

## Role of Foliar Application of Some Microelements in Management of Onion Basal Rot

Abd-El-Baky, A.A.; Morsy, S.M.A. and Khalifa, M.M.A.

Plant Pathology Research Institute, Agricultural Research Center, 12619, Giza, Egypt.

**T**he microelements boron, iron and zinc were tested for their effect on the incidence of onion basal rot caused by *Fusarium oxysporum* f.sp. *cepae* (FOC) and *Fusarium solani* (FS). The tested microelements inhibited mycelial growth and spore germination of the causal pathogens and their counts in the soil and rhizosphere of onion plants. In field experiments, the results of the two seasons indicated that spraying of microelements resulted in significant reduction to the disease during storage with significant increase to bulbs yield and their diameter. Generally, spraying with the tested microelements at the rate of 50 g/ 100L.water was more effective in reducing onion basal rot than 100 g/100L.water.

The percentages of onion basal rot after five months of bulb storage were determined. In storage experiments, results indicated that boron was the least effective in reducing the disease, which resulted in 19.75% disease infection, on the average at the rate of 50 g/100L.water. On the other hand, iron treatment was the most effective one in this respect 10.0%, on the average followed by zinc 11.25%, on the average during 2008/2009 and 2009/2010 growing seasons. The activity of the oxidative enzymes, *i.e.*, peroxidases and polyphenol-oxidase and phenolic compounds were higher in treated onion plants by the microelements compared with control.

**Keywords:** Onion, *Allium cepae*, Basal rot, chemical inducers, *Fusarium oxysporum*, *Fusarium solani*, peroxidase, phenols, polyphenol-oxidase.

Onion (*Allium cepae* L.) is a crop of great importance in Egypt for local consumption and exportation. It is liable to attack by several diseases, which cause great losses in the field, during transportation and in storage. Basal rot caused by *Fusarium oxysporum* f.sp. *cepae* (FOC) is a serious disease on onion. Several studies have been conducted for controlling this disease chemically (El-Shehaby *et al.*, 1997) and biologically (Abd-El Baky, 2005). On the other hand, several investigators found that microelements had antibacterial and antifungal properties (Mashaal and El-Zawily, 1984; Ibrahim *et al.*, 1987; El-Gamili *et al.*, 1997; Abd- El Moneem *et al.*, 2005 and El-Shehaby *et al.*, 2009). Mahanshi and Stradhaua, (1988) also reduced downy mildew on muskmelon by foliar spray with copper and zinc. Keel *et al.* (1989) enhanced biocontrol potentiality of *Pseudomonas fluorescens* strain CHA0 for controlling tobacco black rot caused by *Thielaviopsis basicola* by addition of Fe Cl<sub>3</sub> to soil. On the other hand, the effect of microelements on some crop diseases and yield was studied by Metwally (1986) and Sadat (2002) compared with the untreated control.

Hanafi (2004) found that the linear growth of *Macrophomina phaseolina*, *Sclerotium rolfsii* and *Fusarium moniliforme* (the causal of peanut root and pod-rot diseases) was decreased by increasing the concentration of zinc, manganese, and copper as sulphate. Also, she found that sporulation and sclerotia formation of the tested fungi were decreased by increasing the concentration of all tested microelements from 500 to 1000 ppm.

The aim of this work was to study the effect of boron, iron, and zinc as fungistatic materials on the linear growth and spore germination of basal rot fungi under laboratory conditions. Also, the role of these microelements on management of the disease under field conditions.

### Materials and Methods

Trials were carried out to study the effect of boron, iron, and zinc on *Fusarium oxysporum* f. sp. *cepae* (FOC) and *F. solani* (FS) under laboratory conditions. Also, field trails were carried out at Malawy, Minia Governorate. during 2007-2008 and 2008-2009 seasons for management of the disease. The isolated fungi were purified using single spore technique. The purified fungi were identified depending on their morphological features and the description of Booth (1971).

#### 1. Laboratory experiments:

##### 1.1. Effect of different microelements on mycelial growth and spore germination:

Boron (Sodium borate) 24.8% (Elgomhoria chemical co.), Iron [ferrous sulphate ( $\text{FeSO}_4$ )] and Zinc [zinc sulphate ( $\text{Zn SO}_4$ )] supplied by Sigma-Aldresh Co. at the concentrations of 125, 250, 500, 750 and 1000 ppm were evaluated for their effect on mycelial growth and spore germination of the two tested fungi. Each concentration was added to 100 ml PDA before solidification, poured in Petri plates and inoculated at the center with 0.5 cm. in diameter discs of 7days - old cultures of any of the two tested fungi and incubated at  $26\pm 2^\circ\text{C}$  for 5days. Medium free from the microelements was used as control. Three replicates were used for each treatment. Mycelial growth was measured when fungal growth covered the surface of any plate. The number of the germinated spores was counted 48 h. after inoculation. A disc of 0.5 cm in diameter was transferred into sterilized 100 ml of distilled water in sterilized Jar, then one ml of 1/100 dilution was distributed on PDA medium, incubated at  $26\pm 2^\circ\text{C}$  and then the colonies of each fungus were counted.

#### 2- Field experiments:

Field trials were conducted at Mallawy Exp. Res. Station (planted on mid of December) during 2008-2009 and 2009-2010 growing seasons to investigate the effect of spraying of the tested three microelements on the natural infection by basal rot under field conditions. The field was divided into plots of  $42\text{ m}^2$  ( $7\times 6$  m and 9 rows) and planted with Giza 20 onion c.v. transplants (aged 60 days). All agricultural practices were followed at the proper time and rates throughout the growing season. Total count of fungal spores was assessing at the end of the experiment (Johanse *et al.*, 1960). The bulbs were harvested when about more than of 50% of leaves top were down.

Microelements were applied as foliar spray at the rate of 50 and 100 g / 100L. water, 30 and 60 days after planting. The untreated plots served as control. Four replicate plots were used for each treatment. Diameter and weight of 100 bulbs sampled from each treatment were recorded.

### 3- Storage experiment:

Two hundred bulbs were sampled from each field replicate at harvest and stored for five months in plastic net bags under room temperature. The percentages of rotted bulbs were recorded at the end of storage period. Also, biochemical changes associated with the treatment with the microelements were assessed.

### 4. Biochemical changes associated with the development of onion bulb after spraying with microelements:

#### 4.1. Determination of enzymes activity.

Leaf samples taken from each treatment were collected 3 days after spraying the microelements. Enzyme activities were determined according to Allam and Hollis (1972).

#### 4.2. Determination of phenolic compounds.

Free, conjugated, and total phenols were determined in treated and non-treated onion bulbs with the tested microelements. Phenolic extractions were carried out according to a modified method suggested by Johnson and Schall (1957).

### 5. Statistical analysis:

All experiments were designed in complete randomized blocks. Data were statistically analyzed using "F" test and treatments were compared by L.S.D. values according to Gomez and Gomez (1984).

## Results and Discussion

### 1- The *in vitro* effect:

Data shown in Table (1) show that the tested microelements *i.e.*, boron, iron and zinc resulted in significant inhibition to the linear growth of the two tested fungi. This inhibition was more pronounced when iron was used, which both fungi failed at 1000 ppm followed by zinc then boron. The metal element may be bound directly to the protein or chelated by a porphyrin or flavin prosthetic group. Iron, molybdenum or zinc are common components of metalloenzymes (Ross,1975). Also, Hanafi (2004) found that number of sclerotia of *Macrophomina phaseolina* and *S. rolfsii* and number of spores of *A. flavus* and *F. moniliforme* increased by increasing concentration from 25 to 50 ppm of ZnSO<sub>4</sub> and it returned to decreasing at 100 ppm.

On the other hand, increasing the concentrations increased the inhibition degree. Duffy and Defago (1997) found that Fusaric acid (FA) production was completely repressed by zinc sulphate as low as 10 µg/mL. Also, they added that one or more of the pathogenicity factors may have been insensitive to be suppressed by zinc or may even have been stimulated by zinc. These results are in accordance with that obtained by Mahmoud *et al.* (2009), which found that the highest occurrence of aflatoxigenic fungi and seeds aflatoxin contaminations were increased by increasing zinc sulphate from 100 to 200 ppm in both greenhouse and field trials during 2007 and 2008 growing seasons.

In fact, all elements could be toxic when used at high concentrations, whereas they considered essential at trace concentrations. Observation indicates that the 125ppm concentration of iron and /or zinc are likely to encourage spore germination of FOC and FS. These results are in according with that obtained by Hanafi (2004).

**Table (1): The *in vitro* effect of certain boron, iron, and zinc concentrations on linear growth of *F. oxysporum* f.sp. *cepae* and *F. solani*, 5 days after incubation at  $26 \pm 2^\circ\text{C}$**

Concentration (ppm)	Linear growth in (cm) of microelements					
	Sodium borate		Ferrous sulphate		Zinc sulphate	
	FOC*	FS**	FOC	FS	FOC	FS
1000	5.65	5.90	0.00	0.00	5.55	4.88
750	6.53	5.28	1.75	1.30	4.05	3.50
500	7.38	4.60	1.72	1.95	3.25	3.33
250	8.28	9.00	2.75	2.76	3.81	3.97
125	9.00	9.00	6.38	5.43	6.38	8.35
Control	9.00	9.00	9.00	9.00	9.00	9.00
L.S.D. (0.05)	0.46	0.33	0.31	0.10	0.32	0.27

\* FOC= *F. oxysporum* f.sp. *cepae*, \*\* FS = *F. solani*

## 2. Field experiments:

Data presented in Table (2) show clearly that PDA medium amended with zinc sulphate and sodium borate at 125, 250 and 500 ppm significantly reduced spore germination of *F. oxysporum* f.sp. *cepae*, except at 125 ppm for zinc sulphate. However, an increase was occurred at 750 ppm and then returned to reduce at the high rate (1000 ppm). Ferrous sulphate led to complete inhibition of spore germination at all concentrations used except at 125 ppm. Low concentration of element may be benefit to physiology of fungal cells, meanwhile at high concentration the fungal cell receptors occurs with saturation of element and then becomes toxic to the fungus. These results can be explained according to Ross (1975) who reported that a metal may have toxic effect if it impairs growth or metabolism of an organism above a certain concentration.

The count of the spores of the two tested fungi under field conditions are shown in Table (3). In general, treatment with boron, iron and zinc at the two tested rates resulted in high reduction in the total count of both fungi in the soil and the rhizosphere of onion plants compared with control treatment. However, it was noticed that boron at 50g/100 L. water resulted in high reduction in the total count of *F. solani* only compared with high concentration 100g/100 L. water.

This could be explained by the fact that micro-and microelement amendments have been used commercially on a limited scale to manage certain soil-borne diseases including Fusarium wilt of tomato and other vegetable crops (Engelhard, 1989).

Disease reduction is most often attributed to the improve of nutrition that boosts host defenses or to direct inhibition of the fungal growth and activity. Pathogen suppression may also result indirectly from amendment - mediated modification of

chemical and physical properties like soil and rhizosphere pH (Simon and Sivastithamparam,1989) or from alternation of host root exudates to disfavor pathogenic activity (Huber, 1989). Meanwhile, Mandal and Sinha (1992) reported that zinc and other minerals reduce Fusarium wilt of tomato by inducing host resistance.

**Table (2): Effect of boron, iron, and zinc on the number of germinated spores of *F. oxysporum* f. sp. *cepae* and *F. solani***

Concentration (ppm)	No. of germinated spores ( $\times 10^3$ ) of microelement					
	Sodium borate		Ferrous sulphate		Zinc sulphate	
	FOC*	FS**	FOC	FS	FOC	FS
1000	4.67	0.00	0.00	0.00	11.67	0.00
750	14.00	0.66	0.00	0.00	31.67	0.33
500	12.67	0.33	0.00	0.00	23.33	0.66
250	11.00	0.33	0.00	0.00	17.67	0.00
125	18.33	0.33	70.33	3.66	64.33	4.33
Control	64.33	3.00	64.33	3.00	64.33	3.00
L.S.D. (0.05)	3.35	1.02	1.57	0.48	4.48	1.45

\*FOC= *F. oxysporum* f.sp. *cepae*, \*\* FS = *F. solani*

**Table (3): Effect of boron, iron and zinc on fungal spore counts in the soil and the rhizosphere under field conditions.**

Treatment (ppm)	Count of spores ( $\times 10^3$ ) of microelements											
	Sodium borate				Ferrous sulphate				Zinc sulphate			
	FOC*		FS**		FOC		FS		FOC		FS	
	Rhizosphere	Soil	Rhizosphere	Soil	Rhizosphere	Soil	Rhizosphere	Soil	Rhizosphere	Soil	Rhizosphere	Soil
50	113	12	56	6	25	6	25	6	22.7	6	0.0	0.0
100	300	12	200	7	50	6	50	0.0	333	12	83	6.1
Control	333	35	229	24	333	36	229	24	333	36	229	24

\*FOC= *F. oxysporum* f.sp. *cepae*, \*\* FS = *F. solani*

Table (4) shows that the treatments with the tested elements at the rates of 50 and 100 g./100L. water resulted in significant reduction to the disease when onion bulbs were storage in a storer during seasons of 2007/2008 and 2008/2009 compared with control treatment. In addition, the low concentration was more efficient in this regard compared with the high concentration.

This may be attributed to fungicidal effect of these microelements when their concentration exceeds a certain limit, Sadat (2002). On the other hand, iron treatment was the most effective followed by zinc. Spraying of onion plants with iron reduced mean of basal rot infection to 10.0 and 12.5 % at 50 and 100 g./

100L.water, respectively. Meanwhile, when the plants were sprayed with zinc, the reduction was 11.0 and 13.75 %, respectively. In a few cases, though mineral amendments appear to reduce the disease by stimulating indigenous populations of microorganisms indirectly that are beneficial to plant growth and antagonistic to pathogens (Huber, 1989).

**Table (4): Effect of microelements foliar spray on the percentages of basal rot infection in Malawi station during 2008/2009 and 2009/2010 growing seasons.**

Treatment	Percentage of infection at concentrations (ppm) during					
	50		Mean	100		Mean
	2008/2009	2009/2010		2008/2009	2009/2010	
Boron	17.5	22.0	19.8	20.0	27.5	23.75
Iron	10.0	10.0	10.0	12.5	12.5	12.5
Zinc	12.0	10.0	11.25	15.0	12.5	13.75
Control	22.5	32.5	27.75	22.5	32.5	27.5
L.S.D. at 5%	4.9	4.9	----	3.4	5.2	---

Table (5) show clearly that the diameter of onion bulbs treated with elements were greater than that of non-treated ones in the two seasons. However, the weight of onion bulbs treated with elements was higher than the non-treated at the different concentrations.

Onion plant treated with iron at concentration of 50 g /100L.water gave the highest mean weight (266.5kg) followed by the same element (253 kg) at concentration of 100 g / fed of the two successive growing seasons 2007/2008 and 2008/2009. This led to larger bulbs as the absorption of water and nutrients was more efficient (Sadat, 2002).

**Table (5). Effect of foliar application with boron, iron and zinc at different concentrations on diameter and weight of onion bulb**

Treatment	Dose (ppm)	Weight of Bulbs (Kg) / plot			*Mean diameter of bulbs in (cm)		
		2008/09	2009/10	Mean	2008/09	2009/10	Mean
Boron	50	257	235	246.0	7.6	7.9	7.8
	100	232	210	221.0	7.7	7.5	7.6
Iron	50	270	263	266.5	8.2	8.1	8.3
	100	255	251	253.0	7.8	8.0	8.0
Zinc	50	260	243	251.5	7.9	8.0	8.0
	100	250	231	240.0	7.7	7.6	7.7
Control	50	192	198	195.0	7.4	7.6	7.5
	100						
Mean	50	245.1	233	-----	7.8	7.8	-----
	100						
L.S.D. at 5%	----	7.0	5.0	-----	0.4	0.7	-----

\*Values are mean of 100 bulbs for each treatment.

4. Biochemical changes associated with the development of onion bulb after spraying with the microelements:

4.1. Determination of enzyme:

Data presented in Table (6) showed that the different tested systemic resistance inducing agents caused increase in the activities of peroxides and polyphenoloxidase in bulb tissues compared with control.

Zinc caused the highest increase in the activities of peroxides and polyphenoloxidase enzymes at the two doses (50 and 100 g / 100 L. water) followed by iron and boron which showed low activities for the two enzymes. This explains the results obtained that treatment with zinc was the best in decreasing the percentage of infection during storage (Table 4).

Concerning this point, many investigators explained the role of oxidative enzymes on disease. Byrde *et al.* (1960) indicated that resistance to several plant diseases appeared to be due to inhibition of fungal enzymes by polyphenols and their oxidation products. Badawi *et al.* (1986) treated onion seedlings with zinc and mixture of it with copper, molybdenum, and manganese. They found that all treatments increased peroxides activity specially zinc + copper + manganese and zinc + copper + molybdenum followed by zinc alone. Meanwhile, zinc alone slightly increased polyphenoloxidase activity. Avdiushko *et al.* (1993) found that many plant enzymes are involved in defense reaction against plant pathogens.

**Table (6): Effect of different concentrations of microelements peroxides and polyphenoloxidase activity (as optical density /g. fresh weight)**

Element	Enzymatic activity at two doses (g/100 L. water)			
	Peroxides		Polyphenoloxidase	
	50	100	50	100
Boron	4.28	2.815	0.188	0.169
Iron	4.86	3.813	0.195	0.174
Zinc	5.43	4.44	0.41	0.311
Control	4.02	4.02	0.069	0.069

4.2. Determination of phenolic compound:

Data tabulated in Table (7) reveal that zinc treatment at 50 g/100L.water recorded the highest amount of free phenols (4.28 mg), conjugated phenols (13.6 mg.) and total phenols (13.31 mg.), meanwhile, no clear differences were found between boron and ferrous treatments compared to control.

The present data showed that activation of the oxidative enzyme (peroxides and polyphenoloxidase) and phenols was obviously higher in treated onion plants compared to control. These results agree with those recorded by Abdou *et al.* (2001) and El-Fiki *et al.* (2004).

**Table (7): Effect of different concentrations of microelements on free, conjugated, and total phenols (mg/g. fresh weight).**

Element	Phenolic compound at two doses (g /100 L. water)					
	Free phenols at		Conjugated phenols at		Total phenols at	
	50	100	50	100	50	100
Boron	1.92	1.41	9.31	5.65	11.23	7.06
iron	2.06	1.73	9.45	6.05	11.51	7.78
Zinc	4.28	3.85	13.6	9.46	9.32	13.31
Control	1.97	1.24	10.73	6.46	8.76	7.71

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Corresponding author: Abd-El-Baky, A.A.  
E-mail: [ahmad\\_abdelbaky2000@yahoo.com](mailto:ahmad_abdelbaky2000@yahoo.com)

## دور الرش ببعض العناصر الصغرى في تقليل الإصابة بمرض عفن القاعدة في البصل

أحمد على عبد الباقي ، صابر محمد على مرسى ، و ممدوح محمد عبد الفتاح خليفة

معهد بحوث أمراض النباتات ، مركز البحوث الزراعية ، الجيزة ، مصر

أجرى اختبار لدراسة تأثير الرش بأى من العناصر الصغرى البورون والحديد والزنك على الإصابة بعفن القاعدة في البصل المتسبب عن الفطر *Fusarium oxysporum f.sp. cepae* and *Fusarium solani* ، حيث أوضحت النتائج المعملية أن لهذه العناصر القدرة على تثبيط نمو *Mycobacterium* وإنبات جراثيم الفطرين. في التجارب الحقلية ، أوضحت النتائج أن عنصر الحديد أعطى أعلى تأثير في تقليل المرض حيث انخفض متوسط الإصابة الى 10% يليه الزنك فكان 11.25% اما البورون فقد كان أقل تأثيرا على خفض نسبة الإصابة بعفن القاعدة حيث قلل الإصابة بالمرض الى 19.75% عند استخدام معدل 50 جم / 100 لتر ماء عند نفس المعدل السابق خلال موسمي النمو 2008/2007 و 2009/2008 مقارنة بالكونترول ، كما تم حساب عدد الجراثيم في التربة والمنطقة المحيطة بجذور البصل (الريزوسفير) معمليا ووجد ان المعدل 50 جم / 100 لتر ماء اعطى أقل عدد من المستعمرات الفطرية لكلا الفطرين. كما كان لاستخدام العناصر الصغرى السابقة تأثيرا إيجابيا معنوي علن الصفات المحصولية للإبصال الناتجة وتقليل عفن القاعدة عند التخزين.

أدى الرش بالعناصر الصغرى لنباتات البصل إلى زيادة نشاط أنزيمات البيروكسيداز والبولي فينيول أكسيداز عند الرش بتركيزات 50 ، 100 جرام / 100 لتر ماء مقارنة بالكونترول. أيضا أدى إلى زيادة في الفينولات الكلية عند استخدام معدل 50 جم / 100 لتر ماء أما عند معدل 100 جم / 100 لتر فكانت الزيادة عند استخدام الزنك فقط - ولم تكن هناك فروق معنوية عند استخدام البورون أو الحديد مقارنة بالكونترول. وبصفة عامة وجد ان الرش بالعناصر الصغرى بمعدل 50 جم / 100 لتر ماء ذو تأثير معنوي في تقليل النسبة المئوية لإصابة البصل بعفن القاعدة مقارنة بمعدل 100 جرام / 100 لتر ماء.