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## Does treating faba bean seeds with chemical inducers simultaneously increase chocolate spot disease resistance and yield under field conditions?

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**Abstract:** The use of chemical inducers for the development of host resistance is an alternative approach to plant disease management and is assumed to be much more environmentally sound than traditional pesticides. Five chemical inducers, salicylic acid, oxalic acid, ascorbic acid, dipotassium hydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ), and calcium chloride ( $\text{CaCl}_2$ ), all at concentrations of 10 mM, and an untreated control treatment were applied by seed soaking to compare their effectiveness in inducing resistance against chocolate spot disease (*Botrytis fabae* Sard.) in faba bean under greenhouse and field conditions, and to investigate whether use of these chemicals in this way is associated with a reduction in plant growth and yield under field conditions. A field experiment was conducted over 2 years (2007 and 2008) using a randomized complete block design with 4 replications. Results indicated that, under greenhouse and field conditions,  $\text{KH}_2\text{PO}_4$  and  $\text{CaCl}_2$  treatments were either moderately or less effective in reducing disease severity of chocolate spot. However, both inducers significantly increased the growth and yield of faba beans under field conditions compared with the untreated control treatment. The greatest reduction in disease severity of chocolate spot under greenhouse and field conditions was observed in treatments of ascorbic acid and oxalic acid. The decrease in disease severity ranged from 70.6% to 79.9% for oxalic acid and from 68.1% to 80.2% for ascorbic acid, as compared with the untreated control under greenhouse conditions. Under field conditions, the decreases in disease severity with ascorbic acid and oxalic acid treatments relative to the untreated control were 79.4% and 71.3% on day 30, 62.4% and 41.8% on day 45, and 60.1% and 41.1% on day 60, respectively. The treatments of salicylic acid and oxalic acid produced the lowest values for growth and yield parameters. These results led to the conclusion that, for field application of chemical inducers to develop host resistance, it is important to study their effects on growth and yield in addition to their ability to control diseases.

**Key words:** Induced resistance, growth parameters, seed soaking, seed yield, *Vicia faba*

### Introduction

Faba bean (*Vicia faba* L.) is one of the most important legume crops in the global human diet, especially for poorer sections of the population.

However, this crop is subjected to many abiotic and biotic stresses that seriously compromise the final yields. Among the menacing biotic stresses, the chocolate spot disease caused by *Botrytis fabae* Sard.

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is one of the most economically important diseases that damage the foliage, limit photosynthesis activity, and reduce faba bean production globally (Torres et al. 2004). For example, in the Maghreb, Nile Delta, or African regions, losses in yield due to chocolate spot disease can reach 60%-80% among susceptible cultivars and up to 34% among tolerant cultivars (Bouhassan et al. 2004; Sahile et al. 2008). Most importantly, the disease can also cause total crop failure under severe epidemic conditions (Torres et al. 2004). Therefore, it is necessary to protect the faba bean from this disease to bridge the gap between production and consumption of this crop in Egypt.

Substantial progress has already been made in control of this disease through the use of fungicides and the breeding of resistant genotypes. However, the use of potentially hazardous fungicides in agriculture has been the subject of growing concern because of their possible adverse effects on the environment. Moreover, frequent application of fungicides leads to the risk of developing new resistant strains of pathogens (Smith and Littrell 1980). Williams (1990) also reported that the resistance expressed by a set of cultivars in one geographical region may not be effective in another region due to the existence of pathogens and/or the variability in environmental conditions. Therefore, the current trend in crop protection against diseases is to apply different chemical inducers that will stimulate the inherent defense mechanisms of the host plant. Such chemical inducers are assumed to be much more environmentally sound than synthetic fungicides, to have a lower economic cost for farmers, to lack environmental and toxicological risks, and to create induced systematic resistance in the hosts against several pathogens.

Various chemical inducers have been considered for their potential to induce systematic resistance in the host plant to protect them from different pathogens. For example, pearl millet seeds treated with several dibasic and tribasic potassium phosphates have been shown to demonstrate induced systemic resistance against *Sclerospora graminicola* (Chaluvaraju et al. 2004). Pearl millet seeds treated with different vitamins have been reported to display induced resistance against downy mildew disease

under greenhouse conditions (Pushpalatha et al. 2007). Similarly, salicylic acid has been extensively studied for its role in disease resistance and has been demonstrated as a resistance inducer in several plant species, including in barley against *Erysiphe graminis* (Walters et al. 1993) and in rice against *Pyricularia oryzae* (Manandhar et al. 1998).

Although many studies have recommended the use of chemical inducers for controlling plant diseases, to our knowledge, there has been little research on the adverse effects of these chemical inducers on plant growth and yield, especially under field conditions. Furthermore, seeming inconsistencies in results have been observed with respect to the effects of these inducers on crop growth and yield. For example, Heil et al. (2000) applied the analog of salicylic acid, BTH, to wheat in the absence of pathogens. They found that plants treated with BTH had lower biomass and numbers of ears and grains per plant than nontreated plants, and the effects were most pronounced when the nitrogen supply was limited. Redman et al. (2001) induced resistance in tomato by jasmonic acid and showed that it caused a 25% reduction in seed yield. Similar results were observed in sunflower seeds treated with acibenzolar-S-methyl (ASM), which reduced shoot fresh weight (Prats et al. 2002). In contrast, Iriti and Faoro (2003) found that when beans were treated with BTH there was a statistically insignificant reduction in seed yield. Aminuzzaman and Hossain (2007) induced resistance in wheat with Bion (benzothiadiazole) and found that it caused a 53.3% higher grain yield in comparison with the untreated control. An increase in yield was also apparent when resistance was induced in barley by application of various chemical agents (Dehne 1984). These results led to the suggestion that, for field application of chemical elicitors of induced resistance, an important consideration, in addition to their ability to control diseases, is to study their effects on growth and yield. In this vein, the objective of this study was to examine the effectiveness of nonconventional chemicals as inducers of resistance against chocolate spot disease in faba bean and determine whether use of these chemicals in this way is associated with a reduction in plant growth and yield under field conditions.

## Materials and methods

### Experimental site and conditions

Greenhouse and field experiments were conducted at the experimental farm of the Faculty of Agriculture, Suez Canal University, Ismailia, Egypt (30°58'N, 32°23'E; 13 m above mean sea level) during the 2007 and 2008 growing seasons. The climate in the study area is semiarid, with an average annual rainfall of about 33 mm and an average temperature of about 35.5 °C in summer and 13.0 °C in winter. The soil texture at the site is predominantly sandy throughout its profile (79.9% coarse sand, 15.7% fine sand, 2.7% silt, and 1.7% clay) with low organic matter content (0.35%). Soil texture (particle size distribution) was analyzed using the pipette method (Gee and Bauder 1986).

### Chemical inducers and preparation of chemical solutions and seed treatments

The chemical inducers used in the study, salicylic acid, oxalic acid, ascorbic acid, dipotassium hydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>), and calcium chloride (CaCl<sub>2</sub>), were obtained from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Aqueous solutions were prepared at concentrations of 10 mM for each chemical inducer by dissolving a known amount of inducers in sterile distilled water at room temperature (25 °C) and mixing with a stirrer for a few minutes to ensure complete solubilization, or until no granules were left. In the greenhouse and field experiments, the inducers were tested as seed soaking treatments. The seeds of faba bean cultivar Giza 843 (seeds obtained from the Agricultural Research Center, Giza, Egypt) were soaked in aqueous solutions of different chemical inducers and in distilled water as a control for 24 h and allowed to dry on filter paper for 3 h. This genotype is moderately resistant to chocolate spot disease; however, at the same time, it is tolerant to *Orobanche foetida*.

### Greenhouse experiment

#### Inoculation preparation

A single spore of *Botrytis fabae* was isolated from naturally infected faba bean plants during the 2006 growing season. Purified cultures were identified according to the methods of Morgan (1971). An isolate of *B. fabae* was grown on faba bean leaf

dextrose agar medium containing 250, 30, 20, and 20 g of faba bean leaves, dextrose, sodium chloride, and agar in 1 L of distilled water, respectively, and then incubated at 20 °C in a cycle of 12 h darkness and 12 h under near-UV light (Philips TL/65/80W/05, maximum emission 450 nm) to induce sporulation (Tivoli et al. 1986). After 14 days of growth, an inoculum suspension was prepared by adding sterile distilled water and stirring with a sterile loop. Spore suspensions were then adjusted to  $2.5 \times 10^5$  spores mL<sup>-1</sup> with sterile distilled water using a hemocytometer, as detailed by Derckel et al. (1999).

### Infection procedures

This experiment was carried out in a greenhouse under conditions of the winter growing season of Egypt to evaluate the efficacy of chemical resistance inducers in reducing chocolate spot disease in faba bean cultivar Giza 843. Five soaked seeds from each chemical inducer and distilled water were sown in pots, each 25 cm in diameter, containing sterilized soil that was similar to the soil of the experimental field. After 21 days, the pots were divided into 2 groups. The first group was sprayed with a spore suspension of *B. fabae* at a concentration of  $2.5 \times 10^5$  spores mL<sup>-1</sup>. However, the second group was left without inoculation and sprayed with distilled sterilized water as a control treatment. All pots were covered with polyethylene bags for 24 h to maintain the high humidity required for spore germination. The pots were arranged in a randomized complete block design in the greenhouse with 4 replicates. Disease severity was recorded for inoculated and uninoculated plants at 24, 48, and 72 h after artificial inoculation using a 0-9 scale, where 0 indicates no visible symptoms and 9 represents disease covering more than 80% of the foliar tissue (ICARDA 1986).

### Field experiment

#### Experimental design, agronomic practices, and measurements

A randomized complete block design with 4 replications was used in each season. The experimental plot contained 6 ridges, 60 cm apart and 5 m in length (18 m<sup>2</sup> in total area). The soaking seeds were planted in hills on 2 sides of the ridges with 20 cm between hills. Two seeds in each hill were sown on 13 October 2007 and 2 November 2008 to obtain

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a final plant population of about 33.3 plants m<sup>-2</sup>. Fertilization consisted of 50 kg ha<sup>-1</sup> of N as ammonium sulfate (20.5% N), 31 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> as calcium super phosphate (15.5% P<sub>2</sub>O<sub>5</sub>), and 50 kg ha<sup>-1</sup> of K<sub>2</sub>O as potassium sulfate (48% K<sub>2</sub>O). The phosphorus fertilizer was applied at the time of sowing. The nitrogen fertilizer was added before the first irrigation. Potassium fertilizer was added in 1 dose at 40 days after sowing.

At 90 days after sowing, 5 plants from each plot were harvested at random to determine the plant height, number of leaves and branches per plant, leaf area per plant, and total dry weight per plant. Plant samples were separated into leaves and stems after the plant height was recorded. Leaf area was measured using a LI-3000 Area Meter (LI-COR, Walz Co., OR, USA). After the leaf area was determined, the samples were dried in a forced-air oven at 75 °C for 48 h, and then their dry weights were determined. The total dry weight was obtained by the summation of the corresponding fractions.

After physiological maturity at about 170 days after sowing, an additional 5 plants from each plot were harvested at random to determine the number of pods and seeds per plant, the 100-seed weight, and the weight of pods, seeds, and straw per plant. Seed yield was determined by hand harvesting an area of 2 internal ridges, each 5.0 m in length (6.0 m<sup>2</sup> in total area), from each plot. Seed samples were collected from the yield samples to determine the moisture content, and seed yield was adjusted to a moisture content of 15.5%.

#### Disease assessment

The severity of chocolate spot was assessed 3 times at 15-day intervals, starting from day 30 after sowing. Fifteen randomly selected and tagged plants from each plot were used for chocolate spot severity assessment. Severity was rated on leaves from the same 15 randomly selected representative plants from each plot using a 0-9 scale, where 0, 1, 2, 3, 4, 5, 6, 7, and 8 represent no visible leaf infection (0) or disease covering less than 10%, 20%, 30%, 40%, 50%, 60%, 70%, or 80% of the foliar tissue, respectively; 9 represents disease covering more than 80% of the foliar tissue (ICARDA 1986). Disease severity (DS) values were calculated using the following formula, according to Wheeler (1969) and ICARDA (1986):

$$DS (\%) = \frac{\sum (\text{disease grade} \times \text{number of plants in each grade})}{(\text{total number of plants} \times \text{highest disease grade})} \times 100$$

#### Laboratory analysis

##### Determination of peroxidase activity and total phenols content

Leaf samples from each treatment under greenhouse conditions were collected at 0, 12, 24, and 48 h after inoculation for determination of peroxidase activity assay, and then 3 g of fresh leaf was ground in a precooled mortar and pestle containing 9 mL of 0.1 M phosphate buffer (pH 7.1). The extract was centrifuged at 3000 rpm at 6 °C for 20 min. Peroxidase activity was expressed as changes in absorbance min<sup>-1</sup> at 425 nm, according to the methods of Thimmaiah (1999).

Total soluble phenols in fresh leaves were determined by using the colorimetric method described by Folin and Ciocalteu (AOAC 1985).

##### Statistical analysis

Disease severity data were normalized using the arcsine transformation before analysis of variance. Data of all measurements were then subjected to analysis of variance (ANOVA) appropriate for a randomized complete block design (CoStat system for Windows, version 6.311, CoHort Software, Berkeley, CA, USA). Mean separation among chemical inducers for each measurement was determined using the least significant differences (LSD) test. Probability levels lower than 0.05 were categorized as significant.

#### Results

##### Effect of chemical inducers on chocolate spot disease severity under greenhouse conditions

The efficacy of different chemical inducers on chocolate spot disease severity at 24, 48, and 72 h after inoculation with *B. fabae* under greenhouse conditions is presented in Figure 1. The data revealed that the disease severity of chocolate spot was significantly different ( $P \leq 0.05$ ) among the tested

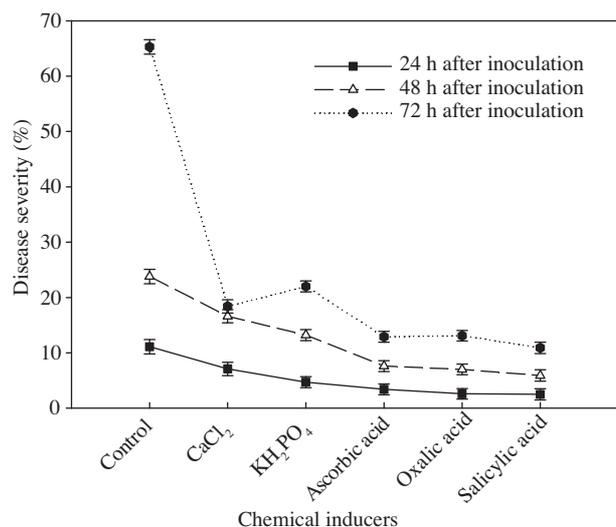


Figure 1. Effects of different chemical inducers by seed soaking on disease severity of chocolate spot in faba bean at 24, 48, and 72 h after inoculation with *B. fabae* under greenhouse conditions. Error bars represent standard errors of the mean.

chemical inducers. The highest reduction in disease severity of chocolate spot at different periods of inoculation was observed with salicylic acid, oxalic acid, and ascorbic acid treatments, whereas seeds treated with  $\text{KH}_2\text{PO}_4$  and  $\text{CaCl}_2$  showed moderate effects. The reduction in chocolate spot disease severity ranged from 75.2% to 83.3% for salicylic acid, from 70.6% to 79.9% for oxalic acid, from 68.1% to 80.2% for ascorbic acid, from 44.5% to 66.3% for  $\text{KH}_2\text{PO}_4$ , and from 36.0% to 71.8% for  $\text{CaCl}_2$ , as compared with the untreated control.

#### Effect of chemical inducers on disease severity under field conditions

Seeds treated with different chemical inducers at 10 mM had significantly ( $P \leq 0.05$ ) reduced chocolate spot disease severity when measured at 30, 45, and 60 days after sowing, as compared with the untreated control (Figure 2). However, seeds treated with ascorbic acid showed the highest reduction of chocolate spot disease severity, followed by oxalic acid. Averaged over the 2 seasons, disease severity decreases for ascorbic acid and oxalic acid treatments relative to the untreated control were 79.4% and 71.3% on day 30, 62.4% and 41.8% on day 45, and 60.1% and 41.1% on day 60, respectively. Treatments of  $\text{KH}_2\text{PO}_4$  and salicylic acid were found to be less

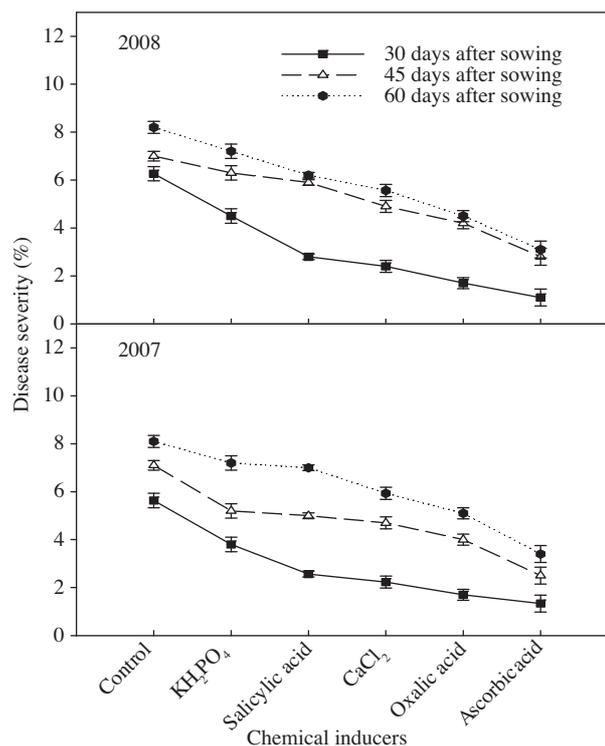


Figure 2. Effects of different chemical inducers by seed soaking on disease severity of chocolate spot in faba bean at 30, 45, and 60 days after sowing during the 2007 and 2008 growing seasons under field conditions. Error bars represent standard errors of the mean.

effective in reducing disease severity under field conditions. For instance, averaged over the 2 seasons, disease severity with  $\text{KH}_2\text{PO}_4$  and salicylic acid treatments were only 18.4% and 22.6% less on day 45 and 11.7% and 19.0% less on day 60 than those of the untreated control, respectively.  $\text{CaCl}_2$  treatment was found to be moderately effective for control of chocolate spot as it afforded an average protection over the 2 seasons of 61.0%, 31.9%, and 29.4% at 30, 45, and 60 days after sowing, respectively (Figure 2).

#### Effect of chemical inducers on peroxidase activity and phenolics content

The total activity of peroxidase was considered increased at periods of 0, 12, and 24 h after inoculation with *B. fabae*, and then it decreased at 48 h. In addition, seeds treated with different chemical inducers resulted in a significant increase in peroxidase activity when compared with the untreated control (Table 1). For example, averaged over 4 measurement times and compared with the

untreated control, the seeds treated with ascorbic acid, oxalic acid, salicylic acid,  $\text{KH}_2\text{PO}_4$ , and  $\text{CaCl}_2$  demonstrated increased peroxidase activity in leaf extracts by 5.0, 4.1, 3.1, 2.3, and 1.2 times, respectively.

Data in Table 1 reveal that seeds treated with oxalic acid, salicylic acid, and  $\text{KH}_2\text{PO}_4$  showed maximum accumulation of total phenols (respectively, 573.5, 591.2, and 584.2  $\text{mg g}^{-1}$  of fresh weight), whereas the lowest accumulation observed was in the untreated control, followed by seeds treated with  $\text{CaCl}_2$ .

### Effect of chemical inducers on growth parameters under field conditions

All vegetative growth parameters at 90 days after sowing (plant height, number of leaves and branches per plant, leaf area per plant, and total dry weight per plant) were significantly affected by the different chemical inducers (Table 2). Fisher's protected LSD test for vegetative growth parameters placed  $\text{KH}_2\text{PO}_4$  and  $\text{CaCl}_2$  treatments in the first position, ascorbic acid treatment in the second position, the untreated control in the third position, and salicylic acid and oxalic acid treatments in the fourth position, with quite similar results for all parameters (except branch number per plant) in both seasons. Tillering capacity was superior in the oxalic acid treatment. Averaged over the 2 seasons,  $\text{CaCl}_2$ ,  $\text{KH}_2\text{PO}_4$ , and ascorbic acid treatments increased plant height by 5.0%, 5.4%, and 12.2%; increased leaf number per plant by 20.1%, 17.9%, and 11.5%; increased leaf area per plant by 26.3%, 26.3%, and 15.0%; and increased total dry

weight per plant by 30.6%, 31.2%, and 15.1%, respectively, when compared with the untreated control. However, salicylic acid and oxalic acid treatments decreased plant height by 12.6% and 13.6%, leaf number per plant by 10.1% and 12.5%, leaf area per plant by 18.3% and 19.7%, and total dry weight per plant by 25.6% and 26.5%, respectively, when compared with the untreated control (Table 2).

### Effect of chemical inducers on yield and yield components

The data presented in Table 3 show that all chemical inducers under study, except salicylic acid and oxalic acid, significantly ( $P \leq 0.05$ ) increased yield and yield components (the number of pods and seeds per plant, the weight of pods, seeds and straw per plant, and the seed yield per hectare) compared with the untreated control. Seeds treated with  $\text{CaCl}_2$  and  $\text{KH}_2\text{PO}_4$  had enhanced yield component parameters and seed yield. Moreover, the number of pods per plant and the weight of seeds and straw per plant after ascorbic acid treatment were on par with the  $\text{CaCl}_2$  and  $\text{KH}_2\text{PO}_4$  treatments. However, salicylic acid and oxalic acid treatments demonstrated lower values for yield components and seed yield than the untreated control. Averaged over the 2 seasons, salicylic acid and oxalic acid treatments decreased the number of pods per plant by 25.3% and 28.4%, the number of seeds per plant by 22.8% and 23.1%, the weight of pods per plant by 14.6% and 18.3%, the weight of seeds per plant by 19.0% and 21.6%, and the seed yield per

Table 1. Effect of different chemical inducers by seed soaking on peroxidase activity in the leaves of faba bean after an inoculation period of 0, 12, 24, and 72 h with *B. fabae*, and total phenolic content.

Chemical inducers	Peroxidase activity (changes in absorbance $\text{min}^{-1}$ at 425 nm)				Total phenolics ( $\text{mg g}^{-1}$ fresh weight of leaves)
	0 h	24 h	48 h	72 h	
$\text{KH}_2\text{PO}_4$	0.586 d	0.666 d	0.809 d	0.380 d	584.2 a
$\text{CaCl}_2$	0.315 e	0.367 e	0.462 e	0.199 e	471.7 c
Ascorbic acid	1.311 a	1.423 a	1.569 a	0.932 a	532.0 b
Salicylic acid	0.779 c	0.888 c	1.065 c	0.521 c	591.2 a
Oxalic acid	1.077 b	1.102 b	1.404 b	0.742 b	573.5 a
Control	0.252 e	0.286 e	0.355 f	0.173 f	450.5 d

Means followed by the same letter are not significantly different at  $P \leq 0.05$ .

Table 2. Effects of different chemical inducers by seed soaking on plant growth parameters of faba bean at 90 days after sowing, grown under field conditions in 2007 and 2008.

Chemical inducers	Growth parameters at 90 days after sowing				
	Plant height (cm)	Number of leaves per plant	Number of branches per plant	Leaf area per plant (cm <sup>2</sup> )	Total dry weight per plant (g)
2007					
KH <sub>2</sub> PO <sub>4</sub>	97.8 b	43.2 a	4.4 b	2231.6 a	40.9 a
CaCl <sub>2</sub>	96.1 b	41.3 a	4.4 b	2307.9 a	41.9 a
Ascorbic acid	107.2 a	37.9 b	3.7 b	1970.2 b	31.5 b
Salicylic acid	77.3 c	30.3 d	4.0 b	1301.3 d	21.1 d
Oxalic acid	78.9 c	29.9 d	6.5 a	1290.1 d	20.3 d
Control	93.1 b	34.3 c	4.1 b	1598.3 c	28.1 c
2008					
KH <sub>2</sub> PO <sub>4</sub>	90.4 ab	40.6 a	4.0 b	2031.6 a	36.5 a
CaCl <sub>2</sub>	92.8 ab	40.3 a	4.2 b	1967.9 a	36.3 a
Ascorbic acid	96.6 a	37.7 b	3.7 b	1730.2 b	31.9 b
Salicylic acid	78.7 cd	29.9 d	4.1 b	1261.3 d	18.9 d
Oxalic acid	75.5 d	28.7 d	6.0 a	1230.1 d	19.1 d
Control	85.7 bc	32.7 c	3.9 b	1538.3 c	25.7 c

Means followed by the same letter are not significantly different at  $P \leq 0.05$ .

Table 3. Effects of different chemical inducers by seed soaking on yield components and seed yield of faba bean grown under field conditions in 2007 and 2008.

Chemical inducers	Yield parameters						
	Number of pods per plant	Number of seeds per plant	Weight of pods per plant (g)	Weight of seeds per plant (g)	Weight of straw per plant (g)	100-seed weight (g)	Seed yield (kg ha <sup>-1</sup> )
2007							
KH <sub>2</sub> PO <sub>4</sub>	24.7 a	58.3 a	75.7 a	60.4 a	53.8 a	98.4 a	3640.0 a
CaCl <sub>2</sub>	22.6 a	54.4 b	72.0 a	57.9 a	54.0 a	96.5 a	3705.0 a
Ascorbic acid	21.5 a	48.0 c	65.5 b	55.3 a	49.5 a	98.5 a	3003.6 b
Salicylic acid	12.0 c	31.6 e	42.6 d	31.7 c	23.7 c	97.8 a	1866.8 cd
Oxalic acid	11.7 c	31.4 e	40.4 d	32.3 c	19.0 c	98.6 a	1826.2 d
Control	16.5 b	42.8 d	50.7 c	40.5 b	27.3 b	97.3 a	2324.9 c
2008							
KH <sub>2</sub> PO <sub>4</sub>	20.0 a	51.2 a	66.9 a	57.5 a	46.8 a	99.2 a	3815.6 a
CaCl <sub>2</sub>	21.0 a	52.2 a	67.3 a	58.8 a	47.2 a	96.0 a	3629.1 a
Ascorbic acid	20.6 a	48.1 b	58.3 b	48.3 a	44.4 a	97.2 a	3151.5 b
Salicylic acid	11.9 c	30.9 d	42.5 d	31.5 c	19.7 c	95.4 a	1703.4 d
Oxalic acid	11.3 c	30.8 d	41.0 d	29.0 c	16.3 c	96.6 a	1655.5 d
Control	15.6 b	38.4 c	49.0 c	37.6 b	25.4 b	96.2 a	2269.3 c

Means followed by the same letter are not significantly different at  $P \leq 0.05$ .

hectare by 22.3% and 24.2%, respectively, when both treatments were compared with the untreated control (Table 3). There were no significant effects of the different chemical inducer treatments on 100-seed weight compared with the untreated control, with values ranging from 96.5 to 98.6 in 2007 and from 95.4 to 99.2 in 2008 (Table 3).

## Discussion

Comparative evaluation of the 5 chemical inducers,  $\text{KH}_2\text{PO}_4$ ,  $\text{CaCl}_2$ , ascorbic acid, salicylic acid, and oxalic acid, at concentrations of 10 mM showed varying efficiency in reducing chocolate spot disease severity of faba bean under greenhouse and field conditions. The present study demonstrates that, under greenhouse conditions, the greatest reduction in severity of chocolate spot at different periods after inoculation with *B. fabae* was obtained by oxalic acid, salicylic acid, and ascorbic acid treatments. Under field conditions, ascorbic acid gave the best protection against chocolate spot disease, followed by oxalic acid. However,  $\text{KH}_2\text{PO}_4$  or  $\text{CaCl}_2$  treatments were found to be moderately or less effective for controlling chocolate spot disease under greenhouse and field conditions (Figures 1 and 2). The difference in the efficiency of these chemical inducers in controlling chocolate spot under both conditions may be attributed to the differential mode of action of these chemicals.

The mode of action of chemical inducers for controlling plant diseases may include: (1) acting as second messengers in enhancing the host defense mechanism (Geetha and Shetty 2002); (2) activating resistance by increasing the activity of peroxidase (POD), the synthesis of new POD isoforms, or the accumulation of the phenolic compound (Zhang et al. 1999; Sarma et al. 2007); (3) activating resistance through inhibition of some antioxidant enzymes and catalases, thereby leading to production of elevated amounts of  $\text{H}_2\text{O}_2$  accumulation (Radwan et al. 2008); and (4) enhancing resistance by direct effects on multiplication, development, and survival of pathogens or indirect effects on plant metabolism, with subsequent effects on the pathogen food supply (Reuveni and Reuveni 1998; Khanam et al. 2005). As evident from the differential mode of action of the chemical inducers, the varying efficiencies among

these chemicals in protecting faba bean against chocolate spot disease have been observed under greenhouse and field conditions.

Previous research has suggested that peroxidase (POD) activity is related to induced resistance in plants (Qin et al. 2003). POD activity produces the oxidative power for the cross-linking of proteins and phenylpropanoid radicals, resulting in reinforcement of cell walls against attempted fungal penetration (Kristensen et al. 1999). Tarrad et al. (1993) reported that the increase in peroxidase activity enhanced lignification in response to chocolate spot disease, which may restrict fungal penetration. Another supportive suggestion was made by Nawar and Kuti (2003), who stated that an increase in peroxidase activity is considered to be a preliminary indicator for resistance of broad beans to chocolate spot disease. These findings indicate a positive relationship between resistance and peroxidase activity. Our results indicate that seeds being treated with different chemical inducers resulted in a significant increase in peroxidase activity when compared with the untreated control (Table 1).

As regards phenols, the higher accumulation of phenolic compounds in the treated plants challenged with the pathogen is an important criterion for considering the chemicals for wider use. It is well known that synthesis of phenols occurs as an early response of plants to attempted infection by pathogens, as antimicrobial compounds, signal molecules, and cell wall strengthening components (Kruger et al. 2002). Sarma et al. (2007) revealed a positive correlation between the accumulation of phenolic compounds and the reduction of plant mortality in chickpea when exposed to *Sclerotinia* stem rot. Results of the present study also reveal the role of chemical inducers in the accumulation of phenols. Plants treated with oxalic acid and salicylic acid by seed soaking showed the maximum accumulation of total phenols, as compared with the untreated control (Table 1).

An important finding of this study was that some chemical inducers used had adverse effects on the plant growth and yield of faba bean under field conditions (pathogen-free conditions). It is interesting that the plants treated with salicylic acid and oxalic acid by seed soaking demonstrated lower values for

vegetative growth, yield components, and seed yield parameters than did the untreated control plants (Tables 2 and 3). These decreases in growth and yield may be related to: (1) a metabolic competition for resources between processes involved in plant growth and processes necessary for plant differentiation, like the synthesis of chemicals for plant defense (Herms and Mattson 1992; Heil et al. 2000); (2) some chemical induction of pathogen resistance in faba beans that can lead to a reduced size and number of root nodules, which negatively affects the plants' mutualistic interactions (Martinez-Abarca et al. 1998; Heil 2000); and (3) the fact that some of the molecules involved in the signaling pathway, such as salicylic acid, have already been demonstrated to have, or are likely to have, autotoxic effects on plants, which might negatively affect plant fitness (Rasmussen et al. 1991). The findings obtained in this study were in agreement with those reported by Heil et al. (2000), Redman et al. (2001), and Cipollini (2002), who found that application of chemical resistance inducers often leads to adverse effects on further plant development. In contrast, a number of studies found no negative effects of chemically induced resistance on plant growth and yield (Mitchell and Walters 1995; Iriti and Faoro 2003; Boyle and Walters 2005).

According to Walters and Boyle (2005), the differences in the above mentioned results may reflect not just differences between species, but also in environmental conditions. The latter plays an important role in the apparently contradictory results, which in fact represent different outcomes of a complex interplay of factors (Dietrich et al. 2005). Therefore, these results suggest that environmental conditions should be taken into account when chemical inducers are used under field conditions, because they might have played an important role in the evaluation of inducibility by chemical inducers.

Additionally, it is noteworthy that there were obvious differences in the effects of the various chemical inducers used on vegetative growth and yield under field conditions. Among them, some

inducers increase and others decrease the growth and yield when compared with the untreated control (Tables 2 and 3). This is probably due to the significant differences between chemical inducers in the energy consumption needed for elicitation of pathogen resistance and/or the differential mode of action of these chemicals in controlling plant diseases. In our study, plants treated with  $\text{KH}_2\text{PO}_4$  and  $\text{CaCl}_2$  by seed soaking demonstrated higher values for vegetative growth, yield components, and seed yield parameters than the untreated control. This suggested that these inducing chemicals did not cause a major expenditure of energy. At the same time, ions of  $\text{Ca}^{2+}$ ,  $\text{K}^+$ , and P have vital roles in elevating rates of photosynthesis due to metabolic factors, including photosynthetic pigments, carotenoids, the efficiency of photosystem II, rubisco enzyme concentration and activity, and the supply of ATP and NADPH to the photosynthetic carbon reduction cycle (Sander et al. 1999; Walters and Bingham 2007). However, the decreases in growth and yield of plants treated by salicylic acid and oxalic acid may be related to the differential mode of action of both chemicals in controlling plant disease. For example, salicylic acid is presumed to play a role in controlling ion uptake by roots and stomatal conductivity (Raskin 1992). Mori et al. (2001) reported that application of salicylic acid stimulates stomatal closure in many plants. Doubrava et al. (1988) suggested that the efficacy of oxalate treatment in inducing resistance is due to the sequestering of calcium ions from plant tissues and the inhibiting of abscisic acid that controls the guard cells of the stomata. The removal of  $\text{Ca}^{2+}$  ions may affect cell membranes and destroy cell compartmentalization. All of these mechanisms for salicylic acid and oxalic acid may directly or indirectly affect photosynthesis efficiency. For instance, it is well known that the stomatal opening provides  $\text{CO}_2$  to inner leaf tissues, and so narrowing the stomatal apertures may lead to a significant decrease in the photosynthesis rate. Such decreases in the photosynthesis rate could affect the balance and culminate in reduced growth and yield.

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