J. Plant Prot. and Path., Mansoura Univ., Vol. 3 (1): 23 - 33, 2012

EFFECT OF CERTAIN MICROELEMENTS ON GRAIN SORGHUM STALK ROT CAUSED BY *Fusarium moniliforme* J. SHELD.

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ABSTRACT

Grain sorghum [*Sorghum bicolor* L. (Moench)] is subjected to infection by certain diseases causing great and substantial damage to the crop. *Fusarium* stalk rot of sorghum caused by *F. moniliforme* (J. Sheld) represents the common stalk rot in Egypt and many areas of the world. The tested sorghum cultivars reacted differently to *F. moniliforme* in the two tested seasons. Giza15 sorghum cv. exhibited the highest percentage of infection and disease index followed by Shandaweel 6. Dourado sorghum cv. exhibited the lowest percentage of infection and disease index in both growing seasons. *In vitro*, the microelements (copper, zinc, manganese and their mixture) at 2g L^{-1} significantly reduced the linear growth of *F. moniliforme*. Copper and the mixture treatment exhibited the highest toxic effect on the tested pathogen, followed by zinc. Manganese exhibited the lowest toxic effect. Seed treatments with different microelements significantly increased peroxidase activity in sorghum leaves as compared with untreated ones. The highest peroxidase activity was obtained in both sorghum cultivars with the mixture treatment and copper then, zinc treatment. Treatment of sorghum seeds with manganese exhibited the lowest peroxidase activity in both Giza15 and Dourado sorghum cultivars. Chlorophyll a and b concentrations in the leaves of copper- and zinc- treated plants were significantly higher than those of manganese compared with untreated plants (control). In mixture treatments, the interaction between sorghum cultivars and microelements had significant effect on chlorophyll content. Based on the present data, it is recommended to use a mixture of microelements on grain sorghum to control or at least alleviate the infection by *F. moniliforme*.

Keywords: Microelements, sorghum, stalk rot, *Fusarium moniliforme*

INTRODUCTION

Grain Sorghum (*Sorghum bicolor* L.) considered as economic cereal crop in Egypt as well as in many parts of the world under tropical and sub-tropical climatic conditions. It is cultivated in many locations in Upper Egypt, where the climate is more favorable for plant growth. Its importance comes from its wide usage as an animal food and in some industrial purposes.

Sorghum is subjected to infection by certain diseases causing serious and substantial damage to the crop. *Fusarium* stalk rot of sorghum caused by *F. moniliforme* J. Sheld, represents the common stalk rot in many areas of the world and in Egypt (El-Assiuty, 1982; Pande and Karunakar, 1992 and Asran, 1998). Management of *Fusarium* stalk rot has achieved little success with classical methods as fungicides which have residual effect for human and environment. The pathogen is seed-borne and survives in soil causing reduction of germination through seed decay and seedling blight. Studies of Kedera *et al*., (1992) indicated that *F. moniliforme* spreads

systemically in corn plants and can be isolated from the crown, nodes, cob and seeds of corn. Sorghum and corn cultivars varied in their reaction to stalk rot disease (Asran, 1998 and Asran and Buchenauer, 2002).

Micro- and macroelements have been used commercially on a limited scale to manage certain soil-borne pathogens (Engelhard, 1989 and Asran, 1998). Disease reduction is most often attributed to improved nutrition that boosts host defenses or directly inhibits the fungal growth and activity. Pathogen suppression may also result indirectly from the modification of chemical and physical properties of soil and rhizosphere pH or from modification of host root exudates to disfavor pathogenic activity (Huber, 1989). Moreover, the microelements may enhance the level of plant phenols which play a major role in plant disease defense (Abd El-Hai *et al*., 2009).

The resistance of plant may also be induced when using microelements such as manganese and zinc (Marshner 1986, Abd El-Hai *et al*., 2007, and El Baz 2007).

This work was planned to study the management of *Fusarium* stalk rot of sorghum using certain microelements and to investigate the role of certain plant constituents in plant defense mechanisms to such disease.

MATERIALS AND METHODS

Source of microorganisms:

Fusarium moniliforme isolate (No. 8) was previously tested and found as sever pathogenic to maize plants (Asran and Buchenauer, 2002), so, it was used in this study.

Source of **sorghum cultivars**

Sorghum cultivars (Giza 15, Shandaweel 6 and Dourado) were supplemented from Sorghum Department, Crop Research Institute, Agriculture Research Center, Giza, Egypt.

Pathogenicity of *F. moniliforme* on sorghum cultivars under greenhouse condition:

The tested *F. moniliforme* isolate was examined for its pathogenicity on the sorghum cultivars (Dourado, Giza 15 and Shandaweel 6) under greenhouse conditions in the growing seasons of 2009 and 2010 by using soil infestation method to determine the percentage of infection, the toothpick technique was used to evaluate the disease index for sorghum stalk rot.

Soil infestation by fungal inocula was carried out using sorghum grain medium for growing *F. moniliforme*. Half kilo milk bottles each containing 100 g washed sorghum grains and moistened with a standard amount of distilled water were autoclaved at 1.5 kg/cm^2 for 20 minutes and inoculated with *F. moniliforme*, then incubated at 27 °C for 2 weeks. Sterilization of pots and soil was carried out using 5% formalin solution 15 day before planting date. Four Kg sterilized soil was potted in sterilized pots (25 cm in diameter) and the inoculum was added at 3.5% and thoroughly mixed with the soil in each pot. After one week, each pot was sown with five surface sterilized grains of the tested sorghum cultivars. Five pots were used for each cultivar as replicates.

Plants were fertilized by recommended dose of NPK and irrigated when necessary.

For inoculation of stalks, the toothpick technique was used as described by Jardine and Leslie (1992). Round wooden toothpicks about 6 cm. long were boiled several times (about 1 hr. each time) in tap water to remove any possible toxic substances that might inhibit the growth of the tested fungus. Boiled toothpicks were washed in fresh tap water after each boiling, then dried and kept into glass jars. Potato dextrose broth, supplemented with 0.1% yeast extracts was added to moisten the toothpicks until slight excess of the medium accumulated at the bottom of each jar and autoclaved immediately at 1.5 cm^2 for 20 min. Autoclaved toothpicks were inoculated with 8 mm. discs of *Fusarium moniliforme* cultures, then incubated at 27 °C about 3 weeks until the fungal growth covered the toothpicks.

Five of surface sterilized grains of each tested cultivar were seeded in sterilized pots, containing autoclaved soil. Inoculation was performed at the second internode above the ground surface of fifty-five days old plant by using an ice pick to create a hole at the second internode. A toothpick was then inserted into the hole. Sterile toothpicks were inserted into the second internode of control plants. One month after inoculation, the stalks were cut longitudinally and stalk discoloration was determined using the following rating scale ranging from 1 to 5 (Hooker, 1956): 1= 0-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100% of the inoculated internode diseased and 5 = 100% with infection extending into the adjacent internode. For each replicate a disease index (DI) similar to that described by Liu *et al.*, (1995) was calculated as follows: DI% = $\sum$(1A +2B + 3C + 4D + 5E)/ 5T X 100 where, A, B, C and D are the number of plants corresponding to the numerical grade, 1, 2,3,4 and 5 respectively, and 5T is the total number of plants (T) multiplied by the maximum discoloration grade (5).

In vitro experiment

Effect of the tested microelements on the fungal growth:

The effect of individual manganese, zinc and copper at 2 g L^{-1} and their mixture were mixed in PDA medium before solidification, and then poured in sterile Petri dishes (9 cm). Five plates for each treatment were used as replicates while each plate was inoculated with 5 mm fungal disc. Treatments were incubated at 27 ºC. The linear growth of the fungus was measured when the full growth of tested isolate was observed in the check treatment. The experiment was carried out twice.

Incidences of stalk rot disease as affected by seed treatment with the tested microelements:

Microelements namely zinc as Zn EDITA, manganese as Mn EDITA and copper as $CuSO_4$ were tested for their effect on incidence of sorghum stalk rot disease under greenhouse conditions in 2009 and 2010 growing seasons. Sterilized seeds of Giza 15 cultivar were treated with micronutrients 2 days before sowing by soaking seeds in 2 g L^{-1} of manganese, zinc and copper separately and their mixture for three hours, then treated seeds were dried under room temperature (Ancpok, 1990). Sterilized treated seeds with the previous microelements were sown in sterilized pots (50 cm diameter) containing sterilized soil. Each pot was seeded with 3 seeds. Five pots were

used as replicates. Pots containing non treated seeds were used as control. The above treatments were also used as foliar applications 45 and 60 days after sowing. All pots were fertilized with NPK at the recommended doses for sorghum. For inoculation of stalks, the toothpick technique was used as mentioned above. One month after inoculation, the stalks were cut longitudinally and stalk discoloration was determined as described before.

Determination of peroxidase activity

Leaves samples of sorghum plants were collected after 60 days from sowing date. Leaf tissues were used directly or stored at - 80°C until use. Leaf tissues were homogenized in a per-chilled mortar and pestle with liquid nitrogen in sodium phosphate buffer 0.01 M, pH 7.0 (5 ml/g fresh weight). The homogenates were centrifuged at 15000 rpm for 10 min. at 4 °C. The supernatant was immediately used for assaying peroxidase activity using guaiacol as hydrogen donor according to Hammerschmidt *et al*., (1982). The reaction mixture consisted of 2.9 ml of a mixture of 0.01 M sodium phosphate buffer (pH 6.0) and 0.25 % (v/v) guaiacol and 0.1 M H_2O_2. 0.1 ml crude enzyme (extract) was added at room temperature to initiate the reaction, changes in absorbance at 470 nm during two minutes were recorded. Enzyme activity was expressed as increase in absorbance/min/ g fresh weight.

Determination of Chlorophyll

Chlorophyll a and b of the tested sorghum were detected in plants after 60 days from sowing. Spectrophotometer method recommended by Wolf (1963) was applied. All leaves of each tested plant were mixed together and a sample of 1.0 gram fresh weight of plant leaves was then ground in a porcelain mortar with 85% aqueous acetone. The extract of each sample was quantitatively made up to 50 ml. with acetone (85%). The absorbency of the extract was determined using Spectrophotometer model SPECTRONIC 20 D. at wave lengths of 644 and 633 nm. The extinction was measured against a blank of pure 85 % aqueous acetone. Taking in consideration the dilutions made to determine the concentration of photosynthetic pigment fractions (chlorophyll a and b) as µg/ml using the following equations:

Chlorophyll a = $10.3\ E_{663} - 0.918\ E_{644}$.

Chlorophyll b = $19.7\ E_{644} - 3.87\ E_{663}$.

Finally these pigment fractions were also calculated as mg / g leaves fresh weight of differently treated plant.

Statistical analysis

All data were subjected to statistical analysis and means were compared using L.S.D. range test as described by (Gomez and Gomez, 1984).

RESULTS

Reaction of certain sorghum cultivars to *F. moniliforme* infestation

The soil infestation and toothpick technique for fungal inoculation were used as described before. The experiments were conducted in 2009 and 2010 growing seasons under greenhouse conditions. Percentage of

infection was recorded with the soil infestation experiment, while the disease index was estimated in case of toothpick experiment. Table (1) illustrates the results and shows that the tested sorghum cultivars reacted differently for *F. moniliforme* in the two tested seasons. Giza15 sorghum cv. exhibited the highest percentage of infection and disease index followed by Shandaweel 6. Dourado sorghum cv. exhibited the lowest percentage of infection and disease index in both growing seasons. Figure (1) shows different degrees of Fusarium stalk rot (1-5) in sorghum plants

Table (1): Reaction of sorghum cultivars to stalk rot disease caused by *F. moniliform* in 2009 and 2010 growing seasons under greenhouse conditions.

Cultivars	2009		2010		Reaction*
	Infection %	Disease index	Infection %	Disease index	
Dourado	14.50	17.88	14.00	16.12	R
Giza 15	38.00	48.75	52.10	57.60	S
Shandaweel 6	22.00	27.20	28.20	29.11	MS
L. S. D. (p= 0.05)	6.10	10.50	9.80	15.50	

***S = Highly susceptible, MS = Moderatly susceptible, R = Resistant**

Figure 1: Different degrees of *Fusarium* stalk rot (1-5) in Giza 15 sorghum plants

Effect of microelements on fungal growth

Data presented in Figure (2) indicate that the tested microelements (copper, zinc, manganese and their mixture) at 2g L^{-1} significantly reduced the linear growth of *F. moniliforme In vitro*. Copper and the mixture treatment exhibited the highest toxic effect (3.2 and 3.6 cm, respctivily) followed by zinc (4.2 cm). Manganese exhibited the lowest toxic effect on tested fungi (5.9 cm). On the other hand, the effect of Rizolex –T50 shows that the

concentration 2g L^{-1} significantly dicreased the linear growth of the pathogenic fungus.

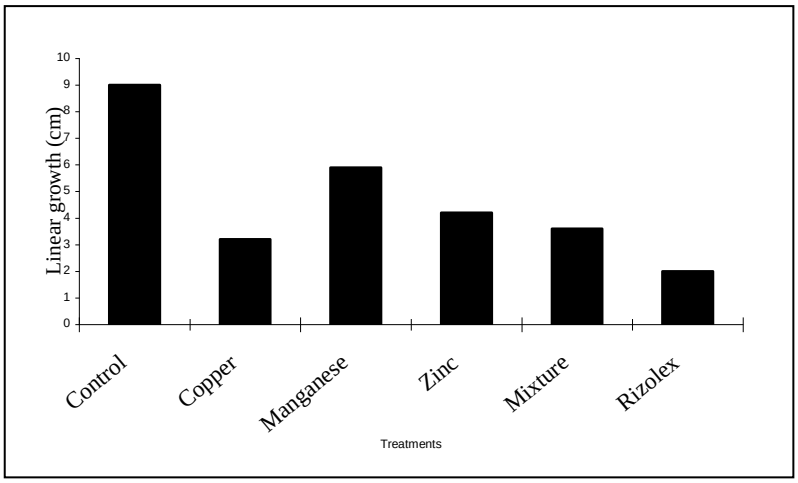

Figure (2): Effect of microelements on the linear growth of the tested pathogenic fungus (*F. moniliforme*).

Effect of the tested microelements on disease incidence

The most suscibtable cultivar (Giza15) was used in the test. Results in Table (2) indicate that treatment of sorghum seeds by 2g L^{-1} copper, manganese, zinc and their mixture decreased significantly disease index. Copper and zinc were superiors in this respect followed by manganese. The combination between copper, zinc and manganese was more effective in this respect followed by treatment of copper, zinc or manganese alone.

Table (2): Effect of the tested microelements on disease incidence of sorghum stalk rot disease caused by *F. moniliforme* after 60 days from Giza15 sowing

Treatments	2009		2010	
	Disease index	Reduction %	Disease index	Reduction %
Copper	21.83	58.02	22.19	56.49
Manganese	40.17	22.75	43.15	15.39
Zinc	24.40	53.08	22.66	55.57
Mixture	19.22	63.04	20.24	60.31
Rizolex-T5	10.33	80.13	9.88	80.63
Control	52.00	-	51.00	-
L. S. D. (*p*= 0.05)	**7.81**		**6.15**	

Effect of the tested microelements on peroxidase activity in sorghum leaves

The efficacy of the tested treatments in disease control was reflected on the peroxidase activity. Results in Table 3 show that seed treatment with different microelements significantly increased peroxidase activity in sorghum

leaves as compared with untreated ones. The highest peroxidase activity was obtained in both sorghum cultivars with mixture treatment, and copper followed by zinc treatment. Treatment of sorghum seed with manganese exhibited the lowest peroxidase activity in both sorghum cultivars. In general, peroxidase activity at cv. Dourado was more active in peroxidase than Giza15 sorghum cultivar.

Table (3): Peroxidase activity in sorghum leaves as affected seed treatment with different microelements.

Treatments	Dourado		Giza15	
	Peroxidase activity units	Increase %	Peroxidase activity units	Increase %
Copper	54.07 a	53.87	44.22 a	45.36
Manganese	47.85 b	36.17	40.14 b	31.95
Zinc	39.19 c	11.53	33.28 c	9.40
Mixture	56.44 a	60.61	46.88 a	54.11
Control	35.14 d	-	30.42 d	-
L.S.D.(*P*= 0.05)	**3.45**		**2.69**	

Chlorophyll a and b contents in sorghum leaves

Chlorophyll a and b contents in leaves of treated sorghum plants of Dourado (R) and Giza15 (S) were determined as mg/g fresh weight. Results in Table (4) indicate that the chlorophyll a and b concentrations in the leaves of the treated plants with copper and zinc were significantly higher than those of manganese compared with untreated plants (control). In mixture treatmentsThe highest content of chlorophyll a and b were recorded with sorghum cv. Dourado plants which treated with copper, zinc or mixture of tested microelements. Data also indicate that chlorophyll a was higher than chlorophyll b in the leaves of both sorghum cultivars.

Table (4): Content of chlorophyll a and b in sorghum leaves as affected by seed treatment with the tested microelements

Treatments	Chlorophyll a		Chlorophyll b	
	Dourado	Giza 15	Dourado	Giza 15
Copper	1.71	1.44	1.53	1.24
Manganese	1.62	1.32	1.49	1.2
Zinc	1.37	1.01	1.12	0.98
Mixture	1.73	1.46	1.54	1.26
Control	1.09	1.01	0.97	0.92
L.S.D. (*P*=0.05)				
Treatment (T)	0.51		0.39	
Cultivars (C)	0.36		0.21	
Interaction (TxC)	0.78		0.43	

DISCUSSION

Fusarium moniliforme may cause seed rot, seedling blight and stalk rot on sorghum and maize plants (El-Assiuty 1982 and Asran and Buchenauer, 2002). The results of this investigation indicate that the tested sorghum cultivars reacted differently to *F. moniliforme* in the two tested

seasons. Sorghum cv. Dourado was more resistant to disease than cv. Shandaweel 6 and cv. Giza 15. Such results provide a practical way for overcoming disease stress to sorghum plants by cultivating the resistant cultivars in heavy infested areas of the pathogen. Such results are in accordance with those reported by Claflin (1991), Jardine and Leslie (1992), Karunakar *et al*., (1993) and Asran (1998). They concluded that differences in sorghum cultivars in their reaction to disease may be due to physiological and / or histological aspects.

Plant resistance to pathogen requires complex metabolic pathways in the infected cells, aimed at recognizing pathogen presence and hindering its propagation within plant tissues (De Gara *et al.,* 2003). In the recent years, there has been an increasing interest in the use of microelements as a protective agent in agriculture (Abd-El-Moneem, 1996; Duffy and Défago, 1997 and Abd-El-Hai *et al*., 2009). Microelements are known to have various activities such as anti-fungal activity against several phytopathogenic fungi (Abd-El-Moneem, 1996 and Abd-El-Hai *et al.,* 2009), increased biocontrol activity of *Psudomonas flurescens* (Duffy and Défago, 1997). In this study, tested microelements (copper, zinc, manganese and their mixture) significantly reduced the linear growth of *F. moniliforme in vitro*. Copper and the mixture treatment exhibited the highest toxic effect followed by zinc, then manganese treatment. These results are in agreement with those reported by Abd-El-Moneem (1990) who mentioned that microelements reduced and inhibited growth of *Fusarium* wilt on cumin and *Fusarium* pathogen of root rot of wheat. Abd-El-Hai *et al.* (2009) reported that zinc, manganese and their combination significantly reduced the linear growth of *Macrophomina phasolina* and *Rhizoctonia solani*.

Treatment of sorghum seeds with 2g L^{-1} copper, zinc, manganese and their mixture decreased significantly disease index of sorghum plants compared with untreated control in both growing seasons. Microelements were previously known as inducer resistance in sunflower (Abd-El-Hai *et al*., 2009). Manganese plays a role in regulating the levels of auxin in plant tissues by activating photosynthesis (Marschner, 1986). Auxin may induced the systemic resistance and encourage the meristemic activity of the plant which resulted in more cell division and cell enlargement (Devlin and Witham, 1983). Manganese and zinc are co-factors of Super Oxide Dismutase, which considered enzymatic antioxidant, hence alleviate the harmful effect of Reactive Oxygen Species (ROS free radicals) caused by fungal stress. These findings are in agreement with Kostas and Christos (2006), they found that the foliar application of microelements can be used to reduce the severity of tan spot disease on durum wheat, however the physiological basis of this pattern still unknown.

Results in Table (3) show that seed treatments with different microelements significantly increased peroxidase activity in sorghum roots as compared with untreated ones. The highest increase percent was obtained on both sorghum cultivars with both the mixture treatment and copper alone followed by zinc treatment. Such findings are similar to those reported by Abd-El-Moneem (1996) who studied the effect of microelements on incidence of sesame charcoal root-rot and wilt disease complex. In addition to the direct

roles of peroxidase in resistance against fungal disease in which peroxidase take a part in several defense pathways *e.g*, it might be involved in the resistance by generation of H_2O_2 as well as by increasing the concentration of free radicals and their polymerization products (Mahdy, 1994). Enhanced peroxidase activity is often associated with resistance phenomena such as lignin production and other plant enzymes (Podile and Laxmi, 1998).

In the present work the content of chlorophyll a and b in the leaves of the treated plants with copper and zinc were significantly higher than those of manganese compared with untreated plants (control). In mixture treatment had a significant effect on chlorophyll content. Increasing chlorophyll concentrations in sorghum plants will increases carbohydrate content in plant tissues. Carbohydrates are the main repository of photosynthetic energy, they comprise structurally polysaccharides of plant cell walls, principally cellulose, hemicelluloses and pectin that consider a barrier against plant pathogens invasion and phenolic compounds which are associated with structural carbohydrates, that plays a major and important role in plant disease (Hahlbrock and Scheel, 1989). In addition, the enhancement in chlorophyll content is resulting from stimulating pigment formation and increasing the efficacy of photosynthetic apparatus with a better potential for resistance as well as decreasing photophosphorylation rate, which occurred after infection (Amaresh and Bhatt, 1998).

Based on the present data, it is recommended to use the mixture of the previous microelements on grain sorghum to control or at least alleviate the infection by *F. moniliforme*.

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تأثير بعض العناصر الصغرى على مرض عفن الساق في الذرة الرفيعة المتسبب عن فطر *Fusarium moniliforme*

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يعتبر مرض عفن الساق والمتسبب عن فطر *F. moniliforme* من الأمراض المعروفة على نباتات الذرة الرفيعة فى مصر ، وقد أجرى هذا البحث بغرض إستخدام بعض العناصر الصغرى مثل النحاس والزنك والمنجنيز بالإضافة لاستخدام خليط من هذه العناصر فى مقاومة هذا المرض

وقد تم التوصل للنتائج التالية:-

- تم إختبار تأثير الفطر على ثلاثة أصناف من الذرة الرفيعة (جيزه 15 ، شندويل 6 ، دورادو) وقد أظهر الصنف دورادو درجة عالية من المقاومة بينما كان الصنف جيزه 15 أكثر الأصناف المختبرة قابلية للإصابة يليه الصنف شندويل 6، وذلك فى موسمى النمو 2009 ، 2010.
- في المعمل تم دراسة تأثير العناصر الصغرى المختبرة منفردة ومخلوطها مجتمعة على نمو الفطر *F. moniliforme* وقد أدت هذه المعاملات لخفض النمو للفطر وكان أكثرها سمية النحاس ثم معاملة الخليط ثم الزنك وكان المنجنيز أقلها تأثيرا على نمو الفطر.
- وجد أن أعلى نشاط لإنزيم البيروكسيديز فى كلا صنفى الذرة الرفيعة (جيزة 15، دورادو) ظهر فى حالة نقع البذور فى مخلوط العناصر الصغرى وكذلك فى حالة نقع البذور فى النحاس يلى ذلك المعاملة بالزنك. وقد أظهرت معاملة نقع البذور بالمنجنيز أقل معدل فى نشاط إنزيم البيروكسيديز.
- وجود زيادة معنوية في تركيز الكلوروفيل أ و ب في أوراق النباتات المختبرة عند معاملة البذور بالنحاس والزنك كلاً منهما على حده أو بمخلوط من العناصر الصغرى المختبرة.

قام بتحكيم البحث

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