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INDUCED RESISTANCE AGAINST CHOCOLATE SPOT DISEASE OF FABA BEAN

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ABSTRACT

Some abiotic treatments effectively induced resistance in faba bean (cv. Giza 3) against chocolate spot disease when were applied as seed soaking or foliar spray. The compounds were di-potassium hydrogen phosphate, ferric chloride, salicylic acid and ethephon. Application of chemical treatments as seed soaking or foliar spray reduced the percentage of browning and spreading of *Botrytis* lesion using detached leaves technique. Foliar spray of faba bean plants with K_2HPO_4 and $FeCl_3$ at concentrations of 50 mM and 20 mM, respectively, significantly reduced chocolate spot under field conditions. Meanwhile, seed soaking was less effective. Application of K_2HPO_4 and $FeCl_3$ significantly reduced flower drops and increased seed yield. SDS-PAGE of protein revealed incidence of various new bands in treated plants.

Keywords: Chocolate spot, Induced resistance, di-potassium phosphate, ferric chloride, salicylic acid and ethephon.

INTRODUCTION

Faba bean (*Vicia faba* L.) is attacked by a wide range of fungal pathogens. Chocolate spot caused by "*Botrytis faba*e (Sard.) and *Botrytis cinerea* (Pers.)" is the main foliar fungal pathogen of faba bean in Egypt (El-Helaly, 1938; Hegazy,1964; Sirry, *et al.*, 1981 and Abou-Zeid *et al.*, 1998). Control of the disease depends mostly on using chemical fungicides (Hanounik and Bisri , 1991). Present ecological concerns about frequent fungicide applications are serious and include worker safety, contamination of drainage water and consumer exposure to fungicide residues. However, it is a problem to obtain effective control as the fungal population has the potential to develop resistance against most used systemic fungicides (DeWaard *et al.,* 1993). Therefore, alternative control measures are needed to fulfill grower's efforts to achieve pesticide-free production.

There are numerous reports demonstrating that resistance can be systemically induced in a number of plants by prior treatment with biotic agent or simple chemical substrates (Kuc´, 1987; Aly and Afifi, 1989; Harfoush and Salama, 1992; Reuveni *et al.*, 1992; Gamil, 1995, and Mosa, 1997) Resistance that developed in the treated leaves was termed localized acquired resistance (Ross, 1961a), and resistance in untreated parts of the plant was termed systemic acquired resistance (SAR) (Ross, 1961 b). SAR was shown to be effective against broad spectrum of diseases including viruses, bacteria and fungi (Kuc´, 1987). It has been reported that di-and mono-potassium phosphate salts could induce local and systemic resistance to various plant diseases (Mosa, 2002)

The objective of this study was to induce resistance in faba bean against chocolate spot disease as alternative to fungicides.

MATERIAL AND METHODES

Inducer treatments.

Four chemical compounds *i.e.* di-potassium hydrogen phosphate (K_2HPO_4), ferric chloride ($FeCl_3$), salicylic acid (SA) and ethephon were tested against chocolate spot disease on faba bean. Chemicals were applied as seed soaking or foliar spray. For seed treatments, pregerminated seeds were soaked for 12 h in aqueous solutions of 50 mM K_2HPO_4, 20 mM $FeCl_3$, 10 mM SA and 500 PPM ethephon. For foliar spray 45-days old faba bean plants were sprayed with aqueous solutions of K_2HPO_4 (50 mM), $FeCl_3$ (20 mM), SA (1 mM) and ethephon (50 PPM). Non-treated plants were sprayed with water.

Pathogen and inoculum production

A virulent isolate of *Botrytis fabae* grown on faba bean extract agar medium (Hanounik, 1986) or on potato dextrose agar medium and incubated on 22 °C for 10 days under near ultraviolet light for a duration of 12 hour daily. A spore suspension (10^5 conidia/ml) was prepared using haemocytometer.

Evaluation of inducer treatment *in vivo.*

The detached leaves technique described by Mansfield and Deverall (1974), followed.

Leaves of faba bean without any signs of damage were detached from the middle of the flowering nodes. The abaxial surfaces of detached leaves were gently washed with distilled water and dried with tissue paper before inoculation. Leaves were placed on moistened filter paper in plastic boxes, and then inoculated on their abaxial epidermis with 20 ml droplets containing 200 conidia of *B. fabae* or 2000 conidia of *B. cinerea*. Incubation was carried out at 18 ± 2 °C under fluorescent lamps. Data were recorded as browning of infection sites and spreading of lesions according to Mansfield and Deverall (1974).

Efficacy of chemical inducers in the field.

Field experiment was carried out at Maryiot Research Station, Desert Research Center during 2001/2002 & 2002/2003 growing seasons to study the effect of four chemical inducers on incidence of chocolate spot disease. Chemicals were applied as seed soaking or foliar spray. For seed soaking, seeds were soaked individually in solutions of K_2HPO_4 50 mM., $FeCl_3$ 20 mM., SA 10 mM and ethephon 500 PPM., for 24 h before sowing. For foliar spray, 45-day old plants were separately sprayed with solutions of K_2HPO_4 50 mM., $FeCl_3$ 20 mM., SA 1 mM and ethephon 50 PPM., and addition spray treatment were applied 10 days later. Incidence of chocolate spot and yield characters were recorded.

Disease assessment

Chocolate spot disease was assessed as the percentage of leaf lamina affected. through 5 times during plant growth after 60, 80, 100, 120 and 140 days from sowing. Disease severity was assessed using the scale described by Anonymous. (1976). The percentages given are proportions of the leaf area covered by the fungal lesions.

Effect of inducer treatments on faba bean growth and yield characters.

Faba bean Plant were randomly chosen from each replicate plot to determine of plant height, number of branches, number of pods/plant, number of seeds/pod, weight of 100 seeds and seed yield/plant.

Determination of phenol content.

Total, free and conjugated phenols were determined in ethanol extract according to the method described by Snell and Snell (1953).

SDS Gel-Electrophoresis for soluble proteins.

SDS-polyacrylamide gel electrophoresis was performed in 10 % acrylamide slab gels following the system of Laemmli (1970) by their protein profile and modified by Studier (1973).

Statistical analysis

All experiments were set up in a complete randomized design. Data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS Institute, Inc., 1996). Means were separated by Duncan's multiple range test at P? 0.05 level.

RESULTS

Evaluation of chemical inducers *in vivo*.

All chemical treatments reduced both percentage of browning and spreading of lesions of *Botrytis fabae*. Data present in Table (1) show that K_2HPO_4 and SA reduced lesion browning and spreading of faba bean detached leaves after 24 and 48 hrs from inoculation, followed by $FeCl_3$ and ethephon either as seed soaking or foliar spray applications.

Table (1). Screening of various chemical treatments against chocolate spot of faba bean caused by *Botrytis fabae* using detached leaves technique[a)].

	Treatments	Concen-tration	24 h		48 h	
			Browning (%)[b)]	Spreading %)[c)]	Browning (%)	Spreading (%)
Seed soaking	K_2HPO_4	50 mM	20.0	5.5	46.0	18.0
	$FeCl_3$	20 mM	22.0	6.0	53.0	23.0
	Salicylic acid	10 mM	20.0	7.0	50.3	22.0
	Ethephon	500 PPM	20.0	6.0	68.2	17.5
Foliar spray	K_2HPO_4	50 mM	18.2	5.0	37.5	15.0
	$FeCl_3$	20 mM	18.5	5.0	52.5	20.0
	Salicylic acid	1 mM	20.0	2.5	40.0	20.0
	Ethephon	50 mM	22.0	4.0	48.0	22.5
	Non-treated		33.0	14.8	82.3	50.0

i) Inoculum droplets containing 20 µ each containing 2000 conidia.
ii) Percentage of browning beneath the inoculum droplet.
iii) Percentage of spreading lesion.

Effect of various chemical treatments on severity of chocolate spot in the field.

Results in Table (2) show the effect of chemical treatments as a seed soaking on chocolate spot disease severity during the growing season starting from 60, 80, 100, 120 and 140 days after sowing. Results indicate that, generally, all treatments significantly reduced severity of chocolate spot, up to 140 days after sowing. Meanwhile, K_2HPO_4 and salicylic acid (SA) at 50 and 10 mM, respectively had the highest reduction of disease severity, followed by $FeCl_3$ and ethephon treatments.

Foliar spray of faba bean plants with chemicals at the tested concentration showed the best reduction of severity of chocolate spot more than seed soaking. K_2HPO_4 and $FeCl_3$ at 50 mM and 20 mM respectively, reduced disease severity up to 70% compared to non-treated plants and followed by SA 2 mM and ethephon 100 PPM Table (3).

Table (2). Effect of chemical treatments as a seed soaking on chocolate spot disease severity for various intervals starting from 60 day after sowing, (data are recorded of two season)[x].

Treatments	Concen-tration	Disease severity (%)[y]				
		Days after sowing				
		60	80	100	120	140
K_2HPO_4	50 mM	3.5 c[z]	8.5 b	11.5 c	19.c	26.83 c
$FeCl_3$	20 mM	5.0 b	10 b	13.5 b	22 b	30 b
Salicylic acid	10 mM	1.0 d	7.5 b	11.5 c	17 d	24.0 d
Ethephon	500 mM	3.5 c	9.67 b	11.5 c	17.5 d	28.0 c
Non-treated		12.5 a	16.83 a	25 a	32 a	40.0 a

xxiv) Seeds were soaked in the tested chemicals for 12 h before sowing.
xxv) Disease severity was assessed using the scale described by Anon. (1979).
xxvi) Values followed by the same letter in each column are not significantly different at P? 0.05 according to Duncan's multiple range test.

Table(3). Effect chemical treatments as a foliar spray on chocolate spot disease severity for various intervals starting from 60 day after sowing (data are recorded of two season)[x]

Treatments	Concen-tration	Disease severity (%)[y]				
		Days after sowing				
		60	80	100	120	140
K_2HPO_4	50 mM	1.0 d[z]	3.3 d	9.3 e	13.0 d	20.6 e
$FeCl_3$	20 mM	2.0 b	5.7 c	11.0 d	17.3 c	22.6 d
Salicylic acid	1 mM	2.0 b	10.0 b	13.0 c	19.6 c	25.0 c
Ethephon	100 PPM	2.0 b	9.3 b	15.0 b	24.3 b	30.6 b
Non-treated		12.5 a	16.83 a	25 a	32 a	40.0 a

xxiv) Plants were sprayed to run-off twice after 45 days from sowing and 10 days later.
xxv) Disease severity was assessed using the scale described by Anonymous. (1976).
xxvi) Values followed by the same letter in each column are not significantly different at P? 0.05 according to Duncan's multiple range test.

Effect of inducer treatments as foliar spray on phenol content.

Results in Table (4), indicate that all chemical inducers tested greatly increased phenol contents of the inoculated faba bean plants with *Botrytis fabae*, compared with non-inoculated plants.

Table (4): Effect of various chemical treatments as foliar spray on phenol content[a] of faba bean plants[b].

Treatments	Concentra-tions	Non-inoculated			Inoculated		
		Total	Free	conjugated	Total	Free	conjugated
K_2HPO_4	50 mM	98.3	60.0	38.3	100.9	59.0	41.9
$FeCl_3$	20 mM	97.1	65.0	32.1	101.0	60.2	40.8
Salicylic acid	1 mM	81.3	63.0	18.3	100.5	60.2	40.3
Ethephon	50 PPM	98.0	63.8	34.2	98.0	59.8	38.2
Non-treated		90.4	58.0	32.4	96.9	54.0	42.9

i) phenol contents were determined, 72 hr after challenge inoculated with *B. fabae*.

ii) Leaves of 50-day-old plants were sprayed with each tested chemical and after 55 days they challenged with suspension of *Botrytis fabae* (10^5 conidiospore)

Effect of chemical treatments as foliar spray on soluble proteins of faba bean plants.

Foliar spray of faba bean plants with chemicals at the tested concentration showed the appearance of new protein bands in treated plants compared with non-treated. K_2HPO_4 and salicylic acid at 50 mM and 1 mM respectively, greatly induced appearance of new protein Fig. (1)

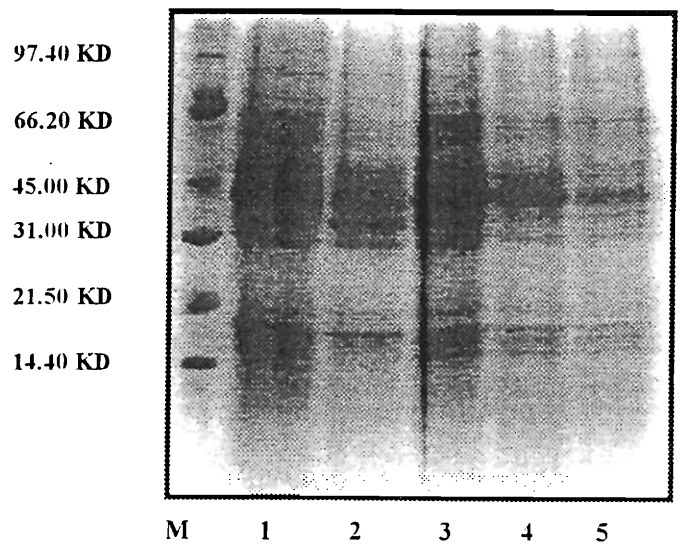

Fig.(2): SDS Gel-Electrophoresis for soluble proteins of faba bean plants (cv. Giza3) sprayed with various chemical inducers twice after sowing, 45 and 55 days. (M) Marker, (1) K_2HPO_4 50

mM, (2) $FeCl_3$ 20 mM, (3) SA 10 mM, (4) Ethephon 500 PPM, (5) Non-treated plants

Effect of chemical treatments on growth characters and yield components.

Generally, all treatments either seed soaking or foliar spray significantly increased plant height, number of pods, 100-seed weight and seed yield per plant. Meanwhile all tested chemicals had no significant effect on number of branches. However, K_2HPO_4 and $FeCl_3$ were the best treatments that highly increased seed yield compared with other treatments Table (5&6).

Table (5). Effect of chemical treatments as seed soaking[x)] on growth, yield and yield component of faba bean[y)].

Treatments	Concen t-ration	Plant height (cm)	No. of branche s/plant	No. of pods/ plant	100-seed weight (g)	Seed yield/ plant (g)
K_2HPO_4	50 mM	123.0 a[z)]	3.0 a	32.6 ab	125.3 a	108.0 a
$FeCl_3$	20 mM	120.3 a	2.7 a	27.3 cd	120.6 c	98.3 b
Salicylic acid	10 mM	101.3 b	2.7 a	31.3 b	123.6 b	66.6 de
Ethephon	500 mM	83.5 d	2.3 a	35.3 a	108.3 d	69.6 d
Non-treated		91.0 c	2.3 a	25.6 d	104.3 e	76.5 c

xxiv) Seeds were soaked in the tested chemicals for 12 h before sowing.
xxv) Data were recorded at maturity of faba bean plants.
xxvi) Values followed by the same letter in each column are not significantly different at P? 0.05 according to Duncan's multiple range tests.

Table (6). Effect of chemical treatments[a)] as foliar spray on growth, yield and yield component of faba bean[b)].

Treatments	Concent-rations	Plant height (cm)	No. branches/plant	No. pods/ plant	100-seed weight (g)	Seed yield/ plant (g)
K_2HPO_4	50 mM	109.6 a	3.0 a	35.6 a	140.6 a	119.3 a
$FeCl_3$	20 mM	101.3 b	2.3 a	26.3 c	135.5 b	102.5 c
Salicylic acid	1 mM	88.3 e	2.0 a	24.0 e	130.6 c	107.0 b
Ethephon	50 mM	95.6 c	3.3 a	30.3 b	129.3 c	101.0 c
Non-treated		91.0 d	2.3 a	25.6 d	104.3 d	76.5 d

i) fourty-five day old plants were sprayed to run-off by each tested chemical under field conditions.
ii) Data were recorded at maturity of faba bean plants.
*) Values followed by the same letter in each column are not significantly different at P? 0.05 according to Duncan's multiple range tests

DISCUSSION

The results of this study concluded that, resistance to faba bean plants against chocolate spot caused by *Botrytis fabae* and *B. cinerea* can be induced by chemical treatments. Similar results in this respect were reported by several investigators (Abu El-Hawa, 1998., Islam *et al.*, 1998 and Walter and Murray., 1992). As it indicated by *in vivo.*, and field experiments, all

either by seed soaking or foliar spray significantly reduced chocolate spot disease severity of faba bean plants. However, treatment of faba bean seeds with K_2HPO_4 and $FeCl_3$ or foliar spray at 50 and 20 mM respectively, was the most effective treatments to reduce disease severity. Meanwhile, salicylic acid was the best as foliar spray at 1 mM. These results suggest that, the induction of resistance of tested chemicals was systemic because, seed soaking could reduce disease development on leaves. Meanwhile, these chemical compounds have no direct antimicrobial activity against many fungal and bacterial pathogens including *Btorytis fabae* and *B. cinerea* as reported by Aly and Afifi, 1989; Salem *et al.*,1992; Walter and Murray, 1992; and Islam *et al.*, 1998.

Seed soaking or foliar spray with mono or di-potassium poshphates. have been shown to induce resistance in different crops against various foliar diseases (Gottstein and Kuc, 1989; Manandhar *et al.*,1998a and Mosa, 2002). The results of the present study are in agreement with Walter and Murray, (1992) who stated that, phosphate and EDTA had inducible effect of faba bean plants against rust disease cause by *Uromyces vciae-fabae*. Meanwhile, Ferric chloride provide it's efficiency either by seed soaking or foliar spray to reduce chocolate spot disease severity.

The results of this study revealed the appearance of new bands of soluble proteins in treated faba bean plants indicates plant response against chocolate spot disease. The appearance of new proteins were reported and identified as Pathogensis-related proteins (PR proteins) which, also may be induced by chemical treatments (Kessmann *et al.*, 1994; Benhamou, 1996; and Sticher *et al.*, 1997).

The results of this study clearly indicate the beneficial effect of K_2HPO_4 and $FeCl_3$ as foliar treatment for faba bean plants. Both treatments have greatly reduced of chocolate spot disease incidence and also, increased seed yield. This study may facilite the application of induced resistance using simple, non-toxic chemical solution to protect faba bean against chocolate spot and other foliar diseases of faba bean.

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المقاومة المستحثة لمرض التبقع البنى فى الفول البلدى.

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يعتبر مرض التبقع البنى (الشيكولاتى) من اخطر الأمراض التى تصيب الفول البلدى فى مصر.و يهدف هذا البحث إلى دراسة حث مقاومة الفول البلدى لمقاومة مرض التبقع البنى الشيكولاتى باستخدام بعض عوامل الحث الغير حيوية , وذلك تحت ظروف تجارب الحقل. وقد شملت هذه العوامل فوسفات البوتاسيوم الثنائية , كلوريد الحديديك , حمض السالسيليك , والايثيفون وذلك إما نقع البذور أو رش المجموع الخضرى بها. أدى استخدام هذه المعاملات الى خفض كلا من نسبة التلون وانتشار بقعة الإصابة بالفطر بوترايتس وذلك بإستخدام تكنيك الأوراق المفصولة. أدى رش المجموع الخضرى بكل من فوسفات البوتاسيوم الثنائية وكلوريد الحديديك عند التركيزات ٥٠ , ٢٠ ميلليمول على التوالى الى اختزال شدة الاصابة بمرض التبقع الشيكولاتى , بينما كانت معاملات نقع البذرة اقل فعالية. كما أدت المعاملة بفوسفات البوتاسيوم الثنائية وكلوريد الحديديك عند التركيزات ٥٠ , ٢٠ ملليمول سواء نقع البذرة أو رش المجموع الخضرى الى تقليل نسبة تساقط الأزهار والى زيادة محصول النبات. وقد اظهر تكنيك التفريد الكهربى الى ظهور حزم بروتين جديدة فى النباتات المعاملة مقارنة بالنباتات الغير معاملة.