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Inducing Resistance against Leaf Rust Disease of Wheat by some Microelements and Tilt Fungicide

¹Somaya Tohamey and ²Seham A. Ebrahim

¹Plant Pathology Research Center, Agricultural Research Center, Egypt

²Department of Agricultural Botany, Faculty of Agriculture, Zagazig University, Egypt

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Corresponding Author:

Somaya Tohamey
Plant Pathology Research Center,
Agricultural Research Center, Egypt

ABSTRACT

Three nutritional elements, i.e., boron at rate 0.1, manganese and zinc at rate 4 g L⁻¹ as well as tilt fungicide 2.5 m L⁻¹, were applied as a foliar spray to evaluate their capabilities to induce resistance against leaf rust disease of wheat caused by *Puccinia triticina* in greenhouse and field experiments during 2014/2015 growing season in Itay El-Baroud and Kafr El-hamam, Egypt. In greenhouse under artificial inoculation, a significant reduction in rust disease incidence and severity was found because of spraying wheat plants with tilt, boron, zinc and manganese, respectively, compared to untreated infected control. Same trend of results recorded in the field experiments conducted under natural infection at two locations, i.e., Itay El-Baroud and Kafr El-hamam. Tested treatments improved spike weight, grain weight spike and 1000 grain weight, over the control. Boron, zinc and manganese application increased activity of enzymes i.e., peroxidase and poly phenoloxidase and concentration of total phenols. Such increases in enzymatic activities and concentration of total phenols may be responsible for controlling leaf rust disease in wheat.

Key words: Wheat, induced resistance, leaf rust, microelements, tilt fungicide, polyphenoloxidase, peroxidase, total phenol

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important cereal crop in all over the world. Wheat is considered the first strategic food crop in Egypt. Its stable diet for the world population and contributes more calories and protein to the world diet more than only other cereal crops. It is grown on roughly 200 million ha with an average production of 600 million t (Rajaram and Braun, 2006). The cultivated area of wheat in Egypt is about 3.2 million fedan with an average production of 9.5 million t (Hasan, 2013). In Egypt wheat plants are liable to attack by many diseases such as; rusts, smuts and some other minor disease. Leaf rust caused by *Puccinia triticina*, is the most widespread diseases in about all the wheat growing area in Egypt. Balanced nutrition does not only help to achieve better yield in crop production but also

allows plants to protect themselves from new infection (Agrios, 2005; Narayanasamy, 2002). Fungicides are currently the only commercially available practices for controlling rust disease of wheat.

The control of plant diseases using classical pesticides raises serious concerns about food safety, environmental quality and pesticide resistance, which have dictated the need for alternative pest management techniques. In particular, nutrients could affect the disease tolerance or resistance of plants to pathogens. Manganese can control a number of diseases, Mn has an important role in lignin biosynthesis, phenol biosynthesis, photosynthesis and several other functions (Dorads, 2008). Boron was found to reduce the severity of many diseases because of the function that B has on cell wall structure, plant membranes and plant metabolism. Abd El-Hai *et al.* (2007), in laboratory and field experiments,

stated that nutritional elements (ferrous, zinc, calcium and manganese), were promising in controlling both rust and chocolate spot diseases in faba bean. Qin *et al.* (2010) revealed that boron strongly inhibited spore germination, germ tube elongation and mycelial spread of *Botrytis cinerea* in the culture medium. Moreover, application of boron at 1% caused the appearance of abnormal spores (disrupted) in some cases. Furthermore, boron led to the leakage of cellular constituents (soluble proteins and carbohydrates) from hyphae of *B. cinerea*. Morsy (2012) studied the effect of three nutritional elements i.e., manganese, zinc and calcium and plantvax 20% EC against faba bean rust disease and found significant reduction disease by plantvax, calcium, zinc and manganese, respectively. Liew *et al.* (2012) evaluated the effects of copper (Cu) and boron (B) foliar applications on the reduction of fungal diseases of rice and found that the foliar application of Cu and B was found to be able to reduce fungal disease infestation in MR219 rice cultivar and subsequently increase rice yield. Tarabih and El-Metwally (2014) found that boric acid 1% and jojoba oil 0.1% treatments gave maximum reduction in linear growth and dry weight of *Penicillium digitatum* and *P. italicum* (the causal blue and green rot of orange) as well as disease infection (0.0%) caused by both fungi in the two season. The present investigation aimed to evaluate the effectiveness of some microelements and fungicides to induce resistance against leaf rust disease of wheat caused by *Puccinia triticina* in greenhouse and field experiments.

MATERIALS AND METHODS

Three microelements, i.e., manganese and zinc at rate 4 g L⁻¹, boron at rate 0.1 g L⁻¹ as well as fungicide tilt 2.5 m L⁻¹, were applied as foliar application to evaluate their capabilities to induce resistance against leaf rust disease of wheat in greenhouse and field experiments during 2014/2015 growing season.

Inocula preparation (leaf rust urediospores): Fresh mixtures of aggressive pathotypes of *Puccinia triticina*, urediospores were collected from infected adult wheat plants in greenhouse of Wheat Dis. Research Department, Plant Pathology Research Institute, ARC. Urediospores were mixed with talc powder (1:20 v/v) in baby cyclone and used in case of artificial inoculation.

Wheat grains were obtained from Wheat Research Department, Crops Research Institute, Agriculture Research Centre, Giza. The active ingredient is propiconazole 41.8 and 58.2% other ingredients, Tilt was obtained from Syngenta Co, Egypt. Recommended dose of chemical fertilizers NPK 75-31-48 kg feddan⁻¹. Recommended dose of chemical fertilizers NPK 0.0006-0.000248-0.000384 kg pot⁻¹, Feddan = 4200 m².

Greenhouse experiments: The experiments were carried out by adult plant stages using genotype Sids-1, susceptible to rust disease. Wheat grains were obtained from Wheat Research Department, Crops Research Institute, Agriculture Research Centre, Giza.

Treatments as follow:

- 1-Zinc (Zn) 4 g L⁻¹ 2-Manganese (Mn) 4 g L⁻¹ 3-Boron (B) 0.1 g L⁻¹
- 4-Tilt fungicide 2.5 m L⁻¹ 5-untreated infected control

Adult stage experiments: Ten wheat grains of susceptible genotype-Sids-1 was sown in plastic pots (25 cm diameter) during the beginning of 25th November and received normal irrigation and fertilization. Plants at 30 days after sowing were thinned into ten plants/pot, after 60 day from sowing, plants sprayed with microelements and tilt fungicide and after 72 h inoculated with urediospores of *P. triticina*. Inoculated plants were immediately transferred to dew chamber pre-conditioned to an air temperature of 15-17°C and incubated for 48 h. The plants were then transferred to the greenhouse bench and grown under the conditions described above. Treatments were applied to the upper and lower surfaces until runoff. Control plants were sprayed with distilled water only. All trials were conducted in a complete randomized block design with three replicates.

Artificially inoculated plants were examined to estimate:

- Disease parameters
- Incubation period (IP): Time from inoculation to commencement of sporulation was recorded according to Katsuya and Green (1967)
- Latent period (LP): Latent period was measured according to Parlevliet (1975) by counting the number of visible pustules on market leaves daily until no more pustules appeared. From these data, time between inoculation and 50% of the pustules just visible was estimated
- Leaf rust severity (%) according to Long *et al.* (1992)

Biochemical changes associated with induced resistance:

This study was conducted to identify some biochemical changes associated with induced resistance by some microelements and fungicide. Activity of oxidative enzymes, i.e., peroxidase (POD), polyphenoloxidase (PPO), as well as total phenols content were determined in wheat leaves of treated and untreated plants.

Determination of oxidative enzymes activity: The extraction procedure was essentially based on the methods described by Biles and Martyn (1993) to determine the activity of peroxidase (PO) and polyphenoloxidase (PPO) as follows: Samples of leaves from each treatment and control plants were collected, 24 h after application. One gram of leaf tissues was ground in 2 mL of sodium phosphate buffer (pH 6.5), using a

mortar and pestle. Samples were transferred to eppendorf tubes and then centrifuged for 20 min at 12000 rpm at 4°C. Supernatant, was stored at -8°C. Three replicates were prepared for each treatment.

Polyphenoloxidase activity: Polyphenoloxidase activity was determined according to the method described by Maliak and Singh (1980). The reaction mixture contained 3.0 mL buffered catechol solution (0.01 M), freshly prepared in 0.1 M phosphate buffer (pH 6.5). The reaction was started by adding 100 µL of the crude enzyme extract. Changes in the absorbance at 495 nm were recorded every 30 sec intervals for 3 min.

Peroxidase activity: Peroxidase activity was determined according to the method described by Hammerschmidt *et al.* (1982). The reaction mixture consisted of 2.9 mL of a 100 mM sodium phosphate buffer (pH 6.5) containing 0.25% (v/v) catechol and 100 mM H₂O₂. The reaction was started by adding 100 µL of the crude enzyme extract. Changes in the absorbance at 495 nm were recorded every 30 sec intervals for 3 min. Enzyme activity was expressed as increase in absorbance min⁻¹ g⁻¹ fresh weight.

Extraction and determination of total phenols: Samples of leaves from each treatment and control plants were collected 48 h after application. One gram of leaf tissue was mixed with 15 mL of ethanol 80% and stored in a dark bottle at 4°C for 72 h. Ethanol was daily changed, then all extracts were combined and filtered for determination using Unicou UV-2100 Spectrophotometer. Total phenol estimation was carried out with folin-Ciocalteu reagent according to the method described by Maliak and Singh (1980).

Field experiments: They were carried out at experimental farm of Etay El-Barood (El-Behera Governorate) and Kafr El-Hamam (El-Sharkia Governorate) stations under natural infection during 2014/2015 growing season. The experiments were designed in a complete randomized block design. Three replicates were used for each treatment. The plot size was 10.5 m², containing 6 rows with 20 cm between rows. Each row was sown by 5 g wheat grains of susceptible genotype-Sids-1. Artificial inoculation using mixtures of *P. triticina* was carried out at booting stage (approximately 92 days after planting). Plants were sprayed, with each treatment separately, immediately starting 3 days before inoculation.

This experiment included the following treatments:

- 1-Zinc (Zn) 4 g L⁻¹ 2-Manganese (Mn) 4 g L⁻¹ 3-Boron (B) 0.1 g L⁻¹
- 4-Tilt fungicide 2.5 mL L⁻¹ 5-untreated infected control

Effect of different microelements and fungicide on disease parameters: Leaf rust severity (%) according to Long *et al.* (1992), disease parameters were expressed by five types; immune = (0), resistance = (R), Moderately Resistance (MR), Moderately Susceptible = (MS) and Susceptible = (S) as described by Roelfs *et al.* (1992).

Physiological analysis

Determination of macro elements: Chemical determination of nitrogen, phosphorous and potassium, concentrations in leaves of were carried out using the acid digest of the dried wheat organs according to Piper (1947).

Total nitrogen and crude protein: Total nitrogen concentration in leaves of wheat was determined colorimetrically using Nessler solution according to the method described by Naguib (1963) (Crude protein = total nitrogen×6.25).

Phosphorus concentration: Total phosphorus concentration in leaves of wheat was determined colorimetrically by the hydroquinone method as described by Snell and Snell (1954).

Potassium concentration: The potassium concentration in leaves of wheat was determined using a Carlzeiss Flamphotometer with acetylene burn as described by Brown and Lilleland (1946).

Determination of micro elements: Boron, Zn and Mn were determined in leaves using the methods described by Chapam and Pratt (1961).

Effect of different microelements and fungicide on yield components: At harvest stage, three replicate samples were randomly collected for crop yield analysis, the mean values of yield components parameters i.e., spike weight (g), grain weight/spike (g) and 1000 grain weight (g) were determined.

Statistical analysis: Data was analyzed with the statistical analysis system SAS. All multiple comparisons were first subjected to analysis of variance (ANOVA) comparisons among means was carried out according to Duncan's multiple range test at (p = 0.05) (Duncan, 1995).

RESULTS AND DISCUSSION

Greenhouse tests

Effect of some microelements and tilt fungicide on the severity of rust disease: Data in Table 1 show that the effect of spaying all microelements and fungicide on severity of leaf rust disease of wheat under greenhouse conditions. A significant reduction of the disease was recorded in the plants pre sprayed with tested treatments compared with the untreated

Table 1: Effect of spraying adult plants cv. Sids-1-with some microelements and tilt fungicide on the severity of leaf rust disease under greenhouse conditions

Treatments	Disease severity (%)		Disease severity (%)		Means	
	45 days	Reduction (%)	60 days	Reduction (%)	D.S	Reduction (%)
Mn (4 g L ⁻¹)	31.6 ^b	58.7	40.0 ^b	52.9	35.8	55.7
Zn (4 g L ⁻¹)	25.0 ^c	67.4	36.6 ^b	56.9	30.8	61.9
B (0.1 g L ⁻¹)	20.0 ^d	73.9	30.0 ^c	64.7	25.0	69.1
Tilt (2.5 m L ⁻¹)	5.6 ^e	92.7	5.9 ^d	93.0	5.8	92.8
Untreated infected control	76.6 ^a	-	85.0 ^a	-	-	-

Values of each column followed by the same letter are not significantly different according to Duncans multiple test (p = 0.05), D.D: Disease severity

Table 2: Effect of spraying wheat plants with some microelements on activity of peroxidase and polyphenol oxidase and total phenols mg catechol/100 g fresh weight

Treatments	POX activity	Increased (%)	PPO activity	Increased (%)	Total phenol mg/100 g fresh weight	Increased (%)
Mn (4 g L ⁻¹)	2.2 ^{ab}	18.2	0.06 ^b	83.3	214.3 ^b	32.8
Zn (4 g L ⁻¹)	2.2 ^{ab}	18.2	0.05 ^c	80.0	237.5 ^a	39.4
B (0.1 g L ⁻¹)	2.3 ^a	21.7	0.06 ^b	83.3	239.4 ^a	39.8
Tilt (2.5 m L ⁻¹)	2.2 ^{ab}	18.2	0.30 ^a	66.7	235.3 ^a	38.8
Untreated infected control	1.8 ^b	00.0	0.01 ^d	00.0	144.0 ^c	00.0

Values of each column followed by the same letter are not significantly different according to Duncans multiple range test (p = 0.05), POX: Peroxidase, PPO: Polyphenol oxidase

Table 3: Effect of spraying wheat plants (cv. Sids-1) with manganese, zinc, boron and tilt, on the severity of rust disease under field condition at Etay EL-Baroud and Kafr El-Hamam location during 2014/2015 growing season

Treatments	Etay El-Baroud			Kafr El-Hamam			Mean			General mean	Reduction (%)
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd		
Mn (4 g L ⁻¹)	20.0	25.0	33.3	25	30	35	22.5	27.5	34.1	28.0	65.7
Zn (4 g L ⁻¹)	13.3	21.6	24.0	15	20	25	14.1	20.8	24.5	19.8	75.8
B (0.1 g L ⁻¹)	11.6	15.0	20.0	10	15	20	10.8	15.0	20.0	15.3	81.3
Tilt (2.5 m L ⁻¹)	6.6	6.7	8.3	5	6	7	5.8	6.3	7.6	6.6	91.9
Untreated infected control	75.0 ^a	76.6	83.0	80 ^a	85	90	77.5	82.5	86.5	81.7	00.0

Values of each column followed by the same letter are not significantly different according to Duncans multiple range test (p = 0.05)

infected control. The fungicide tilt, 2.5 m L⁻¹, showed the highest effect against rust disease occurrence and the best treatment in reducing rust severity followed by boron, zinc and manganese, respectively. While, Mn 4 g L⁻¹ was the least effective treatment. Moreover, reduction of leaf rust severity was higher after 45 day than after 60 days.

Effect of some microelements and tilt fungicide on activity of peroxidase (POX) and polyphenol oxidase (PPO) and total phenols: Results obtained in Table 2 indicate that significant increasing (%) in the activities of POX and PPO and concentration of total phenols in the case of spaying tilt fungicide and microelements.

Spraying wheat plants with all tested microelements individually increased activity of both peroxidase (PO) and polyphenoloxidase (PPO) in wheat leaves compared with untreated infected leaves. Boron showed the highest level of both oxidative enzymes (PO and PPO) activity followed by Mn and Zn. Whereas, the least enzymatic activity was recorded with tilt fungicide. Total phenols were higher in wheat leaves treated with boron, zinc and manganese application than those of the untreated infected control. Boron ranked the best treatment in the increasing concentration of total phenols followed by Zn, tilt and Mn on the respectively. Our results indicate that microelements led to a significant increase in the activity of the defense enzymes PPO and PPO of wheat plants infected by *P. tricina*.

The increase of POX activity due to microelements has been involved in lignification (Lagrimini *et al.*, 1987) as well as specific cell wall peroxidase might be required to generate H₂O₂ (Van Huystee, 1987). Also, Nandakumar *et al.* (2001) published that peroxidase might stimulate the interaction Ca²⁺ signals required for induction of defense responses. The PPO is involved in the oxidation of polyphenols into quinines (antimicrobial compounds) and lignification of plant cells during microbial invasion. A number of studies indicated that phenol-oxidizing enzymes may be participating in the responding defence reaction and hypersensitivity by inducing plant resistance against fungi. The include common potato (*Fusarium sambucinum*) (Ray *et al.*, 1998) and wheat (*F. graminearum*) (Mohammadi and Kazemi, 2002).

Field tests: This experiment was carried out in the experimental farm of Etay El-Baroud (El-Behera Governorate) and Kafr El-Hamam (El-Sharkia Governorate) stations under natural infection during 2014/2015 growing season.

Effect of some microelements and tilt fungicide on the severity of rust disease of wheat plants (cv. Sids-1): Data presented in Table 3 show the efficacy of spaying wheat plants (cv. Sids-1) with the tested microelements (manganese, zinc, boron) and tilt on wheat leaf rust severity under field condition at two locations Etay El-Baroud and Kafr El-Hamam locations during 2014/2015 growing season after 45, 60 and

Table 4: Effect of spraying with manganese, zinc, boron and tilt on the component of macro and microelements of wheat plants (cv. Sids-1)

Treatments	N (%)	Crude protein	P (%)	K (%)	Mn	Zn	B
Mn (4 g L ⁻¹)	0.502 ^b	3.14 ^b	0.184 ^a	0.486 ^b	87.11 ^a	42.23 ^{ab}	11.15 ^{bc}
Zn (4 g L ⁻¹)	0.551 ^a	3.44 ^a	0.198 ^b	0.49 ^a	71.36 ^b	49.43 ^a	15.29 ^{ab}
B (0.1 g L ⁻¹)	0.507 ^b	3.17 ^b	0.158 ^c	0.475 ^c	69.39 ^c	44.16 ^{ab}	17.02 ^a
Tilt (2.5 m L ⁻¹)	0.488 ^b	3.05 ^c	0.146 ^d	0.468 ^d	55.29 ^d	38.65 ^{bc}	10.35 ^{abc}
Untreated infected control	0.338 ^d	2.11 ^d	0.094 ^e	0.317 ^e	48.15 ^e	33.11 ^c	9.14 ^c

Values of each column followed by the same letter are not significantly different according to Duncans multiple range test (p = 0.05)

75 days after sowing. The obtained data results revealed that all the tested treatments reduced significantly disease severity compare to the control. The first spay showed the best protection against the disease. In this respects, tilt was the most effective treatment, where it recorded the lowest degree of disease severity followed by boron, zinc and manganese. All tested substances showed also a significantly protection against the disease over the control.

Effect of some microelements and tilt fungicide on the component of micro and macro elements of wheat plants (cv. Sids-1):

The obtained data in Table 4 show the effect of spraying wheat plants (cv. Sids-1) with manganese, zinc, boron and tilt, on the component of micro and macro elements under field condition at Etay EL-Baroud and Kafr El-Hamam Location during 2014/2015 growing season. The results indicated that N, P, K, Mn, Zn, B concentrations and total protein in shoot of wheat plants were significantly increased by spraying the plants under field conditions by Zn, Mn, B and tilt fungicide compare to the control was sprayed with the water only.

Boron is the least understood essential micronutrient for plant growth and development and at the same time B deficiency is the most widespread micronutrient deficiency in the world (Brown *et al.*, 2002; Blevins and Lukaszewski, 1998; Roemheld and Marschner, 1991). Boron has a direct function in cell wall structure and stability and has a beneficial effect on reducing disease severity. The function that B has in reducing disease susceptibility could be because of (Dorads, 2008), (1) Function of B in cell wall structure, (2) Function of B in cell membrane permeability, stability or function, or (3) Its role in metabolism of phenolics or lignin (Brown *et al.*, 2002; Blevins and Lukaszewski, 1998). Boron promotes stability and rigidity of the cell wall structure and therefore supports the shape and strength of the plant cell (Marschner, 1995; Brown *et al.*, 2002). Furthermore, B is possibly involved in the integrity of the plasma membrane (Marschner, 1995; Brown *et al.*, 2002; Dordas and Brown, 2005). Boron has been shown to reduce diseases caused by *Plasmidiophora brassicae* (Woron.) in crucifers, *Fusarium solani* (Mart.) (Sacc.) in bean, *Verticillium albo-atrum* (Reinke and Berth) in tomato and cotton, tobacco mosaic virus in bean, *Tomato yellow leaf curl virus* in tomato, *G. graminis* (Sacc.) (Graham and Webb, 1991) and *Blumeria graminis* (D.C.) (Speer) in wheat (Marschner, 1995). Manganese, zinc, boron

and tilt reduced the injurious effects of leaf rust of wheat. Boron followed by zinc were the most effective in reducing wheat infection by rust disease, while manganese was most moderate effective under both green house and field conditions. In addition, all of treatments used enhanced plant growth productivity and yield as compared with the control. In the present study, in greenhouse and field condition all the treatments significantly reduced disease severity compared to control (untreated). Abd El-Hai *et al.* (2007) reported that, treating cowpea plants with zinc, ferrous and manganese caused high reduction in cowpea rust and increased seed yield. Boron is one the most elements which enter of the cell wall. The role of zinc in enhancement the growth parameters, which followed by stimulation in resistance of wheat to leaf rust disease might be due to that, zinc is known to be essential constituted of three plant enzymes, i.e., carbonic anhydrase, alcohol dehydrogenas and superoxide dismutase. In addition, zinc has a marked effect on the level of auxin (Ohki, 1978) which in turn encourage the meristemic activity of the plant which resulted in more cell division and cell enlargement (Devlin and Withan, 1983). Manganese plays a role in regulating the levels of auxin in plant tissues by activating photosynthesis especially photosystem two (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 Withan, 1983).

Effect of some microelements and tilt fungicide on yield component of wheat plants (cv. Sids-1):

Also, data in Table 5 show the effect of spraying wheat plants cv. Sids-1 with manganese, zinc, boron and tilt, on yield component under field condition at Etay EL-Baroud and Kafr El-Hamam location during 2014/2015 growing season. All tested treatments increased significantly yield parameters over the control. Tilt fungicide treatment was more effective in increasing yield parameters i.e., spike weight/g, grain spike weight (g) and 1000 kernel weight (g) followed by boron, zinc and manganese, respectively. Zeidan *et al.* (2010) reported that grain yield, straw yield, 1000 grain weight and number of grains/spike, Fe, Mn and Zn concentration in flag leaves and grains as well as, protein content in grain were significantly increased by application of these elements. Potarzycki and Grzebisz (2009) reported that zinc exerts a great influence on basic plant life processes, such as (i) Nitrogen metabolism-uptake of nitrogen and protein quality and (ii) Photosynthesis-chlorophyll synthesis, carbon

Table 5: Effect of spraying wheat plants cv. Sids-1 with manganese, zinc, boron and tilt, on yield components under field condition at Etay EL-Baroud and Kafr El-Hamam location during 2014/2015 growing season

Treatments	Spike weight (g)			Grain spike weight (g)			1000 kernel weight (g)		
	Etay El-Baroud	Kafr El-Hamam	Mean	Etay El-Baroud	Kafr El-Hamam	Mean	Etay El-Baroud	Kafr El-Hamam	Mean
Mn (4 g L ⁻¹)	2.3 ^{ce}	2.4 ^{ce}	2.4	2.0 ^{cde}	2.1 ^{bcd}	2.1	56 ^{bcd}	55 ^{bcd}	55.5
Zn (4 g L ⁻¹)	2.9 ^{ac}	2.5 ^{bd}	2.7	2.4 ^{abc}	2.3 ^{bc}	2.4	60 ^{ad}	58 ^{bcd}	59.0
B (0.1 g L ⁻¹)	3.0 ^{ac}	2.8 ^{ad}	2.9	2.7 ^{ab}	2.4 ^{abc}	2.6	64 ^{abc}	62 ^{ad}	63.0
Tilt (2.5 mL ⁻¹)	3.4 ^{ab}	3.5 ^a	3.5	3.0 ^a	3.0 ^a	3.0	69 ^a	65 ^{ab}	67.0
Untreated infected control	1.9 ^{de}	1.8 ^e	1.9	1.4 ^e	1.5 ^{de}	1.5	54 ^{cd}	52 ^d	53.0

Values of each column followed by the same letter are not significantly different according to Duncans multiple range test (p = 0.05)

anhydrase activity; reported that Zn-deficient plants reduce the rate of protein synthesis and protein content. Morsy (2012) found that Zn, Mn and calcium treatments improved plant growth (plant height) and yield (number of pods/plant and weight of 100 seeds) over the control.

The effects of manganese, zinc and boron on the yield components may be attributed to the increase in spike weight, grain spike weight and 1000 kernel weight. Based on the previous results and discussion it is highly recommended to replace fungicides with microelements (manganese, zinc) as well as moron during the cultivation wheat as they promising trend in controlling of rust disease of wheat it decreased infection, environmental friendly and coast effective compared with the ordinary fungicides.

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