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## Management of Chocolate Spot Disease of *Vicia faba* using Nutritional Elements

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### ABSTRACT

Chocolate spot disease is a major limiting factor of faba bean growth and productivity. In this study, laboratory and field experiments were conducted in 2012/2013 and 2013/2014 winter seasons to determine the effect of molybdenum, boron and calcium in comparison to bio-fungicide (blight stop) and chemical fungicide (Kocide 101) for the management of chocolate spot disease and enhancing growth, physiological activities and yield of faba bean plants. The results of laboratory experiments revealed that boron at 6 ppm was the most effective on decreasing pathogen growth after kocide 101 and blight stop. However, calcium 6 mL L<sup>-1</sup> has no effect on fungal linear growth. In field, kocide 101 and blight stop led to the maximum reduction of chocolate spot disease severity followed by boron at 2 ppm then molybdenum at 4 ppm then calcium at 6 mL L<sup>-1</sup>. The highest values of chlorophyll a and b were recorded with calcium 4 mL L<sup>-1</sup> followed by boron at 2 ppm. Also, the high total phenol and wyceron acid content obtained from the application of boron at 2 ppm after blight stop and kocide 101. From nutritional elements, calcium at 6 mL L<sup>-1</sup> gave highest increase in plant height followed by boron 2 ppm then molybdenum 4 ppm. The highest increase in branches number was recorded with boron at 2 ppm. Calcium at 6 mL L<sup>-1</sup> gave the highest values of pods number, plant seed yield and weight of 100 seed followed by boron at 2 ppm. Nutritional elements (boron at 2 ppm, calcium 6 mL L<sup>-1</sup> and molybdenum at 4 ppm) are promising trend in decreasing of chocolate spot disease of faba bean, where it decreased infection, environmental friendly and coast effective compared with the ordinary fungicides.

**Key words:** Faba bean (*Vicia faba*), chocolate spot disease, *Botrytis fabae*, nutritional elements

### INTRODUCTION

Faba bean (*Vicia faba* L.) is one of important fabaceous crop for human nutritional consumption, due to which it represents an important source of protein in the diet of masses (Nassib *et al.*, 1991). In addition to, faba bean supplies an important benefit to the crop by fixing atmospheric nitrogen which helps improve the soil fertility (Sahile *et al.*, 2011). Seeds of faba bean are rich in protein (28%), carbohydrates (56%) and some other compounds (Tewatia and Virk, 1996). Faba bean is attacked by more than 100 pathogens (Hebblethwaite, 1983). Chocolate spot disease caused mainly by *Botrytis fabae* sard. and to some extent by *B. cinerea* pers. ex. Fr. is an important disease of faba bean causing great losses and sometimes complete crop failures (Koike, 1998; Abou-Zeid and Hassanein, 2000). The use of fungicides is an expensive remedy and

may result in reduction in populations of beneficial microorganisms. This problem has been further complicated by the development of fungicidal resistance, in addition to its negative effects on public health and environmental balance (Khaled *et al.*, 1995). So, using alternative approaches has become more attractive.

Some nutritional elements play important role in plant resistance and decrease the harmful effects of plant diseases e.g., molybdenum (El-Hersh *et al.*, 2011), boron (Abd El-Hai *et al.*, 2007a) and Calcium (Hahlbrock and Scheel, 1989; Abd El-Hai *et al.*, 2007b; El-Baz, 2007).

In recent years, biological control is considered an important approach in agriculture biotechnology. Some fungal and bacterial strains enhance plant growth and being used as potential antagonist for controlling many fungal plant pathogens (Matar *et al.*, 2009; Deshmukh *et al.*, 2010; Bendahmane *et al.*, 2012). The aim of this work is to evaluate the effect of some nutritional elements in reducing chocolate spot disease of faba bean in comparison to bio-fungicide (blight stop) and chemical fungicidal (kocide 101).

## MATERIALS AND METHODS

**Faba bean seeds and tested materials:** Faba bean seeds cv. Giza 843 were obtained from Legume Crops Research Department, Agriculture Research Centre, Giza, Egypt. Molybdenum from ammonium molybdate ( $(\text{NH}_4)_6\text{MO}_7\text{O}_{24}$ ) and boron from boric acid ( $\text{H}_3\text{BO}_3$ ) were obtained from Al-Gamhoria Company, Egypt. Calcium from calciven (calcium chelated on amino and organic acids), blight stop (bio-fungicide which contains some fungal and bacterial strains) were obtained from Department of Environmental and Bio Agriculture, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

**Isolation of *Botrytis fabae*:** *Botrytis fabae* was isolated from naturally infected faba bean leaflets exhibiting symptoms of chocolate spot disease collected from different fields of Dakhlia Governorate. Leaflets were cut into small pieces, each with a single lesion and sterilized by soaking in 2% sodium hypochlorite for 1:2 min. and plated on faba bean dextrose agar medium. The plates were incubated at 20°C for 7 days (Haggag *et al.*, 2006). The isolated fungus was purified and identified on the basis of cultural and microscopic characters following Morgan (1971) and Barnett and Hunter (1987).

**Laboratory experiment (*in vitro*):** This experiment aimed to study the effect of some nutritional elements and bio and chemical fungicides on linear growth, fungal dry weight and sporulation of *Botrytis fabae*. Molybdenum, boron at 2, 4 and 6 ppm and calcium at 2, 4 and 6 mL L<sup>-1</sup> as well as blight stop at 6.25 mL L<sup>-1</sup> and kocide 101 at 3 g L<sup>-1</sup> were separately mixed with PDA before solidification, then poured in sterile Petri dishes. Four plates for each concentration were inoculated in the center with a 5 mm disc of *B. fabae* which had been cut from the periphery of 7 days old culture. Four plates inoculated with *B. fabae* only without any amendment served as control.

The plates were incubated at 20±1°C, linear growth of *B. fabae* measured when particular control filled of Petri dishes with fungal mycelia. The influence of molybdenum, boron at 2, 4 and 6 ppm and calcium at 2, 4 and 6 mL L<sup>-1</sup> as well as blight stop at 6.25 mL L<sup>-1</sup> and kocide 101 at 3 g L<sup>-1</sup> on mycelia dry weight and sporulation of *B. fabae*, were tested on PD broth medium in conical flask containing 20 mL broth medium amended individually with tested concentrations of each addition. A set of similar flask containing chemical free medium served as a check. Four flasks of each concentration were inoculated with 5 mm disc of *B. fabae* taken from 7 days old culture.

After 10 days of incubation at 20°C±1, flasks were shaken (100 rpm) for 1 h, then taken 1 mL from each flask to determine the number of spores using a haemocytometer. The rest of flask was filtered through pre-weighted Whatman No.1 filter paper, washed with distilled water and dried at 70°C in a vacuum oven to constant weight.

**Field experiment (*in vivo*):** Experiments were carried out at Tag El-Ezz, Agriculture Research Station, Dakahlia, Egypt under natural infection during 2012/2013 and 2013/2014 winter seasons. The experiments aimed to study the effects of foliar spray treatments of molybdenum and boron (2, 4, 6 ppm), calcium (2, 4, 6 mL L<sup>-1</sup>), blight stop (6.25 mL L<sup>-1</sup>) and kocide 101 (3 g L<sup>-1</sup>) on reduce faba bean chocolate spot disease and some morphological, yield and physiological aspects.

Seeds of faba bean cv. Giza 843 were sown in 16 and 21 November in the first and second seasons, respectively. All agriculture practices were carried out according to the recommendation of Ministry of Agriculture, Egypt. At the age of 40, 55 and 70 days in the two seasons, the plants were sprayed till dripping using small pressure pump with one of the concentration of treatments above. A complete randomized block design with three replicates was used in the two mentioned seasons.

**Disease assessment:** Disease severity of chocolate spot disease was estimated at 55 and 70 days from sowing according to the scale (class rate from 1-9) of Bernier *et al.* (1993), then calculated using the equation adopted by Hanounik (1986):

$$DS(\%) = \frac{\sum(NPC \times CR)}{NIP \times MSC} \times 100$$

where, NPC is the number of plants in each class rate, CR is the class rate, NIP is the number of infected plants and MSC is the maximum class rate.

**Physiological activities:** After 70 days from sowing the following physiological activities were determination: (1) Photosynthetic pigments (chlorophyll a, b and carotenoides) were determined in the blade of the third leaf from plant tip (terminal leaflet) according to Mackinney (1941), (2) Total phenolic compounds were determined in fresh shoot the Folin-Ciocalteu reagent (Malik and Singh, 1980) and (3) Weyerone acid (phytoalexin) was determined according to the method described by Christian (1990). One gram of fresh tissue was homogenized with 40% methanol and agitated on a shaker for 2 h. The extract was filtered through Whatman No.1 filter paper and the solvent was evaporated in vacuum. The dry residue was dissolved in 1 mL of methanol: 0.1 M methyl acetate buffer (pH 2) (1:1 v/v) and stored in vials. HPLC was used to detect the phytoalexins extracted from the plant tissues. The extract was filtered through a 0.45 µm microfilter and the compounds were separated using a C 18-SAX column (250×4.6 nm) with an HPLC (model HP 1050) equipped with a UV detector. The mobile phase consisted of methanol: 0.1 M acetate buffer (pH 2) (1:1 v/v). The detection was performed at 254 nm and the total run time for the separation was approximately 25 min at a flow rate of 1 mL min<sup>-1</sup>.

**Morphological characters and yield components:** Samples were taken at 90 days from planting to estimate growth parameters per plant (height and number of branches). At harvest, pods number per plant, plant seed yield and weight of 100-seed were recorded.

**Statistical analysis:** Data was subject 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 Steel and Torrie (1980).

## RESULTS

**Laboratory experiment:** Great variation in the linear growth, mycelia dry weight and sporulation of *B. fabae* has been observed (Table 1). Boron and molybdenum at 6 ppm concentration and blight stop completely inhibited the sporulation of *B. fabae*. While, kocide 101 completely inhibited linear growth, dry weight and sporulation of the pathogen. But calcium at 6 ppm had no effect on linear growth of *B. fabae* compared with check. Boron at 6 ppm was most effective on reducing pathogen growth after fungicide (kocide 101) and blight stop. However, calcium at 6 mL L<sup>-1</sup> had no effect on fungal linear growth.

### Field experiments

**Disease assessment:** The effects of nutritional elements on disease severity of chocolate spot disease at two different stages of faba bean were recorded in Table 2. All tested materials decreased significantly faba bean disease severity. Except for kocide 101, the maximum reduction of disease severity at 55 and 70 days from sowing in both seasons was recorded by blight stop followed by boron at 2 ppm then molybdenum at 4 ppm and calcium 6 mL L<sup>-1</sup>.

### Physiological activities

**Photosynthetic pigments:** Chlorophyll is a good parameter reflecting the health condition of any plant and carotenoids are known that as a major endogenous plant antioxidant. In this search, photosynthetic pigments content were determined as chlorophyll a, b and carotenoids as shown in Table 3. Data revealed that, calcium at 4 mL L<sup>-1</sup> give the highest values of chlorophylls a and b followed by boron at 2 ppm then molybdenum at 4 ppm. While the application of kocide 101, molybdenum at 6 ppm and boron 6 ppm came late. On the other side, no significant differences were observed in carotenoids content with all treatments in the first season. Meanwhile, in the second season, the maximum increase in carotenoids occurred under the application of kocide 101 followed by boron at 6 ppm then calcium at 6 mL L<sup>-1</sup> and molybdenum at 6 ppm.

Table 1: Effect of nutritional elements, bio and chemical fungicides on growth of *Botrytis fabae*

Treatments	Linear growth (cm)	Mycelium dry weight (mg)	Sporulation $\times 10^4$ mL <sup>-1</sup>
Control	9 <sup>a</sup>	248.64 <sup>a</sup>	67.33 <sup>a</sup>
Mo 2 ppm	6.6 <sup>b</sup>	187.37 <sup>c</sup>	21.00 <sup>f</sup>
Mo 4 ppm	5.17 <sup>c</sup>	157.07 <sup>d</sup>	16.66 <sup>d</sup>
Mo 6 ppm	3.57 <sup>f</sup>	112.33 <sup>f</sup>	0.00 <sup>e</sup>
B 2 ppm	4.17 <sup>e</sup>	118.17 <sup>f</sup>	0.00 <sup>e</sup>
B 4 ppm	3.33 <sup>f</sup>	103.93 <sup>f</sup>	0.00 <sup>e</sup>
B 6 ppm	2.4 <sup>e</sup>	88.413 <sup>h</sup>	0.00 <sup>e</sup>
Ca 2 mL L <sup>-1</sup>	4.6 <sup>d</sup>	134.37 <sup>e</sup>	23.00 <sup>b</sup>
Ca 4 mL L <sup>-1</sup>	4.27 <sup>e</sup>	114.57 <sup>f</sup>	7.00 <sup>f</sup>
Ca 6 mL L <sup>-1</sup>	9 <sup>a</sup>	226.79 <sup>b</sup>	14.33 <sup>c</sup>
Blight stop	1.27 <sup>h</sup>	79.33 <sup>i</sup>	0.00 <sup>e</sup>
Kocide 101	0.00 <sup>j</sup>	0.00 <sup>j</sup>	0.00 <sup>e</sup>

Values within the same column followed by the same letter's are not significantly different  $p = 0.05$

Table 2: Efficacy of nutritional elements against chocolate spot disease severity on faba bean plant after 55 and 70 days from sowing

Treatments	2012/2013		2013/2014	
	At 55 days	At 70 days	At 55 days	At 70 days
Control	32.60 <sup>a</sup>	36.61 <sup>a</sup>	41.40 <sup>a</sup>	42.70 <sup>a</sup>
Mo 2 ppm	16.39 <sup>f</sup>	19.48 <sup>f</sup>	19.09 <sup>f</sup>	23.04 <sup>f</sup>
Mo 4 ppm	13.58 <sup>f</sup>	16.49 <sup>b</sup>	15.75 <sup>f</sup>	17.86 <sup>b</sup>
Mo 6 ppm	27.51 <sup>c</sup>	27.78 <sup>c</sup>	32.47 <sup>c</sup>	32.50 <sup>d</sup>
B 2 ppm	12.97 <sup>e</sup>	12.79 <sup>j</sup>	13.87 <sup>e</sup>	14.19 <sup>j</sup>
B 4 ppm	24.95 <sup>d</sup>	24.27 <sup>e</sup>	28.67 <sup>d</sup>	27.48 <sup>e</sup>
B 6 ppm	30.39 <sup>b</sup>	31.31 <sup>b</sup>	37.25 <sup>b</sup>	36.48 <sup>b</sup>
Ca 2 mL L <sup>-1</sup>	25.92 <sup>d</sup>	25.85 <sup>d</sup>	23.13 <sup>c</sup>	34.18 <sup>c</sup>
Ca 4 mL L <sup>-1</sup>	19.81 <sup>e</sup>	20.89 <sup>f</sup>	23.62 <sup>e</sup>	25.03 <sup>f</sup>
Ca 6 mL L <sup>-1</sup>	13.30 <sup>f</sup>	15.04 <sup>d</sup>	14.93 <sup>e</sup>	19.14 <sup>h</sup>
Blight stop	10.23 <sup>h</sup>	11.66 <sup>c</sup>	10.06 <sup>h</sup>	13.38 <sup>i</sup>
Kocide 101	4.83 <sup>i</sup>	6.55 <sup>j</sup>	4.18 <sup>i</sup>	6.93 <sup>j</sup>

Values within the same column followed by the same letter's are not significantly different p = 0.05

Table 3: Photosynthetic pigments (mg g<sup>-1</sup> FW) of faba bean plants as a response of nutritional elements after 70 days from sowing during two seasons

Treatments	2012/2013			2013/2014		
	Chl. a	Chl. b	Carotenoid	Chl. a	Chl. b	Carotenoid
Control	0.977 <sup>e</sup>	0.786 <sup>b</sup>	0.370 <sup>ab</sup>	0.843 <sup>f</sup>	0.700 <sup>d</sup>	0.343 <sup>bc</sup>
Mo 2 ppm	1.063 <sup>ef</sup>	0.753 <sup>bc</sup>	0.380 <sup>ab</sup>	1.050 <sup>c</sup>	0.753 <sup>d</sup>	0.300 <sup>c</sup>
Mo 4 ppm	1.293 <sup>c</sup>	0.893 <sup>b</sup>	0.380 <sup>ab</sup>	1.217 <sup>b</sup>	0.850 <sup>c</sup>	0.303 <sup>c</sup>
Mo 6 ppm	0.787 <sup>h</sup>	0.590 <sup>d</sup>	0.407 <sup>ab</sup>	0.763 <sup>h</sup>	0.600 <sup>e</sup>	0.396 <sup>a</sup>
B 2 ppm	1.397 <sup>b</sup>	1.030 <sup>a</sup>	0.370 <sup>ab</sup>	1.336 <sup>b</sup>	0.953 <sup>b</sup>	0.340 <sup>bc</sup>
B 4 ppm	1.120 <sup>e</sup>	0.830 <sup>b</sup>	0.366 <sup>ab</sup>	1.217 <sup>c</sup>	0.770 <sup>d</sup>	0.317 <sup>bc</sup>
B 6 ppm	0.670 <sup>i</sup>	0.553 <sup>d</sup>	0.446 <sup>a</sup>	0.707 <sup>i</sup>	0.570 <sup>e</sup>	0.410 <sup>a</sup>
Ca 2 mL L <sup>-1</sup>	1.207 <sup>d</sup>	0.846 <sup>b</sup>	0.320 <sup>b</sup>	1.190 <sup>d</sup>	0.840 <sup>c</sup>	0.343 <sup>bc</sup>
Ca 4 mL L <sup>-1</sup>	1.533 <sup>a</sup>	1.096 <sup>a</sup>	0.413 <sup>ab</sup>	1.417 <sup>a</sup>	1.097 <sup>a</sup>	0.303 <sup>c</sup>
Ca 6 mL L <sup>-1</sup>	1.030 <sup>f</sup>	0.740 <sup>bc</sup>	0.370 <sup>ab</sup>	0.920 <sup>f</sup>	0.693 <sup>d</sup>	0.397 <sup>a</sup>
Blight stop	1.090 <sup>e</sup>	0.776 <sup>b</sup>	0.370 <sup>ab</sup>	1.150 <sup>d</sup>	0.600 <sup>e</sup>	0.383 <sup>ab</sup>
Kocide 101	0.927 <sup>e</sup>	0.643 <sup>d</sup>	0.473 <sup>a</sup>	0.707 <sup>i</sup>	0.627 <sup>e</sup>	0.413 <sup>a</sup>

Values within the same column followed by the same letter's are not significantly different p = 0.05

**Total phenol content:** It is well-known that plant phenolics, in general, are highly effective antioxidants. Data in Table 4 show that, all treatments at any dose used increased significantly total phenol content in both seasons as compared with a check. The highest values in this parameter were obtained by blight stop followed by kocide 101 then boron 2 ppm and calcium 6 mL L<sup>-1</sup>. Taking in consideration that no significant differences between boron 2 ppm and kocide 101.

**Phytoalexins (wyerone acid):** The phytoalexins (wyerone acid) content in faba bean leaves as affected by nutritional elements and bio and chemical fungicides are illustrated in Fig. 1. The highest amount of wyerone acid occurred under the application of blight stop followed by kocide 101 then boron at 2 ppm.

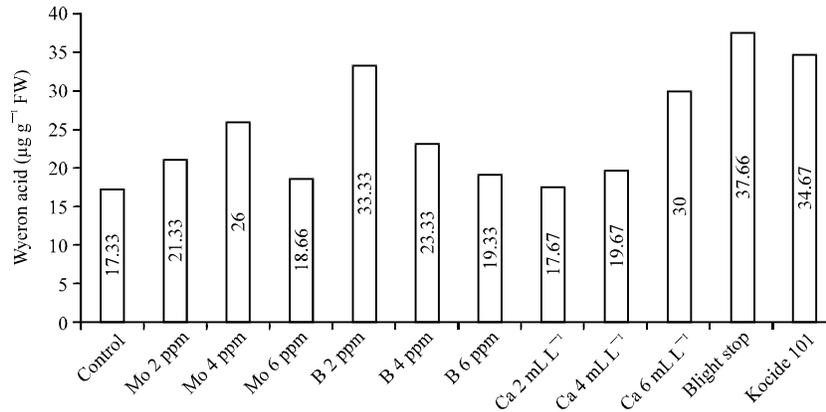


Fig. 1: Effect of nutritional elements on phytoalexin (wyceron acid) level ( $\mu\text{g g}^{-1}$  F.W.) of faba bean. Result are significant at  $p = 0.05$

Table 4: Effect of nutritional elements on total phenol content at 70 days from sowing during two seasons

Treatments	Total phenols (mg/100 g fresh weight)	
	2012/2013	2013/2014
Control	610.07 <sup>h</sup>	558.56 <sup>g</sup>
Mo 2 ppm	743.09 <sup>e</sup>	637.61 <sup>cd</sup>
Mo 4 ppm	780.22 <sup>cd</sup>	662.01 <sup>c</sup>
Mo 6 ppm	642.03 <sup>e</sup>	585.24 <sup>g</sup>
B 2 ppm	808.15 <sup>b</sup>	709.80 <sup>b</sup>
B 4 ppm	771.30 <sup>d</sup>	650.76 <sup>c</sup>
B 6 ppm	701.16 <sup>f</sup>	557.63 <sup>g</sup>
Ca 2 mL L <sup>-1</sup>	631.16 <sup>g</sup>	564.38 <sup>g</sup>
Ca 4 mL L <sup>-1</sup>	790.27 <sup>c</sup>	622.11 <sup>d</sup>
Ca 6 mL L <sup>-1</sup>	731.22 <sup>b</sup>	700.22 <sup>b</sup>
Blight stop	851.15 <sup>a</sup>	759.33 <sup>a</sup>
Kocide 101	813.00 <sup>b</sup>	744.03 <sup>a</sup>

Values within the same column followed by the same letters are not significantly different  $p = 0.05$

**Morphological characters:** The response of faba bean plant growth to the foliar application of nutritional elements and fungicides were determined by means of measuring plant height and number of branches/plant after 90 days from sowing. Data in Table 5 show that fungicide kocide 101 caused significant decrease in plant height in first season and had no significant effect in the second season. While, other treatment caused increase in plant height in both seasons. The highest mean was obtained by the application of blight stop followed by calcium at 6 mL L<sup>-1</sup> then boron 2 ppm and molybdenum 4 ppm. On contrast, Mo 6 ppm, B 4, 6 ppm and Ca 2 mL L<sup>-1</sup> had no significant effect on faba bean plant height compared with check in the first season only. There are a significant differences between all treatments and their effects on number of branches/plant. Blight stop was the most effective followed by boron 2 ppm then calcium 6 mL L<sup>-1</sup>. However, no significant effect in branches number has been obtained under kocide 101, Mo 6 ppm and B 6 ppm treatments compared with a check.

Table 5: Effect of nutritional elements on some morphological characters of faba bean at 90 days from sowing during two seasons

Treatments	Plant height (cm)		No. of branches	
	2012/2013	2013/2014	2012/2013	2013/2014
Control	101.33 <sup>d</sup>	88.67 <sup>h</sup>	3.33 <sup>c</sup>	3.67 <sup>e</sup>
Mo 2 ppm	111.33 <sup>cd</sup>	123.33 <sup>b</sup>	5.00 <sup>ab</sup>	5.67 <sup>a-d</sup>
Mo 4 ppm	122.00 <sup>b</sup>	102.33 <sup>de</sup>	5.33 <sup>ab</sup>	5.33 <sup>b-d</sup>
Mo 6 ppm	104.00 <sup>d</sup>	89.33 <sup>gh</sup>	3.00 <sup>c</sup>	3.33 <sup>e</sup>
B 2 ppm	125.00 <sup>b</sup>	124.33 <sup>b</sup>	5.66 <sup>ab</sup>	6.33 <sup>ab</sup>
B 4 ppm	107.00 <sup>d</sup>	104.67 <sup>d</sup>	5.33 <sup>ab</sup>	5.00 <sup>d</sup>
B 6 ppm	103.00 <sup>d</sup>	92.67 <sup>g</sup>	3.33 <sup>c</sup>	3.33 <sup>e</sup>
Ca 2 mL L <sup>-1</sup>	107.33 <sup>d</sup>	97.33 <sup>ef</sup>	3.67 <sup>c</sup>	4.67 <sup>d</sup>
Ca 4 mL L <sup>-1</sup>	118.33 <sup>bc</sup>	114.00 <sup>f</sup>	4.67 <sup>b</sup>	5.67 <sup>a-d</sup>
Ca 6 mL L <sup>-1</sup>	135.00 <sup>a</sup>	129.33 <sup>ab</sup>	5.33 <sup>ab</sup>	6.00 <sup>a-c</sup>
Blight stop	138.67 <sup>a</sup>	133.67 <sup>a</sup>	6.00 <sup>a</sup>	6.67 <sup>a</sup>
Kocide 101	84.66 <sup>c</sup>	84.66 <sup>h</sup>	3.33 <sup>c</sup>	3.33 <sup>e</sup>

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

Table 6: Efficacy of nutritional elements on yield and its components of faba bean

Treatments	No. of pods/plant		Seed yield/plant		Weight of 100 seeds (g)	
	2012/2013	2013/2014	2012/2013	2013/2014	2012/2013	2013/2014
Control	34.67 <sup>e</sup>	31.33 <sup>f</sup>	61.57 <sup>e</sup>	51.83 <sup>f</sup>	62.07 <sup>fg</sup>	61.69 <sup>f</sup>
Mo 2 ppm	43.33 <sup>de</sup>	49.00 <sup>e</sup>	77.05 <sup>e</sup>	71.34 <sup>e</sup>	63.95 <sup>df</sup>	66.16 <sup>d</sup>
Mo 4 ppm	50.33 <sup>c</sup>	44.67 <sup>d</sup>	80.90 <sup>b</sup>	66.75 <sup>de</sup>	65.15 <sup>e</sup>	62.90 <sup>e</sup>
Mo 6 ppm	36.00 <sup>fg</sup>	33.33 <sup>fg</sup>	62.98 <sup>e</sup>	62.70 <sup>e</sup>	60.36 <sup>f</sup>	61.58 <sup>e</sup>
B 2 ppm	57.33 <sup>b</sup>	60.00 <sup>a</sup>	83.27 <sup>ab</sup>	80.87 <sup>a</sup>	67.19 <sup>b</sup>	65.76 <sup>d</sup>
B 4 ppm	39.67 <sup>ef</sup>	46.00 <sup>d</sup>	70.42 <sup>d</sup>	69.53 <sup>cd</sup>	63.96 <sup>df</sup>	63.67 <sup>e</sup>
B 6 ppm	33.67 <sup>fg</sup>	36.00 <sup>df</sup>	60.15 <sup>e</sup>	62.54 <sup>e</sup>	60.73 <sup>g</sup>	61.26 <sup>e</sup>
Ca 2 mL L <sup>-1</sup>	43.00 <sup>de</sup>	39.67 <sup>e</sup>	76.43 <sup>c</sup>	63.04 <sup>e</sup>	62.81 <sup>ef</sup>	62.41 <sup>e</sup>
Ca 4 mL L <sup>-1</sup>	47.33 <sup>cd</sup>	41.00 <sup>e</sup>	80.90 <sup>b</sup>	64.31 <sup>e</sup>	65.86 <sup>cd</sup>	67.70 <sup>bc</sup>
Ca 6 mL L <sup>-1</sup>	64.00 <sup>a</sup>	54.67 <sup>b</sup>	89.28 <sup>a</sup>	82.35 <sup>a</sup>	72.13 <sup>a</sup>	70.55 <sup>a</sup>
Blight stop	63.67 <sup>a</sup>	53.00 <sup>b</sup>	84.94 <sup>a</sup>	77.00 <sup>b</sup>	68.34 <sup>b</sup>	68.52 <sup>b</sup>
Kocide 101	48.67 <sup>c</sup>	35.33 <sup>f</sup>	75.51 <sup>c</sup>	62.38 <sup>e</sup>	61.76 <sup>fg</sup>	61.74 <sup>e</sup>

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

**Yield components:** Data concerning the average number of pods, seed yield per plant and weight of 100 seed in relation to the effect of nutritional and fungicides are presented in Table 6. Marked differences were detected among all treatments on yield components. Generally, the higher concentration of calcium (6 mL L<sup>-1</sup>) gave the highest values in these parameters followed by blight stop then the low level of boron (2 ppm). However, insignificant effect was noted by kocide 101 on weight of 100-seed compared with check.

## DISCUSSION

The results of this study revealed that all foliar application of microelements (Mo and B), calcium and blight stop decreased the linear growth, dry weight and sporulation of *B. fabae* except for high calcium level (6 mL L<sup>-1</sup>) which showed no effect on linear growth. All treatments decreased the harmful effect of chocolate spot disease. In addition to all of treatments used increased

photosynthetic pigments and phenol contents as well as enhanced plant growth and yield. Blight stop, low level of boron (2 ppm), high level of calcium (6 mL L<sup>-1</sup>) and moderate level of molybdenum (4 ppm) were the most effective.

Blight stop contains some fungal and bacterial strains which considered as a bio-control agents. The effects of bio-control agent including several ways: (1) Nutrient competition, a bio-control agent may grow faster or use its food source more efficiently than the pathogen. (2) Antibiosis, a biocontrol agent may release a product that slows down or kills the pathogens in the vicinity of such a product. (3) Induced resistance, a bio-control agent may cause a plant to make a product that discourages or kills the pathogen. (4) Parasitism, a bio-control agent may feed directly on or in a pathogen. Some bio-control agents use only one of these strategies but the most successful bio-control agents use several of them (Monte, 2001).

The decrease in disease severity of chocolate spot due to molybdenum may attributed to increasing cytokinin level, consequently enhancing the total phenols, calcium content and activity of catechol oxidase which protect plants against pathogen infection (Wahdan, 1991; Chowdhury, 2003). A rapid accumulation of phenols at the infection site is the first step of the defense mechanism in plants which restricts or slows the pathogen growth because of its action as antioxidant, antimicrobial and photoreceptor (Lamba *et al.*, 2008). Such enhancement effective of molybdenum in plant growth and yield might be attributed to increase cytokinin content which induced lateral bud development. Also, molybdenum is an important constituent of nitrate reductase and nitrogenase which induce nitrogen fixation. Nitrogen has an essential role as a constituent of proteins, nucleic acid, chlorophyll and growth hormones (Siddiky *et al.*, 2007; Engelhard, 1993).

In this investigation, the reversing effects of boron on decreasing the harmful effects of chocolate spot disease may be due to the decrease in linear growth, dry weight and sporulation of *B. fabae* as well as increase in phenol content and phytoalexins (wyerone acid). Boron is essential element for translocation of carbohydrates in plants (Siddiky *et al.*, 2007).

Moreover, boron increase photosynthetic pigments due to the increase in cytokinin content in the plant tissues which increase chloroplasts number in leaves by increasing both intensity of cell growth phytohormones and activity of cytoplasm ribosomes consequently, increase in chlorophyll synthesis (Marschner, 1986).

Calcium is the most prominent element which enters in middle lamella structure of the cell wall by combining with pectic acid. There is no doubt that the cell wall is considered as first barrier against plant pathogen invasion, calcium gave strength to cell wall (Saran, 1989). The fungus penetrates cell wall of plant through enzymatic degradation. The enzymatic degradation products of cell wall could act as non specific elicitors of plant defense responses (Hahn, 1996). Elicitors can increase calcium in cell cultures or protoplast (Dmitriev *et al.*, 1996) and may trigger the migration of the plant nucleus to the penetration site in both resistant and susceptible plant cells as the fungus grows through the cell wall (Heath *et al.*, 1997). Moreover, plant cells use calcium as a secondary messenger to control many cell processes, such as stomatal closure (Webb *et al.*, 1996), tropism (Gehring *et al.*, 1990) and many calcium-dependent enzyme activities (Roberts and Harmon, 1992).

## CONCLUSION

Based in the previous results and discussion, it is recommended to replace fungicides with boron at 2 ppm or calcium at 6 mL L<sup>-1</sup> or molybdenum at 4 ppm as a foliar spraying for minimizing faba bean chocolate spot disease and maximizing yield of faba bean plants.

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