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EFFICIENCY OF SOME ECHO FRIENDLY MICROELEMENTS IN CONTROLLING SOYBEAN DAMPING-OFF

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ABSTRACT: Soybean plants doubted to be infcted with damping-off were collected from different naturaly infected districts of Dakahlia Governorate. Frequency of the isolated pathogens revealed that *Fusarium solani*, *Fusarium oxysporum*, *Rizoctonia solani*, *Pythium ultimum* and *Macrophomina Phaseolina.* were the most aggressive pathogenic fungi causing the highest percentage of pre-emergence damping-off in Giza 35and Giza 111soybean cultivars. Among the five isolated fungi, only *F. solani*, *R. solani and P. ultimum* were the most pathogenic ones. *F. solani* exhibit the highest percentage of post-emergence damping-off in both cultivars followed by *R. solani*. Moreover,the highest percentage of haelthy survived soybean plants were obtained when *Pythium ultimum*, was investigated. *In vitro* study boron, cobalt and molybdenum proved microelemnts that *Fusarium solani* was the most affected pathogen when evaluated at any of microelements compared with *R. solani and P. ultimumthe*. Cobalt at 5ppm reveal the highest growth reduction percentage against the 3 fungi where it valued 74%. However, the least inhibitory effect was recorded when molybdenum used at 2ppm being 34.77% for the three pathogenic fungi. Whereas, boron at 2 ppm was apparently equal with molybdenum at 5 ppm. *In vivo* experiments of two consecutive growing seasons 2016 and 2017, revealed that molybdenum(5 ppm) proved to be the most effective where the least percentages of both pre and post emergence (5.66) were obtained followed by cobalt (2 ppm) resulting 8%, meanwhile, boron (2 ppm) was the least effective revealed 9% .Plant morphological parameters in treated soybean showed that plant height ranged from 89 cm for boron (5 ppm) and 111.6 cm for molybdenum cultivar 35 (5 ppm) in growing season 2016 and ranged from 96.0 cm for boron (5 ppm) and 118.6 cm for molybdenum (5 ppm) in 2017 season. The highest chlorophyll a and b values were affected by the application of molybdenum followed by cobalt then boron. Carotenoids and total phenols revealed the highest values when Cobalt (2 ppm), was used. Oil and protein percentages in soybean seeds reported the highest values when molybdenum was applied followed with cobalt.

Key words: Soybean, damping-off, microelements, echo friendly, boron, cobalt, molybdenum.

INTRODUCTION

Glycine max L. Merrill, commonly known as soybean, is a species of legume native of East Asia. The cultivation of soybean in Egypt began in 1970 with an area not exceeded 3,000 acres; it developed to 119,000 acres in 1986.

However, in the last ten years it was noticed that the cultivated area decreased due to the increased in production costs and consequently to the decrease in the return per unit area until the cultivated area in 2018/2019 reached 22.2 thousand acres according to the **Records of Egyptian Ministry of Agriculture (2019)**.

Soybean has great importance, due to it's high contain of protein and oil. It is distinguished from other legume types as it contains all the eight amino acids necessary for the human body. Also seeds of soybean contain the most vitamins that are essential for the

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human body. Several countries of the world extract different kinds of foods from soybean as milk, cheese, yogurt, ice cream. In addition, soybean meal is an important constituent of livestock and poultry feeds (**Rouhi *et al.*, 2011**). Also it considered as a friendly crop to the environment where it supply the soil by nitrogen fixation. Thus, it reduces costs and minimizing impact on the environment (**Akande *et al.*, 2007**).

Damping- off and root rot are most serious and wide spread diseases that infect all cultivated fields with soybean plants allover the world, and cause a high losses in soybean yield (quality and quantity).These diseases are known to be caused by many different pathogenic fungi. *Fusarium solani*., *Rhizoctonia solani* and *Pythium ultimum* are the main causal pathogens of pre and post emergence damping- off as well as root rot of soybean plants (**Haikal, 2008**; **Fayzalla *et al.*, 2009**).

The use of fungicides as the most successful strategy to control causes several problems such as environmental pollution, food contamination and reduces populations of beneficial microorganisms in the soil. The above harmful factors empathize the need for new alternative safe methods to control plant diseases (**Jiang *et al.*, 2012**).

The induced plant resistance against infectious diseases may be defined as the active process resistance dependent on the physical and/or chemical barriers of the host plant which activated by biotic or abiotic agent (**Walters *et al.*, 2007**). Some authors used microelements for induction of plant resistance and alleviated the injurious effects of plant diseases *e.g.*, molybdenum and cobalt (**El-Hersh *et al.*, 2011**) and boron (**Abd El-Hai *et al.*, 2007**). Induced resistance is a promising modern approach with broad spectrum in plant disease control which induced in plants by applying chemical elicitors (**Reglinski *et al.*, 2001**). The present investigation was conducted to study the following aspects:

Collections of soybean root rot fungal pathogens from Dakahilia Governorate as a survey investigation for the diseases. Isolation, purification and identification of pathogenic fungi and their pathogenic capabilities on soybean cultivars. Evaluation of yield components and seed quality. Alsoto investigate the vulnerability of soybean to damping-off diseases and how to reduce their pathogens.

The beneficial effects of the promising microelements on damping-off and root rot diseases incidence, plant growth parameters as well as some physiological and enzymatic, activities of soybean plants grown under field conditions, were also determined after two successful growing seasons.

MATERIALS AND METHODS

Sample Collections

Soybean plant samples doubted to be infected with damping-offgrown under natural infection conditions were collected from nine districts of Dakahlia Governorate, Egypt during the summer season 2015 as shown in Table 1.

Isolation, purification and identification of pathogenic fungi and their pathogenic capability

The infected soybean plant samples which collected from all districts were used. The small parts (1 cm approximately) were surface sterilized using 1% sodium hypochlorite for two minutes, then rewashed several times in sterilized distilled water. The surface sterilized pieces were dried between two sterilized filter papers until dryness then plated on plain agar medium in Petri dishes and incubated at 25 – 28°C for five days. The developed fungi were picked up and purified by the hyphal tip or single spore culture techniques **(Booth, 1977; Barnett and Hunter, 1998)**. The pure fungal isolates were microscopically identified according to their cultural, morphological properties, taxonomic criteria **(Sime *et al.*, 2002)**.

Two cultivars of soybean (Giza 35 and Giza 111) were kindly obtained from Legume Crop Research Department, Field Crops Research Institute, ARC, Giza, Egypt. Seeds (both cultivars) were placed on moistened filter paper using sterile distilled water in sterilized petri dishes then incubated at 25°C to ensure the seeds viability through investigation **(ISTA, 1996)**.

Table 1. Frequency of the isolated fungi of soybean plants of different locations in Dakahlia Governorate

Location	*F. solani*		*F. oxy.*		*R. solani*		*P ultmum.*		*M. phas.*		Total isolation from different locations
	No. of isolates	(%)	No. of isolates	(%)	No. of isolates	(%)	No. of isolates	(%)	No. of isolates	(%)	
Temi El-Amded	**7**	33.33	4	19.05	6	28.57	2	9.52	2	9.52	21
Belkas	**4**	25.00	3	18.75	6	37.50	1	6.25	2	12.50	16
Dekernes	**5**	22.73	8	36.36	5	33.73	3	13.64	1	4.55	22
Aga	**2**	11.11	4	22.22	7	38.89	2	11.1	3	16.67	18
El-Mansoura	**3**	16.67	4	22.22	8	44.44	1	5.56	2	11.11	18
Sherbeen	**2**	15.38	3	23.08	5	38.46	2	15.38	1	7.69	13
El-Senblaween	**4**	18.18	7	31.82	7	31.82	3	13.04	1	4.55	22
Bani-Ebeed	**6**	33.33	5	27.78	4	22.24	2	11.11	1	5.56	18
Talkha	**2**	14.29	4	28.57	5	35.71	2	14.29	1	7.14	14
Total	**35**		42		53		18		14		162

Pathogenicity Test

Pathogenicity of the more frequently isolated fungi (*Fusarium solani* "Temi El-Amdeed", *Rhizoctonia solani* "El-Mansoura" and *Pythium ultimum* "El-Senblaween") using both cultivars of soybean plants (Giza 35 and Giza 111) was performed to induce damping-offdisease on soybean roots at the greenhouse of Plant PathologyResearch Institute, ARC.

The inoculation was performed by growing each individual fungus on Sorghum: Coarse sand: Water (2: 1: 2 *V/V*) medium to obtain fungal mass production. The media contents were thoroughly mixed using glass rod, then bottled and autoclaved. The autoclaved media were inoculated with 5cm agar discs from the periphery of 5 days old colony of the previously isolated fungal genera, then incubated at 28°C.

Plastic bags (25 × 40 cm) filled with autoclaved clay soil were artificially infested singlethe previously prepared fungal inoculum at the rate of 3% *W/W*.

The infested plastic bags and uninfested (as a control) were irrigated and left for 7 days before sowing to insure even the distribution of the inoculated fungi. Three bags were used as replicates and three replicates were used for each particular treatment. Healthy disinfected soybean seeds of both cultivars were sown at the rate of 10 seeds/bag. Data of disease development (percentage of pre-and post-emergence damping-off) as well as healthy survived plants were determined through calculating the percentage of both damping-off and healthy survival after 15, 30 and 45 days of sowing using the following formulas:

Pre-emergence damping-off = No. of non-germinated seeds/ total sown seeds x100 after 15 days.

Post-emergence damping-off = No. of non-germinated seeds/ total sown seeds x100 after 30 days

Healthy survived plants= No. of survived plants/ total No, of sown seeds x100 after 45 days.

Re-isolation has been carried out from the infected plant roots as Koch's postulates, *F. solani* (No. 2), *R. solani* (No. 3), and *P. ultimum* (No. 1) isolates.

These isolated were selected from infected roots for further studies as causal pathogens for soybean damping off causel pathogens.

In vitro effects of different microelements on the pathogenic fungal genera growth

Three different microelements *i.e.* Boron (B) in forms of boric acid (H_3BO_3), Molybdenum (Mo) in form of ammonium molybdate $(NH_4)6$ Mo_7O_{24} and Cobalt (Co) in form of cobalt sulphate (Co SO_4) were evaluated for their capability to suppress fungal growth under Lab. conditions. The microelements were prepared as solutions and dissolved in PDA medium before autoclaving and solidification to obtain the proposed concentrations of 2 and 5 ppm then teemed in sterilized Petri dishes.

Rizolex-T50 as a fungicide was prepared as 3 g/litter, waterthen added to the autoclaved PDA medium before solidification. Three replicates were used for each particular treatment.

After solidification, Petri dishes were inoculated with each of pathogenic fungal discs 5 mm in diameter. All Petri dishes were incubated at 25°C until control treatment completely filled with the fungal growth. Reduction percent was calculated at the end of the experiment.

In vivo effects of different microelements on soybean damping off

Two field experiments were conducted in known naturally heavily infested soybean cultivated areas with the causal soil borne fungal pathogens at Tag El-Ezz Research Station, Dakhlia Governorate, Egypt during the two growing seasons 2016 and 2017.

Soybean seeds were soaked for 15 minutes in the different microelements concentrates then air dried before sowing. In case of control treatment soybean seeds were dressed with Rizolex-T50 at 3g/k. Seeds were sown on 15^{th} May of both seasons. Two seeds per hill were sown at distances of 15 cm. Seeds of the two soybean cultivars were inoculated with *Rhizobium japonicum* before sowing. A split plot design with three replicates was used in the field experiments. The main plots were occupied by cultivars and sub-plots were occupied by different microelement treatments. The experimental plot comprise 5 ridges with 60 cm width and 3.5 m length, occupying an area of 10.5 m^2 (1/400 fad.). The plant received the normal amount of fertilizers and the normal cultural practices for growing soybean as recommended by the Egyptian Ministry of Agriculture and Land Reclamation. Microelements were also foliar sprayed at 30 and 45 days from sowing as active doses.

Damping-off and Disease Assessment

Percentages of germination and pre-emergence damping-off were recorded after 15 days from sowing in both seasons for both cultivars. However, the percentage of post-emergence damping-off was recorded at 45 days from sowing. At the end of the experiment healthy survived plants, were calculated.

At the start of maturity stage (70-90 days), the plants of both soybean cultivars were rated for both disease incidence and severity. The growth parameters of developing soybean plants were measured after collecting samples from all treatments at 70 days from sowing (plant height (cm) and numbers of branches /plant).

Effect of different treatments on some plant growth parameters

The growth parameters of developing soybean plants were measured after collecting samples from all treatments at 70 days from sowing. Collected samples were transferred to laboratory in paper bags to record plant height (cm) and number of branches/plant. Seed germination per cent was determined before sowing.

Biochemical Changes as Affected by Different Treatments

Photosynthetic pigment contents

The photosynthetic pigments (chlorophylls a, b and carotenoids) were extracted and determined spectrophotmetrically according to **Mackinney (1971**). A known fresh weight (0.05 g) of plant leaflet were immersed in 10 ml methanol 90% for 24 hr., and stored closed in a cold dark place after adding a trace of sodium carbonate Na_2CO_3 for reducing acidification **(Robinson *et al.*, 2000**).

Total phenolic compounds

Total phenols were determined at 60 days from sowing in fresh shoot using the Folin-

Ciocalteau reagent according to **Malik and Singh (1980)**. Two grams' fresh weight sample were homogenized in aqueous ethanol 80% at room temperature and centrifuged at 10000 rpm under cooling for 15 min and supernatant, was saved. Re-extraction has been curried out of the residue twice using aqueous 80% and the supernatant were pooled and evaporated to dryness using evaporating dishes at room temperature. The residue was dissolved in a volume of 5 ml distilled water. One hundred microliters (0.1 ml) of this extract was taken and diluted to 3ml with distilled water then 0.5ml Folin-Ciocalteau reagent was added and left for three minutes. Two ml of sodium carbonate 20% concentrate was added then the contents were thoroughly mixed. The developed color was measured photometrically at 650 nm after 60 minutes using catechol as a standard. The results are calculated as mg catechol/100 g fresh weight.

Seed quality

After harvest (110-120day), the percentages of oil, protein and phosphorus of soybean seed were calculated in both cultivars. Seeds were dried at 70^{o}C for 48 hours; finally ground and three replicates were taken to estimate their oil percentage using Soxhlet apparatus according to **AOAC (1970)**. The product of oil was dried at 105^{o}C for 30 minutes and re-weighted. The oil% was calculated using the following formula:

Oil (%) = sample weight before extraction – sample weight after extraction/sample weight before extraction x 100.

Crude protein content was determined by the official Kjeldahl method described in **AOAC (2016)**.

Total phosphorus was determined spectrophotometerically in the samples as described by **Snell and Snell (1967)**.

Statistical Analysis

The obtained data was statistically analyzed according to the technique of analysis of variance (ANOVA) for completely randomized design (laboratory experiments) and split plot design for field experiments as published by **Gomez and Gomez (1984)** by means of MSTAT-C computer software package. Least significant of difference (LSD) method was used to test the differences between treatment means at 5% level of probability as described by **Snedecor and Cochran (1982)**.

RESULTS AND DISCUSSIONS

Sample Collection, Isolation and Identification of Pathogenic Fungi

Soybean samples exhibited typical root rot symptoms were collected from different naturally infected districts of Dakahlia Governorate (Table 2). One hundred sixty two isolates representing five species were isolated and identified in Dept. Plant Pathol. Fac. Agric., Zagazig Univ., Egypt.

Damping off is the most common symptoms where the diseased soybean seedling and plants showing typical symptoms. *Rhizoctonia solani*, *Fusarium oxysporium, Fusarium solani*, *Pythium ultimum*, and *Macrophomina phasolina* were the most frequent isolated fungi that caused the highest percentages of damping off (Table 2).

Results in Table 1 reveal that the high frequent isolated fungi were found in Dekernes district and El-Senblaween. *F. solani* was high frequently isolated fromTemi El-Amdeed and Bani-Ebeed, while the high frequency of *F.oxysporum* was found in Dekernes while, *Pythium ultimum* was highly frequented in Dekernes and El-Senblaween. *R. solani* was the most frequent in El-Mansoura while *M. phaseolina* was most isolated from Aga.

Difference in the percentage incidence of fungal species among districts may be due to the variation of environmental conditions and soil type which affect the interaction between host and the pathogen.

The identified isolates of the different fungal genera were investigated for their pathogenic potentiality to induce damping-off (pre and post) emergence damping off on two soybean cultivar plants (Giza 35 and Giza 111) grown under greenhouse conditions. The results indicated that *Fusarium solani* (No. 2), *Rhizoctonia solani* (No. 3) and *Pythium ultimum* (No. 1) were found to be the most aggressive root rot fungal pathogens based on healthy survived plants of both soybean cultivars. However, the remained isolated fungal genera were neglected due to the least pathogenic capability. These results are in accordance with those recorded by **Rauf (2000) and Wang *et al*. (2008)**.

Table 2. Pathogenic capability of the fungal pathogens to induce damping-off in soybean cultivars

Soybean cultivars				
Giza 35		20.83b	17.33b	62a
Giza 111		25.41a	22.58a	52b
LSD		**4.39**	**1.27**	**4.57**
Treatments				
Control		0	0	100
F. solani (No.2)		23.16b	31.33a	45.83a
R. solani (No.3)		41.83a	28.33b	29.83b
Pythium ultimum (No.1)		27.5b	20.16c	52.33a
LSD		**10.17**	**2.56**	**9.13**
Interaction				
Giza 35	Control	0	0	100
	F. solani (No.2)	21.33d	27.33c	52.00ab
	R. solani (No.3)	36.33b	24.00d	39.66c
	P. ultimum (No.1).	25.66cd	18.00f	56.33a
Giza 111	Control	0	0	100
	F. solani (No.2)	25.00cd	35.33a	39.66c
	R. solani (No.3)	47.33a	32.66b	20.00e
	P. ultimum (No.1)	29.33bc	22.33e	48.33b
LSD		**7.19**	**1.86**	**6.45**

Giza 35 was more tolerant to the infection compared with Giza 111 that might be due to the genetic variations between both cultivars. The injurious of the tested pathogenic fungi on germination and growth might be due to seed rot that consequently increase pre-emergence damping-off and also the damage of root system which reduce the absorption surface for essential nutrients and water uptake (**Porter *et al*., 1990)**. In this connection, **Mahmoud *et al*. (2013)** recorded that the harmful effects might be due to enzymes production which causes rotten lesion on seed cotyledons, in turn, seed rot and plumule soft rot. These results are in agreement with those obtained by **Abd El-Hai *et al* (2016)**.

All tested fungal genera have the potentiality to attack both soybean cultivars causing damping-off. Healthy survived plants of both cultivars proved that Giza 35 was more resistant to the infection comparing with Giza 111.

Such, results of pathogenicity implies that *Rizoctonia solani* (isolate No. 3) *Fusarium solani* (isolate No.2) and *Pythium ultimum* (isolate No.1) were found to be aggressive fungal genera basing on the healthy survived plants being 52.33%, 45.83% and 29.83%, respectively. Thus, *R. solani* (isolate No. 3), *F. solani* (isolate No.2) and *P. ultimum* (isolate No.1) were selected for further studies. Thus, *Fusarium oxysporum* and *Macrophomina phaseolina*, were excluded.

Interaction values reveal that *R. solani* was the most aggressive as it indicate the least survived plants for Giza 35 and Giza 111 cultivars being 39.66% and 20.00% followed by *F. solani* 52.00% and 39.66%, respectively.

However, *P. ultimum* reveals the highest percentage of healthy survived plants being 56.33% and 48.33%, respectively. It could be concluded that soybean seedlings which characterized to be susceptible to the fungal genera are invaded much more than the resistance ones.

In vitro Effects of Different Microelements on Soybean Damping-Off

The inhibitory effects of different abiotic agents (microelements boron, molybdenum, and cobalt) were evaluated *in vitro* against the three pathogenic fungal genera compared with the fungicide (Rizolex-T50)

Results presented in Table 3 indicate that all the tested abiotic agents significantly increased the reduction percent of the three fungal genera with increasing their concentrations. *Rhizoctonia solani* proved to be the most affected compared with the *Pythium* and *F. solani* being 4.9, 4.77 and 3.54, respectively when evaluated with any of the three examined microelements. *Fusarium solani* implies the least reduction value 3.54 % while *R.solani* exhibits the highest value. The different microelements were mostly statistically significant. Cobalt at a concentration of 5ppm reveals the highest reduction percent of the growth of the three different pathogenic fungi where it recorded 74%. However, the least inhibitory effect was recorded when molybdenum at 2 ppm was investigated where it implies reduction percent 34.78% for the three fungal genera *F. solani, R. solani and P. ultimum.* However, Boron at its applied concentrations indicates moderate values while at its concentration 2 ppm was apparently equal with molybdenum at 5 ppm.

However, cobalt 5 ppm proved to have the most toxic effect on the mycelial growth of the three fungal genera followed by boron 5 ppm, meanwhile, molybdenum was the least effective one. These results are in harmony with those obtained by **El-Hersh *et al*. (2011) and Ali *et al*. (2014)** they mentioned that mycelial growth of *F. Solani, R. Solani and P. Ultimum* strongly inhibited by some minerals especially those of boron, cobalt, and molybdenum. Thus, some of the investigated microelements applied as abiotic agents might be act as antimicrobial agents and have been widely used to minimize the activity of the pathogenic fungal genera even for soybean plants **(Abd El-Kareem *et al*., 2004 a and b; El-Gamal *et al.*, 2007; Abd El-Hai *et al.*, 2009)**.

Results obtained from this study also showed that, all tested treatments of microelements, at any concentration used as well as the fungicide (Rizolex-T50) has antifungal activity in laboratory experiments, hence inhibited the fungal linear growth of the three tested pathogenic fungi.

In vivo Effects of Different Microelements on Soybean Damping-Off

The effect of microelements as (boron, molybdenum, and cobalt) as well as fungicide Rizolex-T50 on soybean pre and post-emergence damping-off under natural infection field conditions were investigated. Results in Table 4 show that, Giza 111 cultivar was more susceptible for damping-off symptoms than Giza 35 one.

Regarding the effects of microelements, results indicated that soaking soybean seeds in molybdenum (5 ppm) led to the least pre-emergence in season 2016 being 5.66% followed by cobalt (2ppm) 8.00% then boron at (2 ppm) 9.00%. Pre and post-emergence damping-off reveal the same trend in 2017 season. Thus application of molybdenum at high level decreased pre and post-emergence damping-off compared with the other microelements and significantly differed with the control.

Under natural infection conditions all treatments decreased damping-off disease and enhanced percentage of survived plants, morphological parameters, physiological activities, mineral constituents as well as improved the productivity and seed quality. The application of fungicide Rizolex-T50 as seed treatment is required in the germination stage to help and/or assure an adequate plant stand in the soil for protecting the seeds from seed borne pathogens.

Several microelements suppressed the growth of numerous phytopathogenic fungal genera that attack soybean plants roots. The present study indicate that molybdenum proved to be the most toxic for the investigated three fungal growth followed by boron, meanwhile cobalt was the least effective one. These results

Table 3. Effect of abiotic microelements and the fungicide (Rizolex T-50) on mycelial growth reduction percent of the tested fungal pathogens

Microelement	Mycelial growth (cm)				
	F. solani	*R. solani*	*P. ultimum*	Average	Inhibition (%)
B 2 ppm	4.33b	4.93b	5.73b	4.88b	45.78
B 5 ppm	2.90d	3.33d	4.00e	3.41e	62.11
Mo 2 ppm	5.20a	5.83a	6.60a	5.87a	34.78
Mo 5 ppm	3.46c	4.03c	4.43d	4.15d	53.89
Co 2 ppm	3.43c	4.03c	4.96c	4.14d	54.00
Co 5 ppm	1.90e	2.20e	2.93f	2.34f	74.00
Average	3.54c	4.90b	4.77cd	4.37c	
Rizolex fungicide	0	0	0	0	100
Control	9	9	9	9	9
LSD	**0.28**	**0.29**	**0.34**	**0.17**	

Table 4. *In vivo* effect of abiotic microelements and the fungicide (Rizolex T-50) on damping-off and disease incidence

		Pre-emergence (%)		Average	Post–emergence (%)		Average	Healthy plant (%)		Average
		2016	2017		2016	2017		2016`	2017	
Giza 35	**B 2 ppm**	9.00	13.33ef	11.16g	5.33bcd	8.00de	6.66g	85.67d	78.67e	82.17de
	B 5 ppm	14.00bc	13.33ef	13.66ef	5.66bcd	8.66d	7.16fg	80.34e	78.01e	79.17f
	Mo 2 ppm	11.66cd	11.00fg	11.33g	5.66bc	8.33de	6.99g	82.68e	80.67de	81.67e
	Mo 5 ppm	5.66ef	7.66h	6.66i	4.33cde	6.66def	5.49h	90.01b	85.68c	87.84b
	Co 2 ppm	8.00e	10.66g	9.33h	6.33bc	10.00c	8.16d	85.67d	79.34de	82.50de
	Co 5 ppm	16.33ab	19.00b	17.66c	7.00b	10.66c	8.83cd	76.67fg	70.34g	73.50h
	Rizolex	3.66f	2.66i	3.16l	2.66ef	6.00ef	4.33i	93.68a	91.34a	92.51a
	Control	12.00c	16.00cd	14de	10.00a	13.33ab	11.66b	78.00f	70.67g	74.33h
Giza 111	**B 2 ppm**	12.66c	15.33de	13.99de	4.00cdef	11.00c	7.50ef	83.34e	73.67f	78.50fg
	B 5 ppm	17.33ab	17.66bc	17.49c	4.66cde	12.00bc	8.33d	78.01f	70.34g	74.17h
	Mo 2 ppm	14.00bc	16.00cd	15d	4.00cdef	11.33bc	7.66e	82.00e	72.67f	77.33g
	Mo 5 ppm	8.66d	10.00g	9.33h	3.66def	10.00c	6.83g	87.68c	80.00de	83.84d
	Co 2 ppm	11.33cd	13.33ef	12.33fg	5.00bc	10.00c	7.50ef	83.67e	86.67c	85.17c
	Co 5 ppm	19.00a	22.00a	20.50a	6.00bc	12.00bc	9.00c	75.00g	66.00h	70.50i
	Rizolex	5.33ef	5.00h	5.16i	2.00f	5.66f	3.83l	92.67a	89.34b	91.05a
	Control	18.00a	19.66b	18.83b	11.33a	14.33a	12.83a	70.67h	66.01h	68.34l
LSD		**3.08**	**2.2**	**1.35**	**2.24**	**2.2**	**0.48**	**1.98**	**1.18**	**1.49**

are in harmony with those obtained by **Mahmoud *et al*. (2009) and El-Hersh *et al*. (2011)** who mentioned that cobalt can activate new proteins, chitinase and other pathogenesis related proteins as well asmany other plant defense enzymes. Thus, some of the investigated microelements might consider as barriers against plant pathogen invasion and have been widely used to minimize the activity of the pathogenic fungal genera. **Hahlborck and Scheel (1989**) reported similar explanation concerning boron to be as essential microelement against plant pathogen invasion.

Molybdenum decreased damping-off and root rot when examined under field conditions due to its role in increasing the activity of cytokinins level in turn enhancing total phenols, calcium content and the activation of catechol oxidase; all of these factors lead to the protecting of plants against pathogen infection **(Chowdhury, 2003)** act as inducers **(Cardoso *et al*., 2005)** increased plant growth, photosynthetic pigments and mineral contents **(Datta *et al*., 2011)**.

Also, the enhancement of growth and productivity of soybean by the application of molybdenum might be due to the stimulatory effects on Rhizobium nodule numbers and nitrogenase activity required for organisms utilizing atmospheric nitrogen. In addition to molybdenum it believed to serve as an activator of enzymes involved in nitrogen fixation (**Fageria *et al*., 2003; Siddiky *et al*., 2007)**.

Ahmad *et al*. (2002) stated that the importance of molybdenum and cobalt may be due to their roles as co-factors for some enzymes that play a vital role in protection of plants against pathogen infection through participating in the plant metabolism and nitrogen fixation process.

The effects of microelements, as well as fungicide Rizolex-T50 and their interactions with soybean cultivars on average of plant height and number of plant branches during two successive growing seasons are presented in Table 5. Plant height significantly increased in Giza 111 soybean cultivar compared with Giza 35 in both seasons. While, there is no significant differences between both cultivars in branch number/plant.

The highest average of plant height pointed out 127.60 cm, for molybdenum 5ppm as a microelement for soybean cultivar Giza 111 followed by cobalt 2 ppm being 124.10 cm.

Application of ammonium molybdate concentration of 2ppm significantly increased number of branches/plant of both soybean cultivars 35 and 111compared with the other treatments with an average of 7.67 branches and 8.17 ones for both seasons, respectively.

Biochemical Changes of Soybean Plants as Affected by Different Microelements

Chlorophyll content

The effects of microelements and Rizolex-T50 on average concentration of chlorophyll (a and b) of both soybean cultivars throughout the experimental period during the two growing seasons are presented in Table 6 and Fig. 1.

Results showed that, soybean cultivar Giza 35 indicates higher concentrations of chlorophylls a and b compared to Giza 111 cultivar in both seasons. Results indicate that microelements increased significantly both photosynthetic chlorophyll pigments compared with the control. In general, the maximum values of chlorophyll a and b were obtained due to the application of molybdenum followed by cobalt 2 ppm. However, Rizolex-T50 had no significant effect on photosynthetic pigment parameters compared with untreated control. The obtained results were in the same trend for both soybean cultivars. Average of chlorophyll a and b denote 1.577mg FW and 0.920 mg FW for both seasons compared with control. The same trend was observed with the interaction between treatments and cultivars where the highest value of cholorphyll a and b was showen by the application of molybdenum followed by cobolt then boron. It is worthy to mention that, in this respect the low level of microelements was also effective for both cultivars in both seasons.

Molybdenum and cobalt might act as inducers of the antioxidants enzymes such as glutathione reductive which induced under metal toxicity as well as protect plants from pathogen infection **(Cardoso *et al*., 2005)**. Moreover, **Datta *et al*. (2011)** stated that molybdenum increased plant growth, photosynthetic pigments and mineral contents and ultimately, support plant health. Increasing in photosynthetic pigments

Table 5. Effect of abiotic microelements and the fungicide (Rizolex T-50) on soybean plant height and number of branches as affected by natural infection

		Plant height (cm)		Average	Number of branches/plant		Average
		2016	2017		2016	2017	
Giza 35	**B 2 ppm**	105.0de	114.6de	109.80d	7.66cd	6.66a	7.18d
	B 5 ppm	89.0g	96.0h	92.50f	6.33efg	4.66cd	5.49fg
	Mo 2 ppm	96.3f	106.0g	101.15e	8.00bc	7.33a	7.67d
	Mo 5 ppm	111.6c	118.6c	115.10c	7.00de	5.00bc	6.00e
	Co 2 ppm	108.3cd	116.6cd	112.45cd	7.66cd	7.00a	7.33d
	Co 5 ppm	94.0f	103.6g	98.80e	6.33efg	5.00bc	5.66efg
	Rizolex	85.6g	76.3l	80.90h	6.66ef	5.00bc	5.83ef
	Control	85.0g	76.6l	80.80h	6.00fg	4.33d	5.16gh
Giza 111	**B 2 ppm**	122.3b	122.0b	122.11b	8.33bc	7.00a	7.66cd
	B 5 ppm	103.3e	102.0g	102.65	5.66gh	4.66cd	5.16a
	Mo 2 ppm	113.3c	111.6ef	112.40cd	9.00a	7.33a	8.17b
	Mo 5 ppm	129.3a	126.0a	127.60a	6.00f	5.66b	5.83e
	Co 2 ppm	123.6b	124.6ab	124.10b	8.66b	7.33a	8.00c
	Co 5 ppm	111.3c	109.3f	110.30d	6.00fg	5.66b	5.83ef
	Rizolex	94.6f	85i	89.8g	5.00hi	4.66cd	4.83h
	Control	95.3f	84.6i	89.95g	4.33i	4.00d	4.16i
	LSD	**4.07**	**3.86**	**2.88**	**0.72**	**0.76**	**0.48**

Table 6. Effect of microelements and Rizolex-T50 on concentration of chlorophyll (a and b) of soybean plants as affected by natural infection

		Chlorophyll a (mg g^{-1} FW)		Average	Chlorophyll b (mg g^{-1} FW)		Average
		2016	2017		2016	2017	
Giza 35	**B 2 ppm**	1.533bcd	1.487b	1.510c	0.943ab	0.803de	0.873bcd
	B 5 ppm	1.457ef	1.393d	1.425e	0.920b	0.750	0.835de
	Mo 2 ppm	1.617a	1.537a	1.577a	0.977a	0.863a	0.920a
	Mo 5 ppm	1.553c	1.500a	1.577a	0.953ab	0.817cd	0.885ab
	Co 2 ppm	1.583ab	1.530a	1.557ab	0.970a	0.833bc	0.902ab
	Co 5 ppm	1.493de	1.467bc	1.480d	0.953ab	0.807cde	0.880abc
	Rizolex	1.240g	1.043e	1.142g	0.700e	0.683i	0.692g
	Control	1.223g	1.047e	1.135g	0.697e	0.647l	0.672g
Giza 111	**B 2 ppm**	1.443f	1.453bc	1.448de	0.857cd	0.797ef	0.827e
	B 5 ppm	1.400f	1.357d	1.379f	0.830d	0.723h	0.777f
	Mo 2 ppm	1.570bc	1.493b	1.532bc	0.927b	0.847ab	0.887ab
	Mo 5 ppm	1.483de	1.457bc	1.470	0.853cd	0.773fg	0.813ef
	Co 2 ppm	1.533cd	1.473bc	1.503c	0.877c	0.810cde	0.844cde
	Co 5 ppm	1.440f	1.443c	1.442e	0.837cd	0.743gh	0.790f
	Rizolex	1.180h	0.933f	1.057h	0.647ef	0.607m	0.627h
	Control	1.173h	0.940f	1.057h	0.640e	0.567n	0.604h
	LSD	**0.045**	**0.04**	**0.034**	**0.042**	**0.026**	**0.04**

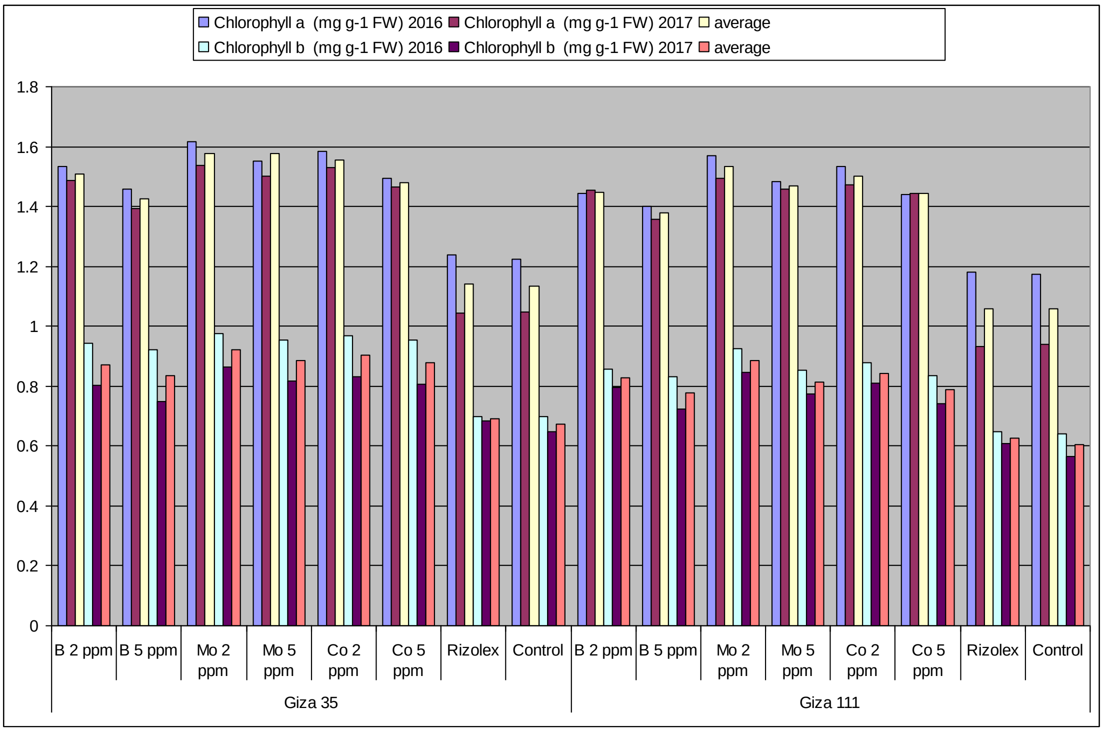

Fig. 1. The effects of microelements and Rizolex-T50 on concentration of chlorophyll a and b of natural infected soybean plants

will lead to increase carbohydrate content that the main repository of photosynthetic energy, they comprise structural polysaccharides of cell walls and principally cellulose, hemicellulose and pectin which consider the first barrier against pathogen invasion.

Carotenoids and Total Phenol Contents

Effects of microelements (boron, molybdenum, cobalt (Table 7 and Fig. 2) indicated the efficacy of the tested microelements on carotenoids of soybean cultivar Giza 35 where cobalt as a microelement was superior in increasing significantly the pigment in both examined seasons compared with seeds even treated with Rizolex-T50 and/or the control one **(Harman, 2006; Reino *et al*., 2008; Abd-El-Khair and El-Gamal 2011)**.

Concerning soybean leaflet cultivar Giza 111 it exhibits similar trend of soybean cultivar Giza 35 where the low concentration (2 ppm) of cobalt pointed out the highest value of leaflet carotenoids (0.367 mg/gFW and 0.427 mg/gFW) for both seasons, respectively.

Results pointed to the efficacy of the different tested stimulants on induction of the total phenols. It is evident that all treatments of both cultivars in both investigated seasons increased significantly total phenols compared with the control one. Cobalt as a microelement at its low concentration (2 ppm) gave the highest values of total phenol contents of soybean leaflet cultivar Giza 35 for both examined seasons 2016 and 2017. Similar trend was noticed concerning those of soybean cultivar Giza 111 for both tested seasons but with significant lower values.

Application of the fungicide Rizolex-T50 was less effective where total phenols value of soybean cultivar 35 were 111 mg/g^{-1}FW and 103.3 mg/g^{-1} FW for both seasons, respectively. However, cultivar G111 pointed out 104.0 mg/g^{-1} FW and 96.0 mg/g^{-1} FW for both seasons, respectively.

Table 7. Effects of microelements and Rizolex-T50 on concentration of carotenoids and total phenols of natural infected soybean plants

		Carotenoids ($\mu g\ g^{-1}$ FW)		Average	Total phenols ($\mu g\ g^{-1}$ FW)		Average
		2016	2017		2016	2017	
Giza 35	**B 2 ppm**	0.327def	0.423b	0.375d	130.6b	133.3c	131.9c
	B5 ppm	0.313ef	0.383d	0.348e	126.3cd	124.6e	125.5ef
	Mo 2 ppm	0.353bc	0.460a	0.407b	132.3ab	136.3b	134.3b
	Mo 5 ppm	0.333cde	0.427b	0.380d	128.0bc	125.6e	126.8de
	Co 2 ppm	0.383a	0.463a	0.513a	136.3a	142.6a	139.5a
	Co 5 ppm	0.373ab	0.457b	0.415b	130.3b	129.0	129.7cd
	Rizolex	0.273g	0.337f	0.305g	111.0h	103.3h	107.2i
	Control	0.207h	0.307g	0.257	104.0i	96.6i	100.3l
Giza 111	**B 2 ppm**	0.310f	0.357e	0.334f	122.6ef	124.6e	123.6fg
	B 5 ppm	0.273g	0.330f	0.302g	118.3b	117.6g	118.0h
	Mo 2 ppm	0.333cd	0.417b	0.375d	124.3de	128.3d	126.3de
	Mo 5 ppm	0.287g	0.367de	0.327f	120.6fb	119.0fg	119.8h
	Co 2 ppm	0.367ab	0.427b	0.397c	127.3cd	135.6bc	131.5c
	Co 5 ppm	0.313ef	0.403c	0.358e	122.3ef	121.0f	121.7g
	Rizolex	0.220h	0.253h	0.237h	104.0i	96.0i	100.0l
	Control	0.177i	0.233i	0.205h	98.30l	85.6l	92.0m
	LSD	**0.02**	**0.018**	**0.011**	**4.60**	**2.49**	**2.68**

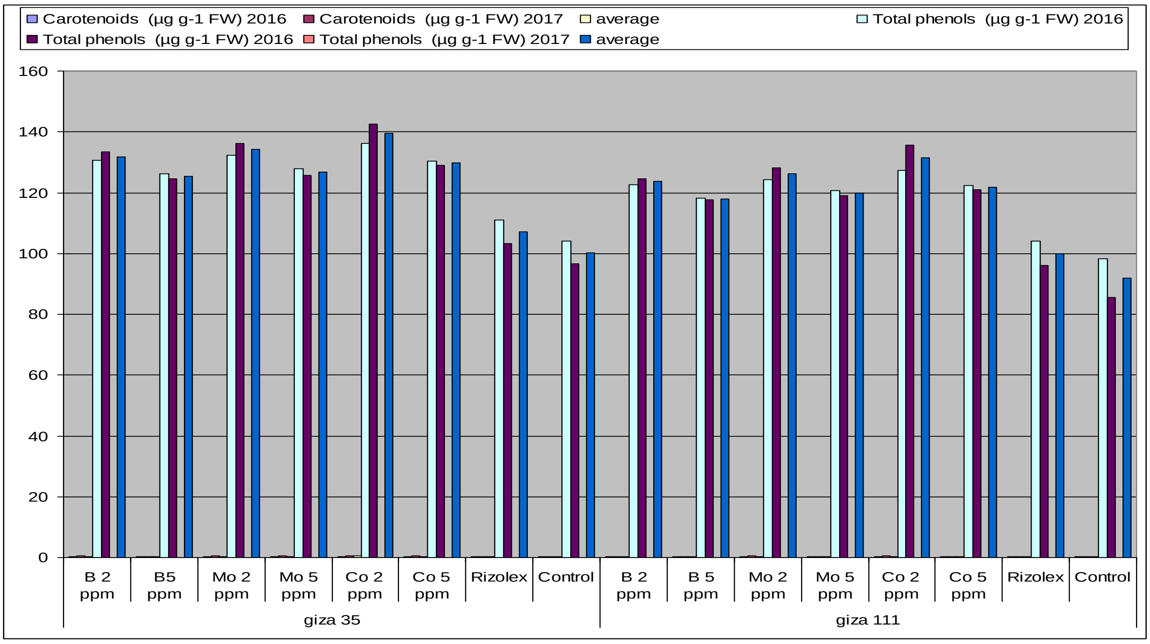

Fig. 2. Effects of microelements and Rizolex-T50 on concentration of carotenoids and total phenols of natural infected soybean plants

Seed Quality

The effect of microelements and fungicide Rizolex-T50 on the percentages of oil, protein and phosphorus of soybean seed cultivars are presented in Table 8 and Fig. 3. It is clear that soybean cultivar Giza 111 display the better seed quality than Giza 35 in both seasons. Moreover, the highest percentages of seed phosphorus occurred under the application of molybdenum.

Oil percent in soybean seeds (cultivars Giza 35) was found to be affected by molybdenum 2ppm with maximum values being 24.25 and 22.75% in both seasons, respectively, oil (%) being 25.00 and 23.91% for both seasons of soybean cultivar G111. Cobalt at 2 ppm followed by molybdenum in this respect.

Concerning seed protein percent, it indicates the same general trend of seed oil for all the investigated molybdenum concentrations as a microelement where both concentrations 2 ppm and 5 ppm revealed the same results with significant differences in both seasons of both soybean cultivars Giza 35 and Giza 111.

The highest percentages of seed phosphorous occurred when molybdenum was applied for both soybean cultivars in both seasons. In this respect, the higher levels of the previously mentioned agent were more effective rather than the lower ones.

Table 8. Effects of microelements and Rizolex-T50 on soybean seed quality plants as affected by natural infection

		Seed oil (%)		Average	Seed protein (%)		Average	Seed phosphorus (%)		Average
		2016	2017		2016	2017		2016	2017	
Giza 35	B 2 ppm	23.22b	22.06cd	22.64	42.14d	38.11	40.13e	0.303f	0.437	0.370g
	B 5 ppm	22.84c	21.42d	22.13	42.99d	38.93	40.96e	0.320e	0.52	0.420e
	Mo 2 ppm	24.25ab	22.75bc	23.5	44.07bc	39.33	41.7d	0.353d	0.537	0.445d
	Mo 5 ppm	23.03c	21.93d	22.48	44.24ab	41.2	42.72c	0.37c	0.573	0.472c
	Co 2 ppm	24.09ab	22.46bc	23.28	41.89d	37.91	39.9	0.25g	0.297	0.274m
	Co 5 ppm	22.98c	21.83d e	22.41	40.73e	37.64	39.19f	0.27g	0.333	0.302l
	Rizolex	19.98e	19.12f	19.95	39.43e	37.23	38.33f	0.313e	0.497	0.405f
	Control	20.3de	19.13f	19.72	40.24e	37.44	38.84f	0.22h	0.277	0.249n
Giza 111	B 2 ppm	24.07ab	23.15abc	23.61	43.16c	41.46	42.31c	0.37c	0.497	0.354h
	B 5 ppm	23.08c	22.35abcd	23.08	44.0 bc	42.18	43.07b	0.39b	0.573	0.482c
	Mo 2 ppm	25.0a	23.91a	24.46	45.0a b	43.1	44.05a	0.40ab	0.59	0.495b
	Mo 5 ppm	23.93b	22.95a	23.44	45.2a	44.21	44.71a	0.42a	0.623	0.522a
	Co 2 ppm	24.22ab	23.62ab	23.92	43.21c	41.26	42.24c	0.3f	0.353	0.327i
	Co 5 ppm	23.53b	22.81abc	23.17	42.39d	41.06	41.73d	0.33e	0.40	0.365gh
	Rizolex	21.31d	20.69ef	21	40.17e	30.28	35.23g	0.353d	0.537	0.445d
	Control	21.77d	20.69ef	21.23	42.11d	40.63	41.37d	0.303f	0.337	0.32i
	LSD	**1.0**	**1.16**	**0.72**	**1.08**	**1.65**	**0.86**	**0.017**	**0.01**	**0.01**

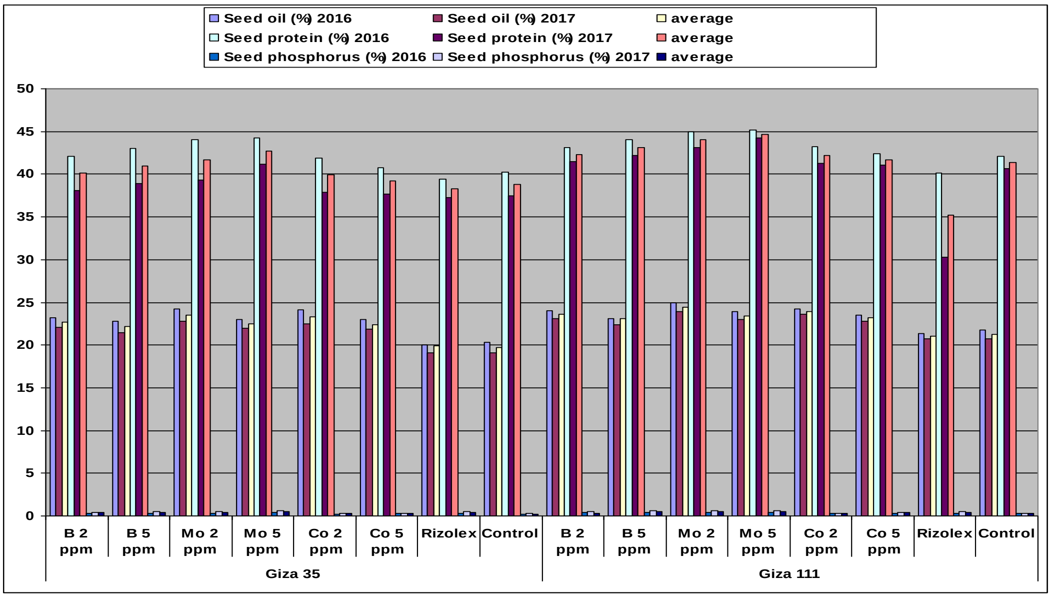

Fig. 3. Effects of microelements and Rizolex-T50 on soybean seed quality plants as affected by natural infection

The role of microelements in inducing the plant resistance might be due to the activation of new proteins and several enzymes that may have a role in disease resistance **(Zaky *et al*., 2002; Mahmoud *et al*., 2009)**. Moreover, cobalt is known to promote many processes of plant growth including leaf expansion as well as the elongation of stem and root (**Atta-Aly *et al*., 1991**). Also, cobalt is a metal component of enzyme B12 which is required for leghemoglobin formation: and also, other important reactions in nitrogen fixation dependent on either vitamin B12 or the coenzyme form **(Marschner, 1986)**.

RizolexT50 as a fungicide resulted in a significant reduction of seed oil and seed protein (%) in the first growing season 2016 while indicated no significant effect in the second one. The fungicide significantly increased seed phosphorus percent of both growing seasons compared with the control.

The chemical control using Rizolex-T50 as seed coating at 3g/kg seeds in this study was more effective in decreasing damping-off diseases which may be due to that the plant seeds are requires treatment with fungicide to assure an adequate plant stand in the field. Similar results were obtained by **Abd El-Kareem *et al*. (2004a) and Abd El-Hai *et al*. (2016b)**.

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فاعليـــة بعـض العنـاصـــر الصغـرى الصــديقـة للبيئه علـــى مقاومة موت بادرات فول الصويا

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يهدف هذا البحث إلى دراسة مدى تأثير العناصر الغذائية الصغرى (البورون – المولبيدنم و الكوبلت) علي مرض موت لبادرات فول الصويا وكيفيه الحد من مسبباتها المرضيه . تم تجميع عينات من نباتات فول الصويا التي ظهرت عليها الإصابه بالمرض من 9 مراكز مختلفه من محافظه الدقهليه خلال الموسم الزراعي 2015، عزلت أنواع مختلفه من الفطريات منها بيثيم ألتيمم، ريزوكتونيا سولاني، فيوزاريوم سولاني، فيوزاريوم اكسيسبوريم وماكروفومينا فاصولينا، أظهر اختبار القدره المرضية أن فطرالريزوكتونيا سولاني كان الأكثر ضراوة كمسبب لموت البادرات قبل الظهور فوق سطح التربة في كلا الصنفين جيزة 35 وجيزة 111 بينما كان فطر فيوزاريوم سولاني الأكثر ضراوة لموت البادرات بعد الظهورفي كلا الصنفين أيضا بينما كان فطر البيثيم اقلهم ضراوة حيث كانت أعلي نسبة للنباتات السليمة، كانت أكثر العزلات ضراوة عزلة الريزوكتونيا رقم 2 ثم الفيوزاريوم رقم 3 وأخيرا البيثيم رقم 1. معمليا وعند دراسة تاثيرالعناصر الصغري وجد أن فطر الفيوزاريوم سولاني الأكثر تأثيرا مقارنة بالفطرين الأخرين (الريزوكتونيا والبيثيم) وقد سجل الكوبلت (5 جزء في المليون) النسبة الأعلى لتثبيط نمو الفطريات الثلاث حيث سجلت 74% بينما الأقل تثبيطا سجل عندما كان المولبيدنم عند تركيز(2 جزء في المليون) 34.77 للثلاث فطريات، كان البورون ذو قيمة متوسطة التأثير عند تركيز (2 جزء في المليون) ويتساوى مع المولبيدنم عند تركيز (5 جزء في المليون) أما في التجارب الحقليه خلال موسمي النمو 2016 و2017 فان نقع البذور 15 دقيقة في المولبيدنم (5 جزء في المليون) أدي الي حدوث أقل نسبة من الموت قبل وبعد الظهور فوق سطح التربة بنسبة 5.66% يتبعه الكوبلت (2 جزء في المليون) 8% ثم البورون (2 جزء في المليون) 9% وذلك عام 2017 ومن الجدير بالذكر أدي تطبيق المولبيدنم عند التركيز الأعلى الي تقليل موت البادرات قبل وبعد الظهور مقارنة بالكنترول، مورفولوجيا كان معدل طول النبات هو 89 سم عند تركيز (5 جزء في المليون) من البورون و111.6 سم للمولبيدنم في موسم 2016 بينما كانت 96 سم للبورون و 118.6 سم للمولبيدنم لموسم 2017، أما كلورفيل أ، ب فان المولبيدنم يتبعه الكوبلت ثم البورون، في حالة الكاروتينات والمحتوى الكلى للفينول كان التركيز الأقل من الكوبلت أعطي أعلى قيمة، في حين أن زيادة نسبة الزيت والبروتين في بذور فول الصويا كان عند المعامله بالكوبلت يتبعه المولبيدنم.

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