

EFFECT OF ZINC, FERROUS, MANGANESE AND BORON ON COWPEA RUST DISEASE

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ABSTRACT

Detection of cowpea rust disease was carried out in 13 districts of Dakahlia governorate. The high disease incidence was in Sherbin district followed by Tmai Elamdid district. While, the low disease incidence was in Aga district. On the other side, the high value of disease severity occurred under Tmai Elamdid, Mit Salsyl and Bilqas districts. While, Minit Elnasr district was came late.

Foliar application of microelements at all tested concentrations and also fungicide (Plantvax 20% EC 1ml/l) significantly decreased the infection with *Uromyces vignae*. The best results were obtained from boron at 0.05 g/l followed by manganese 3 g/l then ferrous 3 g/l.

All tested microelements caused significant increase in plant height and number of branches, leaves and pods/plant. The low concentration was more effective than the higher one except ferrous which gave the reverse. While there was no significant effect due to fungicide treatment on these parameters. The highest values of 100-seeds weight occurred under boron application at 0.05 g/l followed by manganese at 3 g/l then boron 0.025 g/l.

The high total phenol content in cowpea leaves were obtained from the application of boron at 0.05 g/l followed by manganese at 3 g/l then manganese 2 g/l. In all tested microelements treatment a significant increase in chlorophyll a, b and carotenoids content was achieved compared with fungicide and check treatments. This study indicates that, there is a correlation between induced resistance for rust disease and accumulation of phenols.

INTRODUCTION

Cowpea (*Vigna unguiculata*, L.) is one of the most important leguminous crops cultivated in Egypt. Many fungi attack this plant of which, *Uromyces vignae* causes rust disease. Non host resistance to biotrophic fungal pathogens that penetrate epidermal cells directly, the basidiospore derived parasitic stage of rust fungi, is typically expressed as penetration failure (Mellersh *et al.*, 2002). Potentially more experimentally informative, however, is the second parasitic stage of the rust fungi derived from urediospore infection. This parasitic stage is not only the most economically important with respect to rust diseases of crop plants, but it also involves larger defined set of developmental stages during the fungal infection process. After spore germination on a leaf, topographical features of the plant surface may be used by the fungus to locate and recognize stomata prior to going through a defined series of morphological stages required to enter the

stomatal opening and form the first haustorium within a living host mesophyll (Heath, 1997).

Considerable attention has been paid in the last few years to the subject of micronutrients as a limiting factor for increasing agricultural production in Egypt. Nutrients taken up by plants are used for vegetative growth and for the production of storage organs (grain, roots, tubers), and each will now be considered (Mengel and Kirkby, 1982). Plant growth involves a large number of highly integrated enzyme reactions and physiological or metabolic processes, all of which are dependent on adequate supplies of nutrients. The essential micronutrients for field crops are B, Cu, Fe, Mn, Mo, and Zn. The incidence of micronutrient deficiencies in crops has increased markedly in recent years due to intensive cropping, loss of top soil by erosion, losses of micronutrients through leaching and decreased proportions of farmyard manure compared to chemical fertilizers (Fageria et al., 2002).

The aim of this investigation was to study the effect of zinc, ferrous, manganese and boron application on cowpea plants, *i.e.* disease incidence and severity of rust, phenols content, photosynthetic pigments and some morphological aspects as well as yield components.

MATERIALS AND METHODS

The present investigation was conducted at the farm of Tag El-Ezz Research Station, Dakahlia Governorate, Egypt during the summer seasons of 2005 & 2006. The experiment aimed to study the effects of certain microelements in chelate form *i.e.* zinc (16.5%), ferrous (13%), and manganese (13%) at the rate of 2 and 3 g/l as well as boron at 5 and 10 ppm comparing with fungicide Plantvax 20% EC at 1m/l on some morphological and physiological aspects as well as yield components of cowpea. The role of tested treatments to control the rust disease was also considered.

Detection of cowpea rust was carried out in 13 districts of Dakahlia governorate. Five cultivated zone for each district were investigated for disease incidence and disease severity.

The seeds of cowpea (*Vigna unguiculata*, L. cv. Kariem-7) used in the present investigation were supplied from Legume Research Department, Field Crops Institute, Agricultural Research Center, Giza. A randomized complete block design with three replicates was used in this experiment. The experimental plot contained 5 ridges, 70 cm apart and 3 m length, occupying 10.5 m² (1/400 Fed.) which was carried out according to the wet method on one side of the ridges on 3rd May 2005 and 5th May 2006, being subjected to natural infestation.

The plants received the amount of fertilizers recommended by the Ministry of Agriculture.

At the age of 30 and 45 days, the plants were sprayed till dripping using small pressure pump (2 L.) with either microelements or fungicide dissolved in distilled water. Tween-80 was used as a wetting agent at concentration of 0.5%. The control was sprayed by distilled water.

Disease assessment

The disease incidence was recorded as percentage of infection and disease severity of cowpea leaf rust were determined on the traditional method adapted the infection type (Mains and Jackson, 1926) in which 0 (No visible symptoms on the leaf) 1 (minute uredinia surrounded necrotic area) 2 (medium uredinia surrounded by necrotic or chlorotic area) 3 (large uredinia surrounded by chlorotic area) 4 (large uredinia without any chlorosis or necrosis area).

Samples were taken after 60 days from sowing to estimate morphological characters (plant height "cm" and number of branches and leaves/ plant) and physiological aspects (phenolics and photosynthetic pigments in the leaves).

Determination of total phenolics compounds:

Total phenolics were determined after 60 days from sowing in fresh shoot using the Folin-Ciocalteu reagent (Singleton and Rossi, 1965). Samples (2g) were homogenized in 80% aqueous ethanol at room temperature and centrifuged at 10000 rpm for 15 min. under cooling and the supernatants were saved. The residues were re-extracted twice with 80% ethanol and supernatants were pooled, put into evaporating dishes and evaporated to dryness at room temperature. Residues were dissolved in 5 ml of distilled water. One-hundred microlitres of this extract were diluted to 3 ml with water and 0.5 ml of Folin-Ciocalteu reagent was added. After 3 min., 2 ml of 20% of sodium carbonate was added and the contents were mixed thoroughly. The color was developed and absorbance measured at 650 nm. in a Spectrophotometer after 60 min. using catechol as a standard. The results were expressed as mg catechol/ 100 g fresh weight material.

Determination of photosynthetic pigments:

The blade of terminal leaflet of the 3rd leaf tip from plant was taken to determine photosynthetic pigments (chlorophyll a, b and carotenoids) which were extracted with methanol and determined according to (Mackinney, 1941).

For yield components samples were taken at 100 days from sowing to estimate number of pods/ plant and weight of 100 seeds.

Statistical Analysis

Obtained data were subjected to statistical analysis of software CoStat (2005) as the used technique of analysis of variance (Gomez and Gomez, 1984). The means were compared using Least Significant Difference (L.S.D.) at $P=0.05$ as outlined by Duncan (1955).

RESULTS

Data in Table (1) show that, the high disease incidence was in Sherbin distract followed by Tmai Elamdid distract. While, the low disease incidence was in Aga distract. On the other side, the high value of disease severity occurred under Tmai Elamdid, Mit Salsyl and Bilqas distracts. While, Minit Elnasr distract was came late.

Four microelements (boron, iron, zinc and manganese) and fungicide (Plantvax 20% EC) were tested for their effect on rust incidence and severity of cowpea under field conditions as shown in Table (2).

Table (1): Detection of rust disease in cowpea plant in different location in Dakahlia governorate

Locations	Disease Incidence %	Disease Severity
El-Mansoura	24.0	1.8
Talkha	31.0	2.2
Tmai Elamdid	35.2	2.6
El-Manzalah	20.8	1.4
El-Gamalia	23.6	2.0
Mit Salsyl	31.6	2.6
Minit Elnasr	22.6	1.2
Dekmis	25.8	1.6
Aga	19.4	1.8
El-Snblawin	32.4	2.2
Bilqas	34.4	2.6
Mit Ghamr	25.8	2.0
Sherbin	37.8	2.2

Table (2): Microelements against rust disease in cowpea plant 60 days after sowing

Treatments	Disease Incidence %	Disease Severity
Control	88.33 a	3.67 a
Fungicide	18.67 ef	1.67 b-d
Zinc 2 g/l	33.00 c	2.67 a-c
Zinc 3 g/l	23.33 de	1.67 b-d
Ferrous 2 g/l	29.00 cd	2.67 a-c
Ferrous 3 g/l	18.00 ef	1.67 b-d
Manganese 2 g/l	26.00 cd	2.33 a-c
Manganese 3 g/l	15.33 f	1.33 cd
Boron 0.025 g/l	21.67 d-f	1.67 b-d
Boron 0.05 g/l	5.67 g	0.67 d

Values within the same column followed by the same letter(s) are not significantly different ($P=0.05$).

It could be observed that all the tested concentrations of microelements and fungicide decreased significantly the disease incidence of rust disease on cowpea leaves.

Generally, the higher the concentration of the tested element the greater the inhibition of disease incidence. On the other hand, all concentrations of the tested microelements and fungicide led to remarkable reduction in disease incidence compared with the check treatment. Boron at 0.05 g/l was the most effective in this respect followed by manganese at 3 g/l then ferrous at 3 g/l, which did not show any significant differences compared with fungicide on rust disease incidence.

Concerning the effect of the tested microelements on rust disease severity, data in the same table show that, the application of (3 g/l) zinc, ferrous (3 g/l) and the low level of boron (0.025 g/l) caused the same effect and gives the same value (1.67). The lowest value of disease severity (0.67) occurred under the application of the high level of boron (0.05 g/l) followed by 3 g/l manganese (1.33). While, the application of 2 g/l zinc, ferrous and manganese had no significant effect on rust disease severity compared with control. Zinc or ferrous (3 g/l) or 0.025 g/l of boron had the same effect and gave equal values to that recorded by fungicide treatment (1.67).

Morphological characters

Data concerning the average plant height, number of leaves and branches/plant of cowpea in relation to the effect of either fungicide (Plantvax 20% EC) or microelements are presented in Table (3).

Table (3): Effect of microelements on some growth parameters of cowpea plant 60 days after sowing

Treatments	Plant height (cm)	No. of branches	No. of leaves
Control	77.67 g	5.00 g	32.67 g
Fungicide	78.33 g	5.33 g	34.00 fg
Zinc 2 g/l	99.67 bc	7.00 c-e	55.67 c
Zinc 3 g/l	97.33 cd	6.67 de	50.00 de
Ferrous 2 g/l	87.00 f	5.67 fg	37.67 f
Ferrous 3 g/l	95.00 de	6.33 ef	47.00 e
Manganese 2 g/l	102.67 ab	8.00 ab	64.33 b
Manganese 3 g/l	91.66 e	7.33 b-d	55.00 c
Boron 0.025 g/l	106.00 a	8.33 a	71.00 a
Boron 0.05 g/l	85.66 f	7.67 a-c	55.33 cd

Values within the same column followed by the same letter(s) are not significantly different ($P=0.05$)

Data indicate that fungicide caused insignificant increase in plant height, number of leaves and branches/plant compared with control. While, all microelements caused significant increase in these parameters, the highest means of the studied parameters were obtained by the application of low levels of boron (0.025 g/l), manganese (2 g/l) and zinc (2 g/l) in decreasing order. However, the low level of ferrous came at the end compared with other microelements. It is worthy to mention that, the low concentration of all microelements was more effective than the higher concentration except for ferrous.

Yield components

The effect of microelements as well as fungicide (Plantvax 20% EC) on number of pod/plant and weight of 100 seeds (g) of cowpea at 100 days after sowing are presented in Table (4).

It can be easily notice that the fungicide caused insignificant increase in number of pod/plant and weight of 100 seeds, at the same time gave significant lower values compared with the tested microelements.

Table (4): Effect of microelements on yield components of cowpea plant after 90 days from sowing

Treatments	Number of pods/ plant	Weight of 100 seed (g)
Control	50.00 f	12.19 e
Fungicide	52.00 f	12.24 e
Zinc 2 g/l	70.33 c	12.77 bc
Zinc 3 g/l	66.00 d	12.56 cd
Ferrous 2 g/l	59.33 e	12.39 de
Ferrous 3 g/l	64.33 d	12.71 bc
Manganese 2 g/l	80.33 b	12.75 bc
Manganese 3 g/l	72.66 c	12.91 ab
Boron 0.025 g/l	89.33 a	12.89 ab
Boron 0.05 g/l	70.00 c	13.09 a

Values within the same column followed by the same letter(s) are not significantly different ($P=0.05$)

Data also show that, the low level of boron (0.025 g/l), manganese (2 g/l) and zinc (2 g/l) recorded the highest values followed by manganese (3 g/l) then boron at 0.05 g/l. While, both concentrations of ferrous came at the end. The weight of 100 seeds increased significantly under the application of any of the microelements. The highest values in this parameter were obtained by boron at 0.05 g/l followed by manganese at 3 g/l then boron at 0.025 g/l, taking in consideration the non significant differences among these treatments.

Total phenol content

It is well known that plant phenolics, in general, are highly effective antioxidants. The total phenols in fresh cowpea plants were determined colorimetrically and calculated as catechol equivalents. Boron (0.05 g/l), manganese (2 g/l) and manganese (3 g/l) showed significant increases in total phenols contents compared with other treatments as shown in (Table 5). Treatments showed significant increases in total phenols contents compared with fungicide and check treatments.

Table (5): Effect of microelements on phenol content and photosynthetic pigment contents of cowpea plant 60 days after sowing

Treatments	Phenol mg/100g fresh weight	Chlorophyll A mg/g fresh weight	Chlorophyll B mg/g fresh weight	Carotenoids mg/g fresh weight
Control	187.66 c	0.957 g	0.76 e	0.40 f
Fungicide	200.55 bc	1.08 fg	0.89 e	0.42 f
Zinc 2 mg/l	241.03 b	1.40 de	1.31 cd	0.43 f
Zinc 3 mg/l	245.7 b	1.26ef	1.45 c	0.60 de
Ferrous 2 mg/l	234.68 b	1.49 de	1.20 d	0.57 e
Ferrous 3 mg/l	238.55 b	2.06 c	1.91 b	0.77 c
Manganese 2 mg/l	355.83 a	2.38 b	2.06 b	0.89 b
Manganese 3 mg/l	376.20 a	2.89 a	2.56 a	1.08 a
Boron 0.025 g/l	218.45 bc	1.63 d	1.28 cd	0.69 cd
Boron 0.05 g/l	378.54 a	1.99 c	1.47 c	0.76 c

Values within the same column followed by the same letter(s) are not significantly different ($P=0.05$)

Photosynthetic pigments content

The photosynthetic pigment contents in fresh plants were determined as chlorophyll a, chlorophyll b and carotenoids as shown in (Table 5). Manganese (2 and 3 g/l), ferric (3 g/l) and boron (0.025 and 0.05 g/l) showed a significant increase in chlorophyll a content compared with other treatments. Moreover, all treatments showed significant increase in chlorophyll a content compared with fungicide and check treatments.

Chlorophyll b content showed a significant increase in case of manganese at 2 and 3 g/l, ferric at 3 g/l, boron at 0.05 g/l and zinc at 3 g/l. Moreover, all treatments showed significant increase in chlorophyll b content compared with fungicide and check treatments.

Both concentrations of manganese, ferrous (3 g/l) and boron (0.05 g/l) showed a significant increase in carotenoids content compared with other treatments. All treatments showed significant increase in carotenoids content compared with fungicide and check treatments.

DISCUSSION

The results of this study indicate that resistance to cowpea rust disease could be induced by foliar application of microelements. All tested microelements significantly reduced disease incidence and disease severity of cowpea rust. Boron followed by manganese then ferrous were the most effective microelements in reducing cowpea infection while zinc came lately. In this investigation, the induced resistance against rust disease by using microelements may be due to the increase in phenol contents in cowpea leaves.

The exact role of boron in plants appears to be obscure. However, a variety of physiological changes, are induced which differ mainly with the species. Boron may be essential for translocation of carbohydrates in plants. Its deficiency may cause a breakdown of proteins, serious injuries to cells of the apical meristems of stems and roots. Boron concentration in meristems, roots and fruits is much lower than that of leaves (Mayer, 1960).

Carbohydrates are the main repository of photosynthetic energy, they comprise structural polysaccharides of plant cell walls, principally cellulose, hemicellulose and pectin. Also associated with the structural polysaccharides are phenolic compounds, which play an important role in plant defense such, phenols are essential for biosynthesis of lignin, which is considered an important structural component of plant cell walls (Hahlbrock and Scheel, 1989).

Some of the enzyme reactions in the tricarboxylic acid cycle, notably decarboxylases and dehydrogenases activated by manganese. It plays a role in regulating the levels of auxin in plant tissues by activating the auxin oxidase system (Marschner, 1986). The positive effects of iron on reducing rust disease infection may be due to the active iron occurs in numerous enzyme systems either as a structural component of prosthetic groups or a constituent of the protein itself. The best known prosthetic groups are the iron porphyrins which, when attached to specific proteins, are known as haem

proteins. These include peroxidase, catalase and some dehydrogenases. There is a correlation between these enzymes and control of plant diseases (Kabata and Pendias, 1984). This is due to the role of peroxidase activity in disease development that has been correlated with the expression of resistance in different host-pathogen system (Coffey and Cassidy, 1984 and Cadena-Gomez and Nichololson, 1987).

The role of peroxidase is the oxidative cross-linking of pre-existing hydroxyproline-rich structural proteins in cell wall, making it more resistant to degradation by microbial enzymes (Bradley *et al.*, 1992). Peroxidase has another function which increases plant resistance such as lignin production (Hammerschmidt and Kuc, 1982), phenylalanine ammonia lyase activity, and phenol accumulation (Tena and Valbuena, 1983).

Zinc is known to be an essential constituent of only three plant enzymes, namely carbonic anhydrase, alcohol dehydrogenase and superoxide dismutase. Also, it has long been known that zinc has a marked effect on the level of auxin. It appears to be required in the synthesis of intermediates in the metabolic pathway, through tryptophan to auxin (Ohki, 1978).

The stimulatory effects of microelements on yield components may be attributed to the increase in photosynthetic pigments. Results of similar nature were obtained by (Ohki, 1977) and (El-Samnoudi, 1990) who reported that the application of micronutrients (Zn, Mn and Fe) accelerated flowering and thus increased flowering capacity, yield of seeds and pods of faba bean.

Concerning the effects of microelements on photosynthetic pigments, it has been found that boron increase photosynthetic pigments due to the increase in cytokinin content in the plant tissues, which increases chloroplasts number in leaving by increasing both intensity of cell growth phytohormones and activity of cytoplasm ribosomes thus chlorophyll synthesis was stimulated (Brzenkova and Makronozov, 1976). On the other side, the main role identified for manganese is the one it plays in the evolution of oxygen in photosynthesis (Marschner, 1986). Some of the iron can be stored in the leaves as a ferric phosphoprotein, phytoferritin, which serves as a reserve for developing plastids and hence for photosynthesis (Kabata and Pendias, 1984).

Based in the previous results and discussion, it is highly recommended to replace fungicides with microelements (Zinc, Ferrous, Manganese and Boron) during the cultivation of cowpea where they maximize the yield and its components as well as minimize cowpea rust disease. In addition to the beneficial effects on the environment and health.

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تأثير الزنك والحديد والمنجنيز والبورون على مرض الصدا في اللوبيا
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لقد تم تقدير مرض صدا اللوبيا في ١٣ مركز في محافظة الدقهلية. لوحظ أن أعلى نسبة حدوث للمرض كانت في مركز شربين يليه مركز تمي الأمديد. بينما كان أقل نسبة حدوث للمرض كانت في مركز أجا.

وعلى الجانب الآخر كان أعلى قيمة للشدة المرضية بمراكز تمي الأمديد وميت سلسيل وبلقاس. بينما كان مركز منية النصر أقل المراكز في الشدة المرضية.

أدى الرش بالعناصر الصغرى بجميع تركيباتها المختبرة وكذلك المعاملة بالمبيد الفطرى إلى نقص معنوى للإصابة بمرض صدا اللوبيا. وسجلت المعاملة بالبورون ٠,٠٥ جم/لتر أفضل النتائج تبعها المنجنيز ٣ جم/لتر ثم الحديد ٣ جم/لتر.

جميع العناصر الصغرى أدت إلى حدوث زيادة معنوية لصفات إرتفاع النبات وعدد الأفرع وعدد الأوراق وعدد القرون للنبات وكان التركيز المنخفض هو الأكثر تأثيراً في جميع العناصر المختبرة فيما عدا الحديد الذى أظهر عكس ذلك. فى حين لم يظهر أى تأثير معنوى للمعاملة بالمبيد الفطرى على هذه الصفات. وكانت أعلى قيم لوزن ١٠٠ بذرة ناتجة عن المعاملة بالبورون ٠,٠٥ جم/لتر تبعها المعاملة بالمنجنيز ٣ جم/لتر ثم اللورون ٠,٠٢٥ جم/لتر.

تحققت أعلى قيم لمحتوى الأوراق من الفينولات الكلية بالمعاملة بالبورون ٠,٠٥ جم/لتر تبعها المنجنيز ٣ جم/لتر ثم المنجنيز ٢ جم/لتر. ولقد لوحظ فى جميع معاملات العناصر الصغرى زيادة معنوية فى محتوى الكلورفيلات والكاروتنويدات مقارنة بكل من المبيد الفطرى والكنترول. وأظهرت هذه الدراسة العلاقة بين إستحداث المقاومة لمرض الصدا فى اللوبيا وزيادة المحتوى من الفينولات الكلية.