tested fungicides and micro-elements, were tested according to the results obtained from the previously mentioned tests. According to these results , it is quite evident that carboxin / thiram fungicide was the most fungitoxic mixture against pathogenic fungi . Carboxin / thiram was combined with the other tested compounds. The concentrations used for each compound was the dose required to inhibit mycelial growth of the pathogenic fungi by 25% as compared with control treatment (IC<sub>25</sub>). Data are presented in Table (3)

**Table (3): Percentage inhibition (I%) and Co-Toxicity Factor (C.T.F) of vitavax 200 combined with the tested compounds against pathogenic fungi (IC<sub>25</sub> for each).**

Fungi Treatments	<i>R.solani</i>		<i>S.rolfsii</i>		<i>M.phoseolina</i>		<i>P.debrynum</i>	
	I%	C.T.F	I%	C.T.F	I%	C.T.F	I%	C.T.F
-Rizolex-T / thiram	57.78	+14.06	36.11	-27.78	8.52	-82.96	30.88	-38.24
Topsin -M	18.52	-62.96	41.74	-16.52	55.18	+10.36	53.74	+7.48
Monceren	47.03	-5.94	46.25	-7.5	13.70	-72.60	64.37	+28.74
- Monceren / thiram	41.11	-17.78	45.13	-9.74	22.22	-55.56	43.11	-13.78
-Zn	68.16	+36.22	52.37	+4.74	45.18	-9.64	48.44	-3.12
-Cu	59.55	+19.10	47.98	-4.04	43.70	-12.60	66.35	+32.70
-Mn	60.67	+21.34	50.24	+0.48	47.77	-4.46	42.33	-15.34
-Fe	71.16	+42.32	38.93	-22.14	53.70	+7.40	60.24	+20.48

**a- Joint toxic action against *R.solani*:**

Data presented in Table (3) showed the joint effects of compounds in pairs on the tested fungus. The values of Co-toxicity factor indicated that synergistic effects were obtained in case of adding Vitavax 200 to Zn, Mn and Fe as elements. Additive effects were also observed in case of adding Vitavax 200 to Rizolex-T/ thiram, Monceren and Monceren / thiam as fungicides, as well as Cu as element. Meanwhile antagonistic effects were observed for the combinations of Vitavax 200 + Topsin -M. Many investigators evaluated the joint toxic effects of different compounds against the fungus. Zein (1990), Ahmed and Ali (1990), Mohamed and Abo-Raya (1993b) and Abdel-Aziz et al., (1996).

**b- Joint toxic action against *S.rolfsii*:**

Results in Table (3) showed the joint toxic effects of tested compounds in pairs on the tested fungus *S.rolfsii*. Additive effects were obtained in case of mixing vitavax 200 with Topsin -M, Monceren and Monceren /thiram as fungicides and Zn, Cu and Mn as elements. Vitavax 200 when mixed with Rizolex-T / thiram (fungicide) and Fe (element). Many workers studied the joint toxic effects of different compounds on this fungus( Zein et al., 1990; Mohamed and Abo-Raya ,1993b and Abdel-Aziz et al., 1996).



**c- Joint toxic action against *M. phaseolina***

Data in Table (3) showed that antagonistic effects were observed in case of vitavax 200 added to Rizolex -T/ thiram, Monceren and Monceren / thiram as fungicides, vitavax 200 when mixed with Topsin -M (fungicide), Zn, Cu, Mn and Fe(elements). More studies were carried out to evaluate the joint toxic effect of different compounds against *M. phaseolina*. (Zein et al., 1990; Mohamed and Abo-Raya , 1993b and Abdel-Aziz et al., 1996).

**d- Joint toxic actions against *P. debaryanum***

Data in Table (3) showed the toxic effects of compounds in pairs on the tested fungus. The values of co-toxicity factors indicated that synergistic effects were observed for the combinations of vitavax 200 when mixed with Monceren(fungicide) and Cu and Fe (elements) . On the other hand additive effects were observed in the case of vitavax 200 added to Rizolex and Monceren /thiram (fungicides) and Zn and Mn (elements) . Antagonistic effects were observed for the combinations of Vitavax 200 Rizolexl / thiram ( fungicide ).

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### الملخص العربي

السمية البيولوجية لبعض المبيدات الفطرية والعناصر الصغرى ومخاليطها على بعض فطريات التربة المسببة لمرض عفن الجذور وموت البادرات في فول الصويا

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أجريت هذه الدراسة نظرا لأهمية محصول فول الصويا و التي تتزايد يوما بعد يوم سواء في مصر أو في جميع أنحاء العالم. تتعرض بادرات محصول فول الصويا للأصابة بالعديد من الفطريات الممرضة الكامنة في التربة المصرية والتي من أهمها فطريات الريزوكتونيا سولاني ، سكليروشيم رولفزياي ، ماكروفومينا فاسولينا ، بيتيم ديباريانم والمسببه لمرض عفن الجذور وموت البادرات مما يؤدي لنقص عدد النباتات في الفدان وبالتالي إنخفاض كبير في المحصول . لذا تهدف هذه الدراسة إلى تقدير سمية بعض المبيدات الفطرية والعناصر الصغرى و كذلك تقييم التأثير المشترك لخلائط هذه المعاملات بهدف الحصول على مخاليط لها خواص إبادية قوية على بعض الفطريات المسببة لمرض عفن الجذور وموت البادرات. وقد أجريت الدراسة على أربعة فطريات ممرضة هي : ريزوكتونيا سولاني ، سكليروشيم رولفزياي ، ماكروفومينا فاسولينا ، بيتيم ديباريانم . وفي هذه الدراسة استخدمت خمسة مبيدات معروفة تجاريا وهي ريزولكس - نى / ثيرام ٥٠% ، فيتافاكس ٧٥% ، توبسين - إم ٧٠% ، مونسرين ٢٥% ، مونسرين/ثيرام ٤٧%.. وأربعة عناصر صغرى هي : نحاس ، حديد ، زنك ، منجنيز ، وكلها على صورة كبريتات وقد استخدمت تركيزات مختلفة من كل من المبيدات الفطرية والعناصر الصغرى . وأجريت هذه التجربة في المعمل كما اشتملت الدراسة أيضا على دراسة تأثير الخلط للمبيدات والعناصر الصغرى على الفطريات الممرضة و توصلت الدراسة الى نتائج واضحة أهمها ما يلي:

ثبت من إختبار القدرة المرضية أن الفطر سكليروشيم رولفزياي كان أكثر الفطريات قدرة مرضية لاحداث مرضى موت البادرات وعفن الجذور وتقليل نسبة النباتات الحية لفول الصويا. كما يقلل ارتفاع النبات والاوزان الجافة والرطوبة للمجموعين الجذري والخضري ومحتوى الكلورفيل . المبيد الفطري ريزولكس - نى / ثيرام كان أكثر المبيدات الفطرية سمية للفطرين ريزوكتونيا سولاني ، بيتيم

ديباريانم ، يليه في التأثير المبيد الفطري فيتافاكس ٢٠٠ ، وكان المبيد الفطري ريزولكس - تى / ثيرام أكثر سمية على فطر ماكروفومينا فاسوليننا يليه المبيد الفطري توبسين إم ، بينما المبيدان الفطريان فيتافاكس ٢٠٠ ومونسرين / ثيرام أكثر سمية للفطر سكليروسيم رولفزياي يليهما في التأثير المبيد الفطري ريزولكس - تى / ثيرام .عنصر النحاس كان أكثر العناصر الصغرى سمية للفطريات ريزوكتونيا سولاني ، ماكروفومينا فاسوليننا ، بيثيم ديباريانم يليه في التأثير عنصر الحديد ، بينما كان عنصر الحديد أشد سمية ضد الفطر سكليروسيم رولفزياي يليه عنصر النحاس .عند دراسة التأثير المشترك لمخاليط المبيد الفطري فيتافاكس ٢٠٠ مع المركبات الاخرى نجد أن هناك تأثيرا تنشيطيا في حالة الفطر ريزوكتونيا سولاني عند خلط المبيد الفطري فيتافاكس ٢٠٠ مع كل من العناصر الصغرى الحديد ، المنجنيز ، الزنك .