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Changes in Peroxidase Activity Due to Resistance Induced Against Faba Bean Chocolate Spot Disease

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Citric, benzoic, salicylic and oxalic acids and ribavirin revealed remarkable induced resistance effects against chocolate spot disease of faba bean, caused by *Botrytis fabae* and/or *B. cinerea* when compared with control. *Botrytis cinerea* was much more sensitive to the tested inducers.

Resulted disease reduction was accompanied with a gradual increase in peroxidase activity during experiment period. Among the inducers tested, citric and benzoic acids were the most effective one, since they recorded the lowest percentages of disease severity and the highest levels of peroxidase activities. This increase reached up to two-folds of the control treatment.

Moreover, pretreated faba bean plants showed some new isozymes and increment in the density of original isozymes, especially in infected plants.

Keywords: *Botrytis cinerea*, *Botrytis fabae*, chocolate spot, faba bean, induced resistance, isozymes and peroxidase activity.

Botrytis fabae Sard. is the main cause of chocolate spot disease of faba bean (*Vicia fabae* L.), while *B. cinerea* Pers.ex.Fr. was also reported to cause the same disease problem to lesser degree (Harrison, 1988 and Rahman *et al.*, 2002). Considering the importance of faba bean crop to the Egyptian economy, great attention should be given to control disease problems of such crop.

In the field of plant disease control, with the environmental concern, several promising modern approaches have been developed recently away from pesticides use. Among them is the induced resistance approach, which could be induced in plants by applying chemical elicitors (Reglinski *et al.*, 2001). Chemical elicitors (inducers) seem to predispose the original defence mechanisms in plants against diseases or produce some new compounds supporting it. Ziadi *et al.* (2001), Dmitrier *et al.* (2003) and Achuo *et al.* (2004) and many others used several chemical or natural compounds known to induce plant resistance including salicylic, benzoic, citric and oxalic acids. Other investigators such as Nawar and Kuti (2003) stated that there are positive relationships between peroxidase (enzymes and isozymes) and resistance development in plants. Furthermore, Caruso *et al.* (2001) experimentally supported the idea that peoxidases play a defence role against invading pathogens of wheat kernels.

Current investigation was planned to determine changes in peroxidase activity and newly developed peroxidase isozymes in pre-sprayed plants with salicylic, benzoic, citric and oxalic acids, and ribavirin as inducers applied to artificially inoculated faba bean plants with any of the two pathogens tested. Moreover, to find out the possibilities of enhancing the defence mechanism of faba bean plants by defining the most suitable time of applying inducers to gain maximum reduction in disease severity.

Materials and Methods

Seeds of the highly susceptible cv. Giza-40 were used in this investigation. One isolate of *Botrytis fabae* and another one of *B. cinerea* were isolated from naturally infected faba bean plants, purified, identified according to Morgan (1971), sub-cultured, propagated and kept in refrigerator until use. A spore suspension of each pathogen was prepared fresh immediately before application at 2.5 x 10^5 spore/ml concentration.

Five chemical substances known to induce resistance in plants were used in two concentrations. Inducers under investigation (Table, 1) were evaluated as sources of resistance induced in faba bean plants against chocolate spot disease caused by *B. fabae* and/or *B. cinerea*.

Table 1. Chemical substances, their compositions, known effect(s) and tested concentrations for inducing resistance against *Botrytis fabae* and/or *B. cinerea*

Substance	Chemical composition	Known effect(s)	Tested concentration (mM)
Salicylic acid	2-Hydroxybenzoic	Antiseptic and antifungal	0.7 and 2.1
Benzoic acid	Benzenecarboxylic	Topical antifungal	0.8 and 1.6
Ribavirin	1-β-d-Ribofuranosyl-1H-1,2,4-triazole-3-carboxamide	Antiviral	0.1 and 0.2
Citric acid	2-Hydroxy-1,2,3-propanetricarboxylic	Preservative	0.5 and 1.5
Oxalic acid	Ethanedioic	Reducing agent	1.0 and 2.0

Two major identical groups of 30-cm-diam. pots, each group was prepared for one concentration. The first group (the first concentration) consisted of five sub-groups representing the mentioned inducer treatments. Each treatment consisted of three replicates, each of (5 seeds/pot):

a- Nil plants (treated with designated inducer with no inoculation applied).
b- Bf plants (treated with designated inducer and inoculated with *B. fabae*).
c- Bc plants (treated with designated inducer and inoculate with *B. cinerea*).

Plants developed ftom sub-groups were treated by spraying inducers 15 and 45 days after sowing, each with the defined inducer in a certain concentration. Fifty-three days after sowing, treated plants in each treatment were divided into three groups and exposed to artificial inoculation following the aforementioned procedures. Plants sprayed with tap water only served as control. All plants were covered with polyethylene bags for 24 h. to maintain high relative humidity, then kept under greenhouse conditions for other tests. The major control treatments were assigned as follows:

a- Nil plants (sprayed with tap water with no inoculation applied).
b- Bf plants (sprayed with tap water and inoculated with *B.fabae*).
c- Bc plants (sprayed with tap water and inoculated with *B. cinerea*).

Evaluation of disease reduction:

Disease symptoms were examined 48 h. after inoculation and best resulted effects were illustrated in photographs. Disease reduction percentages were calculated relative to the control treatment as recorded by the same workers (Hassan, *et al.*, 2006).

Evaluation of internal plant reaction:

Biochemical expression of pre-treated faba bean plants was evaluated to determine and emphasis the mechanism(s) developed by the tested chemicals inducing resistance against *B. fabae* or B. cinerea. The concentration of each inducer which gave the best reduction in disease severity was used to study the activity of peroxidase and its isozymes shown in Tables (2a and 2b).

a. Determination of peroxidase activity:

Leaves samples ftom each faba bean treatment, healthy or infected, were collected 0, 3, 6, 24 and 48 h. after inoculation for peroxidase activity assay. Also, untreated healthy or infected leaves were used as control. Enzyme extract was obtained by grinding leaf tissues in 0.1 M sodium phosphate buffer at pH (7.1) (2 ml/g leaf tissues) in a porcelain mortar. The extracted tissues were strained through four layers of cheesecloth. Filtrates were centrifuged at 3000 rpm for 20 min. at 6°C. Peroxidase activity was expressed as changes in absorbance/min at 425 nm according to the method of Allam and Hollis (1972).

b. Peroxidase isozymes:

Extraction, preparation and detection of peroxidase isozymes:

Detection and quantitative changes of peroxidase isozyme patterns were analyzed in pre-treated faba bean leaves with chemical inducers (healthy or infected with *B. fabae or B. cinerea*) at 0, 3, 6, 24 and 48 h. after inoculation using polyacrylamide gel electrophoresis. Where preparation of pathogens and plant samples was carried out as mentioned before by Hassan *et al.* (2006). Protein was extracted according to method described by Khalil (1981). Protein content was measured according to Bradford (1976) and the supernatant was stored at -80°C until use. Electrophoresis of native protein was carried out by using 7.5% acrylamide for the resolving gel and 3.5% for stacking polyacrylamide gel. Detection of peroxidase isozymes were recognized by using benzidine dihydrochloride according to Vallejes (1983). Although peroxidase isozyme patterns of the fungal pathogens were determined and found negative, no results related to this point were presented.

Results

Effect of pre-treatment on disease reduction:

Comparing with the untreated infected control, considerable reduction percentages were recorded when chemical inducers used to predispose faba bean plants to confront and control chocolate spot disease caused by *B. fabae* or *B. cinerea*, as illustrated on Fig. (1). Citric acid (at the lower concentration) and benzoic acid (at higher concentration) were superior in their effects by recording the highest reduction values of chocolate spot disease (Fig. 2), followed by oxalic and salicylic acids, while ribavirin compound was the least effective one.

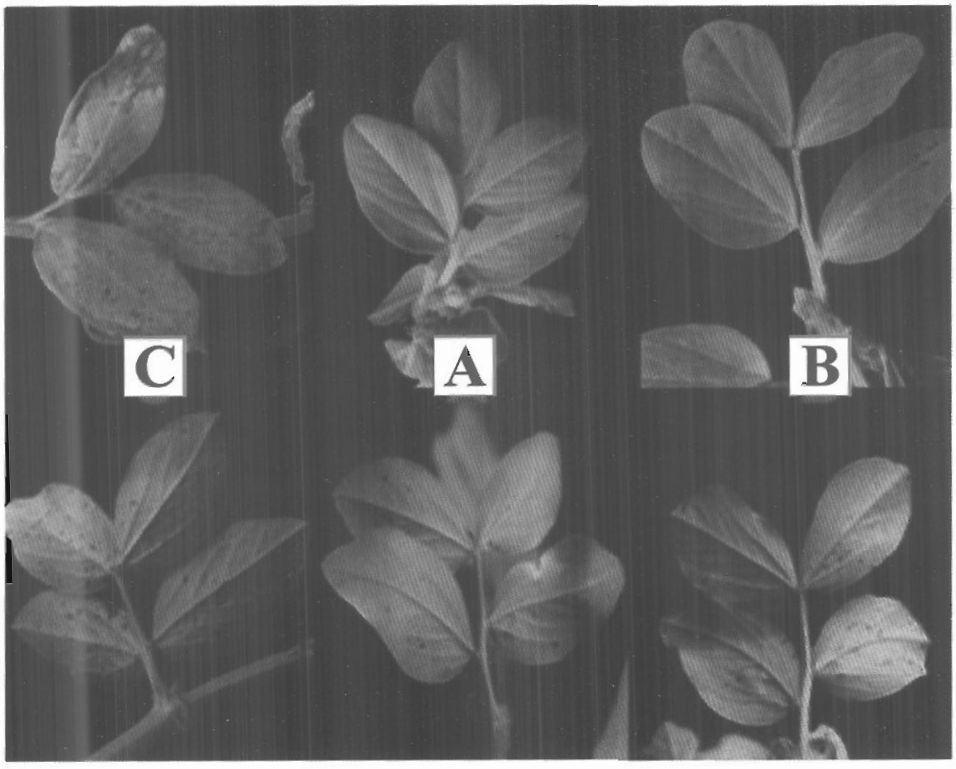


Fig. 1. Effect of spraying faba bean plants with: (A) benzoic and (B) citric acids on chocolate spot disease symptoms caused by *Botrytis fabae* (upper row) and *B. cinerea* (lower row). (C) untreated plants (control).

Effect of pre-treatment on peroxidaSe activity:

Applying chemical inducers resulted in a gradual increase in peroxidase activity in faba bean plants pre-sprayed and inoculated with B. *fabae* or B. *cinerea* during examination periods with pronounced differences according to the tested inducers. Increases in peroxidase activities were higher compared with untreated inoculated plants (control).

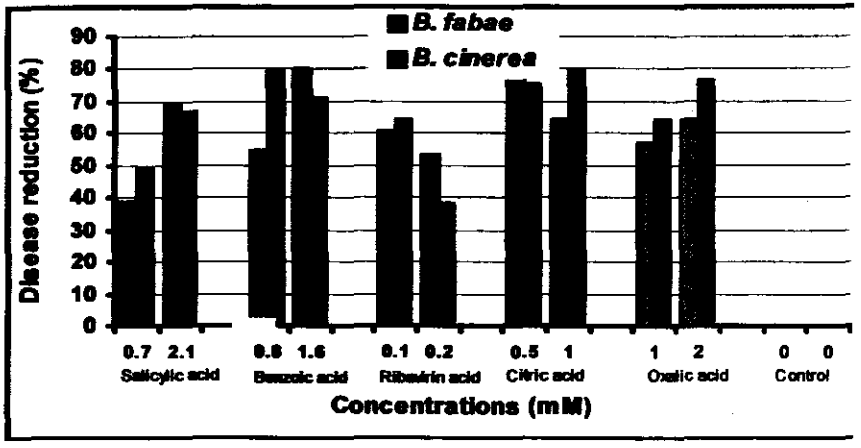


Fig. 2. Reduction percent of chocolate spot disease caused by *Botrytis fabae* or *B. cinerea* as affected by spraying faba bean plants with different chemical compounds.

Data presented in Table (2a) and also illustrated in Fig. (3), indicate that maximum increase in peroxidase activity was recorded against *B. fabae* infection after 24 h. then the activity decreased, except benzoic acid, which resumed and was the most effective inducer judged by the total peroxidase activity during 3, 6, 24 and 48 h., which was more than 2-folds of the control (untreated infected plants), followed by citric acid, salicylic acid and oxalic acid, respectively.

Table 2a. Activity of peroxidase in leaves of faba bean plants pretreated with chemical inducers and inoculated with *B. fabae*

Treatment	Conc. (mM)	Peroxidase activity/minute after (h.)									
		Infected with *B. fabae*					Healthy				
		0	3	6	24	48	0	3	6	24	48
Salicylic acid	2.1	0.307	0.467	0.798	1.889	0.960	0.307	0.468	0.613	1.274	0.937
Benzoic acid	1.6	0.259	1.128	0.997	1.324	2.965	0.259	0.396	0.671	1.303	0.474
Ribavirin	0.1	0.187	0.605	0.709	1.547	0.634	0.187	1.135	0.871	0.614	0.571
Citric acid	0.5	0.550	0.763	0.935	2.281	1.962	0.550	0.962	0.507	0.524	0.242
Oxalic acid	2.0	0.415	0.452	0.677	1.744	1.181	0.415	0.331	0.402	0.315	1.279
Control	--	0.305	0.542	0.607	1.340	0.795	0.305	0.948	0.463	0.926	0.513

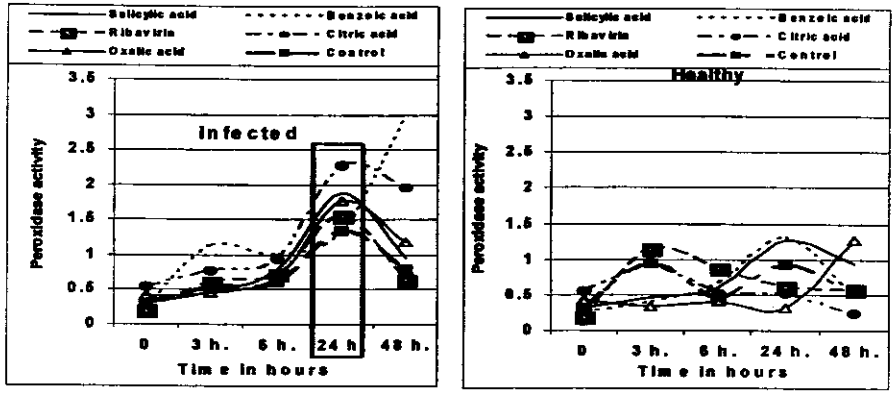


Fig. 3. Variations in peroxidase activities determined from pre-treatments with five inducers in faba bean leaves inoculated with *B. fabae* or healthy ones.

On the other hand, in *B. cinerea* (Table 2b) and Fig. (4), maximum increase in peroxidase activity was recorded after 48 h. in all treatments, except ribavirin, which recorded maximum increase in peroxidase activity after 24 h. then, the activity was decreased. Citric acid, salicylic acid and benzoic acid were the most effective inducers as they recorded the highest increase in peroxidase activity during 6, 24 and 48 h. Such increase in peroxidase activity was more than 2-folds of the control (untreated infected plants).

At the same time, fluctuation in peroxidase activity (during examination periods) was found in healthy faba bean plants sprayed with chemical inducers (Tables 2a and 2b), except, healthy faba bean plants sprayed with salicylic and benzoic acids (Table 2a), and salicylic acid in Table (2b), which recorded maximum increase in peroxidase activity after 24 h. then, the activity decreased.

Table 2b. Activity of peroxidase in leaves of faba bean plants pretreated with chemical inducers and inoculated with *B. cinerea*

Treatment	Conc. (mM)	Peroxidase activity/minute after (h.)									
		Infected with *B. cinerea*					Healthy				
		0	3	6	24	48	0	3	6	24	48
Salicylic acid	2.1	0.307	0.498	1.031	1.462	1.909	0.307	0.468	0.613	1.274	0.937
Benzoic acid	0.8	0.259	0.703	1.336	1.351	1.416	0.259	0.403	0.356	0.397	0.315
Ribavirin	0.1	0.187	0.189	0.452	0.726	0.641	0.187	1.135	0.871	0.614	0.571
Citric acid	1.0	0.550	0.787	1.006	1.222	2.027	0.550	0.486	0.513	0.522	0.397
Oxalic acid	2.0	0.405	0.550	0.589	0.703	0.817	0.405	0.331	0.402	0.315	1.279
Control	--	0.305	0.388	0.414	0.601	0.728	0.305	0.948	0.463	0.926	0.513

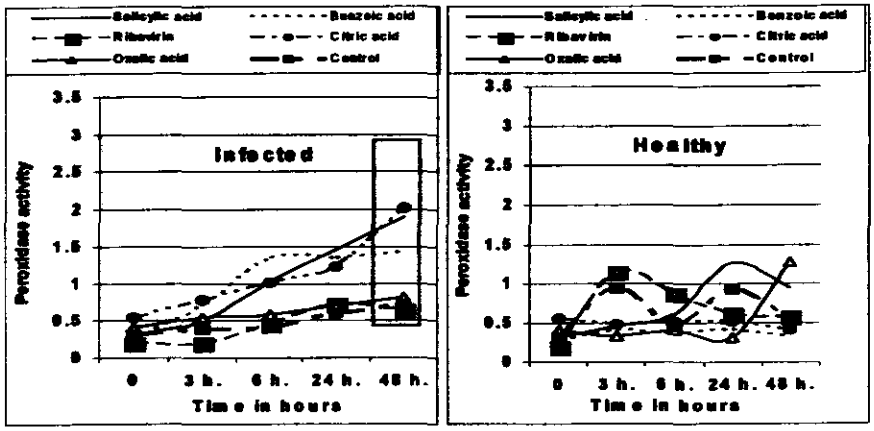


Fig. 4. Variations in peroxidase activities determined from pre-treatments with five inducers in faba bean leaves inoculated with *B. cinerea* or healthy ones.

Maximum increase in peroxidase activity was detected after 24 h. in *B. fabae* and 48 h. in *B. cinerea*, resulted in remarkable increase in disease reduction resulted from the inoculation of both pathogens.

Effect of pre-treatment on peroxidase isozymes:

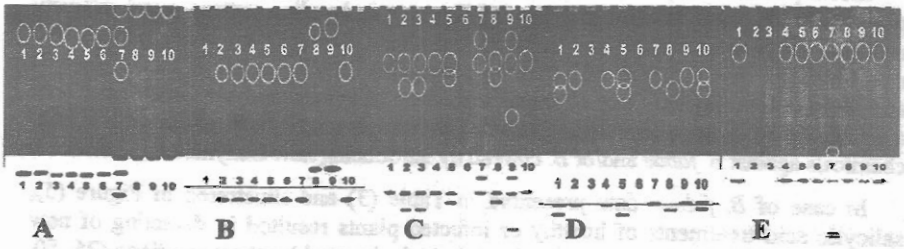
Electrophoretic study of peroxidase isozymes on pathogens and pretreated faba bean plants with chemical inducers indicated differences in banding pattern, band intensity and also between inoculated and uninoculated plants during examination periods. No new isozymes were detected with the two pathogens. Data collected in Tables (3 & 4) and illustrated in Figures (5 & 6) show peroxidase isozymes analysis which gave only a possibility of defence mechanism induced by spraying faba bean plants with certain chemicals against *B. fabae* and/or *B. cinerea* by introducing new isozymes of peroxidase.

In case of *B. fabae*, data presented in Table (3) and illustrated in Figure (5), salicylic acid treatments of healthy or infected plants resulted in detecting of new isozymes or disappearing of some other bands designated by their positions (26, 50, 54 and 112). Isozyme at position (26) was detected in healthy and infected plants after 24 and 48 h. with high concentration in infected plants (100%) compared with healthy one (30.32) after 24 h., at the same time, isozyme at position (50) disappeared during examination periods after though it existed at the beginning. While, the isozyme band recorded at position (54) was absent at zero time, then detected in healthy and infected plants after 3 and 6 h. with 100 % concentration. After 24 h., its concentration started to decline in healthy plants then disappeared. Isozyme at position (112) was detected only in healthy plants after 24 h. In benzoic acid treatment, four isozymes bands were detected at positions (24, 30, 128 and 130). The first one was found only in healthy plants after 48 h. Also, new

Table 3. Peroxidase isozymes in faba bean plants pre-sprayed with chemical inducers and infected with *Botrytis fabae* (as percentages of total amount of isozymes [% AMT])

Treatment	Position	Peroxidase isozymes detected after (h.)									
		(0)		(3)		(6)		(24)		(48)	
		H*	I**	H	I	H	I	H	I	H	I
		1	2	3	4	5	6	7	8	9	10
Salicylic acid	26	-	-	-	-	-	-	30.32	100	100	100
	50	100	100	-	-	-	-	-	-	-	-
	54	-	-	100	100	100	100	21.16	-	-	-
	112	-	-	-	-	-	-	48.52	-	-	-
Benzoic acid	24	-	-	-	-	-	-	-	-	100	-
	30	-	-	-	-	-	-	-	100	-	-
	128	-	-	100	100	100	100	100	-	-	100
	130	-	100	-	-	-	-	-	-	-	-
Ribavirin	24	-	-	-	-	-	-	72.50	-	28.60	-
	40	100	78.97	64.40	100	61.43	-	-	55.91	-	100
	72	-	-	-	-	38.57	-	-	44.09	-	-
	100	-	26.03	35.60	-	-	-	-	-	-	-
	110	-	-	-	-	-	-	-	-	41.87	-
	184	-	-	-	-	-	-	27.50	-	29.53	-
Citric acid	16	37.58	100	-	100	85.98	-	100	-	100	30.89
	42	62.42	-	-		14.06	-	-	100	-	69.11
Oxalic acid	34	100	-	-	100	100	100	41.29	100	100	100
	328	-	-	-	-	-	-	27.03	-	-	-
	344	-	-	-	-	-	-	31.68	-	-	-

* H= Healthy plants.
** I = Plants infected with *B. fabae*.



Lane 1= isozyme pattern in healthy leaves at zero time.	Lane 6= isozyme pattern in infected leaves after 6 h.
Lane 2= isozyme pattern in infected leaves at zero time.	Lane 7= isozyme pattern in healthy leaves after 24 h.
Lane 3= isozyme pattern in healthy leaves after 3 h.	Lane 8= isozyme pattern in infected leaves after 24 h.
Lane 4= isozyme pattern in infected leaves after3 h.	Lane 9= isozyme pattern in healthy leaves after 48 h.
Lane 5= isozyme pattern in healthy leaves after 6 h.	Lane 10= isozyme pattern in infected leaves after 48 h.

Fig. 5. Photographs and diagrams of peroxidase isozyme patterns of pre-sprayed faba bean leaves (healthy or infected with *B. fabae*) with chemical inducers detected after different periods, where: (A) Salicylic acid, (B) Benzoic acid, (C) Ribavirin, (D) Citric acid and (E) Oxalic acid.

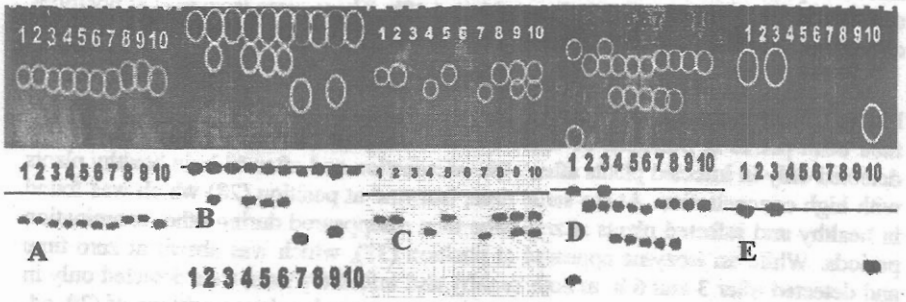
isozyme was detected at position (30) only in infected plants after 24 h. At the same time, isozyme positioned at (128) was not found at zero time but detected after 3 and 6 h. in both healthy and infected plants. The same isozyme disappeared after 24 h. in infected plants then was detected after 48 h. The isozyme positioned at (130) disappeared from both healthy and infected plants during 3, 6, 24 and 48 h., while it was existed only with (100%) concentration in infected plant at zero time. Six different peroxidase isozymes were detected at the positions of 24, 40, 72, 100, 110 and 184 in faba bean plants following treatment with ribavirin. Isozyme at position (24) was detected only in healthy plants after 24 and 48 h. Isozyme position at (40) was detected at 0 and 3 h. in both healthy and infected plants with its high concentration (100%) in infected plants after 3 h. compared with healthy one, the same isozyme appeared also after 24 and 48 h. only in infected plants. The new isozyme was detected at position (72) in healthy plants after 6 h. and in infected plants after 24 h. Other isozymes were detected only in healthy plants, except isozyme at position (100) detected in infected plant at zero time and in healthy plants after 3 h. Citric acid treatment showed isozymes bands at two positions (16 and 42). The first one positioned at (16) which was found in healthy and infected plants at zero time, detected only in infected plants after 3 h. of infection then detected in both healthy and infected plants after 48 h. While, the second isozyme band positioned at (42) was present only in healthy plants at zero time then detected in infected plants after 24 and 48 h. of inoculation. Applying oxalic acid resulted in new three isozymes at positions of 34, 328 and 344. Isozyme positioned at (34) was absent in infected plants at zero time then detected after 3 h. of inoculation in infected plants with high concentration (100 %). Moreover, its concentration resumed high in infected plant after 6, 24 and 48h. There were isozymes at positions of 328 and 344 detected only in healthy plants after 24 h.

Regarding the *B. cinerea* pathogen, data recorded in Table (4) and presented in Fig. (6) revealed that in salicylic acid treatment, three isozymes bands were detected in faba bean plants at positions of 4, 28 and 37. Isozyme recorded at position (4) was detected only in infected plants after 24 h. of infection and after 48 h. in healthy plants with high concentration. At the same time, isozyme at position (28) which was found in healthy and infected plants at zero time then disappeared during other examination periods. While an isozyme appeared at position (37), which was absent at zero time and detected after 3 and 6 h. in both healthy and infected plants, then detected only in infected plants after 48 h. of inoculation. Four isozymes bands at positions of (20, 64, 120 and 126) were detected in faba bean plants pretreated with benzoic acid. Isozyme at position (20) was common in both healthy and infected plants during examination periods 0, 3, 6, 24 and 48 h. Moreover, its concentration was increased in infected plants compared with healthy ones during examination periods after 6, 24 and 48 h. Isozyme (64) was common in infected plants at 0, 3 and 6 h. At the same time, isozyme at position (120) was detected only in healthy plants after 48h. Isozyme recognized at position (126) was detected in healthy plants after 24 h. In ribavirin treatment, 3 isozymes bands were detected during examination periods at positions (22, 78 and 84). Isozyme band at position (22) was detected in both healthy and infected plants at zero time. Its concentration increased only in infected plants after 24h. New isozyme was only detected at position (78) in infected plants after 3 h. of

Table 4. Peroxidase isozymes in faba bean plants pre-sprayed with chemical inducers and infected with *Botrytis cinerea* (as percentages of total amount of isozymes [% AMT])

Treatment	Position	Peroxidase isozymes detected after (h.)									
		(0)		(3)		(6)		(24)		(48)	
		H*	I**	H	I	H	I	H	I	H	I
		1	2	3	4	5	6	7	8	9	10
Salicylic acid	4	-	-	-	-	-	-	-	100	100	-
	28	100	100	-	-	-	-	-	-	-	-
	37	-	-	100	100	100	100	100	-	-	100
Benzoic acid	20	100	40.55	100	64.70	34.26	52.31	59.48	100	74.67	100
	64	-	59.45	-	45.30	65.74	47.69	-	-	-	-
	120	-	-	-	-	-	-	-	-	25.33	-
	126	-	-	-	-	-	-	40.52	-	-	-
Ribavirin	22	100	100	-	-	100	-	-	100	50.61	47.56
	78	-	-	-	100	-	-	-	-	49.39	52.44
	84	-	-	-	-	-	-	100	-	-	-
Citric acid	24	100	-	95.06	-	-	-	-	-	-	-
	48	-	-	-	-	-	-	83.30	79.73	100	100
	56	-	100	4.97	77.44	59.07	68.97	-	-	-	-
	166	-	-	-	22.56	40.93	31.03	16.70	20.27	-	-
Oxalic acid	4	100	-	100	-	-	-	-	-	-	-
	108	-	-	-	-	-	-	-	-	-	100

* H = Healthy plants.
** I = Plants infected with *B. cinerea*.



Lane 1= isozyme pattern in healthy plant at zero time.
Lane 2= isozyme pattern in infected plant at zero time.
Lane 3= isozyme pattern in healthy plant after 3 h.
Lane 4= isozyme pattern in infected plant after3 h.
Lane 5= isozyme pattern in healthy plant after 6 h.
Lane 6= isozyme pattern in infected plant after 6 h.
Lane 7= isozyme pattern in healthy plant after 24 h.
Lane 8= isozyme pattern in infected plant after 24 h.
Lane 9= isozyme pattern in healthy plant after 48 h.
Lane 10= isozyme pattern in infected plant after 48 h.

Fig. 6. Photographs and diagrams of peroxidase isozyme patterns of pre-sprayed faba bean leaves (healthy or infected with *B. cinerea*) with chemical inducers detected after different periods, where: (A) Salicylic acid, (B) Benzoic acid, (C) Ribavirin, (D) Citric acid and (E) Oxalic acid.

inoculation with high concentration (100 %) then detected after 48 h. in both healthy and infected plants. While, isozyme (84) was found in healthy plants during 24 h. Four isozymes bands at positions (24, 48, 56, and 166) were detected in faba bean plants treated with citric acid. Isozyme band at position (24) was detected only in healthy plants at 0 and 3h. New isozymes at position 48 were detected in both healthy and infected plants after 24 and 48 h. with high concentration after 48 h. (100 %). Isozyme at position 56 was detected in healthy and infected plants after 3 and 6 h. with high concentration in infected plants. New isozyme at position (166) was detected only in infected plants after 3h. then detected in both healthy and infected plants after 6 and 24 h. Two isozymes bands at positions (4 and 108) were detected in faba bean plants pretreated with oxalic acid. Isozyme positioned 4 was detected only in healthy plants at zero time and 3 h., respectively. While, isozyme (108) was detected only in infected plants after 48 h. of inoculation.

Discussion

Current findings revealed that citric and benzoic acids were superior in reducing leaf spot severity of *B. fabae* and *B. cinerea* regardless its concentration. Citric and benzoic acids have significantly reduced infection with *Botrytis cinerea* on different plant hosts, Elad (1992), while, salicylic acid induced resistance to grey mould (*Botrytis cinerea*) on tomato and tobacco plants (Achuo *et al.*, 2004).

A rapid increase in peroxidase activity was found in pretreated infected faba bean plants (during examination periods). Maximum increase in peroxidase activity was detected after 24 h. in case of *B. fabae* and after 48 h. in case of *B. cinerea*, which means that each target pathogen should be studied individually to gain maximum possible control of certain pathogen.

Also, application of chemical inducers resulted in changes in peroxidase isozymes in both healthy and infected plants during examination periods. Some treatments introduced new isozymes and/or increase in concentration of some isozymes especially after infection with the pathogen. The role of oxidative enzymes such as peroxidase could be explained as an oxidation process of phenol compounds to oxidized products (quinones) which may limit the fungal growth. Vance *et al.* (1980) and (Fry, 1982) stated that peroxidase is known to be involved in the oxidation of polymerization of hydroxycinnamyl alcohols to yield lignin and cross-linking isodityrosine bridges in cell wall. Another explanation was achieved by Ride (1983) and Tarrad *et al.* (1993), who reported that increase in peroxidase activity enhance lignification in response to chocolate spot infection which may restrict the fungal penetration. These findings indicate a positive relationship between resistance and peroxidase activity. Peroxidase also produces free radicals and hydrogen peroxide which are toxic to many microorganisms (Pena and Kuc, 1992). Another supportive suggestion was brought by Nawar and Kuti (2003) who stated that an increase in peroxidase activity is considered as a preliminary indicator for resistance of broad beans to chocolate spot disease. These compounds act as barriers against pathogen invasion.

Regarding the determined peroxidase isozymes in the current investigation, appearance of new isozymes during examination periods in pretreated infected faba bean plants was closely related to the high reduction in disease severity of chocolate spot disease. Aly and Afify (1989) pointed out that the resistance induced in barley plants showed new peroxidase isozymes in infected plants pre-treated with ethephon. Bargabus *et al.* (2002) indicated that the increase in peroxidase specific activity following benzoic acid derivatives (acibenzolar-S-methyl) treatment was due to the production of two unique isoforms not found in untreated plants.

Generally, data obtained through this investigation showed a positive relation between reduction in severity of chocolate spot disease and the increase in peroxidase activity and peroxidase isozymes as the results of application of chemical inducers especially in pre-sprayed infected faba bean plants with benzoic or citric acids. No doubt these chemicals are environmentally safe, economically cheaper and easy to use on the level of small farms with much less hazards comparing with ordinary fungicides. Although of the aforementioned advantages, contrarily, many points of study related to chemical inducers should be investigated before being commercially accepted and released.

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تغيرات فى نشاط إنزيم البيوكسيديز نتيجة للمقاومة المستحثة ضد مرض التبقع الشيكولاتى فى الفول البلدى

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أدى رش نباتات الفول البلدى بالمستحثات الكيميائية حامض الستريك ، حامض البنزويك ، حامض السيلسيلك و حامض الأوكساليك بالإضافة لمادة الريبافيرين قبل العدوى بالمسبب المرضى إلى انخفاض ملحوظ فى شدة الإصابة بمرض التبقع الشيكولاتى المتسبب عن أى من الفطرين بوتريتس فابى أو بوتريتس سينارويا وذلك مقارنة بالنباتات غير المعاملة. وقد وجد أن الفطر بوتريتس سينارويا كان الأكثر تأثرا بالمستحثات المستخدمة.

لوحظ أن إنخفاض شدة الإصابة كان مصحوبا بزيادة تدريجية فى نشاط إنزيم البيروكسيديز خلال فترات الإختبار فى التجربة. وكان حامض الستريك وحامض البنزويك أكفأ المستحثات المختبرة حيث سجل كلا الحامضين أقل شدة إصابة وأعلى نشاط لإنزيم البروكسيديز حيث وصلت الزيادة لنشاط الإنزيم لأكثر من ضعفى النشاط المسجل فى حالة المقارنة.

كما أدت معاملة المستحثات الكيميائية رشا على نباتات الفول البلدى إلى ظهور مشابهات إنزيمية جديدة وزيادة كثافة المشابهات الإنزيمية الأخرى خاصة فى النباتات المعاملة بالمستحث ومعدية بالفطر الممرض.