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Research Article

Effect of some chemical inducers on chocolate spot disease of faba bean in Tunisia

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Abstract: *Botrytis fabae* is one of the most important fungal pathogens attacking the leaves and the stem of faba bean *Vicia faba* L. and causes severe yield losses. This study was carried out to evaluate the effect of four chemical inducers (salicylic, citric, ascorbic and oxalic acids) and one fungicide (Carbendazim) against *B. fabae* in field and glasshouse conditions. Under field conditions for two seasons and glasshouse experiments, plants treated with salicylic acid showed substantial and significant decrease in the disease severity on the leaves and the stem compared with the control and the fungicide. Salicylic acid was highly effective and controlled the disease better than Carbendazim which provided only partial protection. *In vitro*, the inhibition of fungal growth was investigated and showed that salicylic acid was the best inhibitor of fungal growth (48%) followed by oxalic (39%), ascorbic (33%) and citric (10%) acids 6 days after incubation. An important increase of total phenols was recorded in treatment by salicylic acid in the healthy and infected leaves of faba bean 12, 24, 48, 72, 96 and 120 hours after inoculation. These promising results on the control of the main fungal disease damaging faba bean in Tunisia and other regions will have an important impact on faba bean production.

Keywords: *Botrytis fabae*, chemical inducers, total phenols, *Vicia faba*

Introduction

Chocolate spot, due to *Botrytis fabae* Sard., is an important disease of faba bean *Vicia faba* L. in several regions of the world (Bouhassan *et al*., 2004), which causes great yield loss (Stoddard *et al*., 2010). In the North Africa region, the yield losses due to chocolate spot disease can reach 60-80% among the susceptible cultivars (Bouhassan *et al*., 2004; Sahile *et al*., 2008). The use of resistant cultivars is the best way to control diseases. Unfortunately, no high yielding resistant faba bean varieties to chocolate spot are available (Fernandez-Aparicio *et al*., 2011; Sillero *et al*., 2010; Villegas-Fernandez *et al*., 2010). Other strategies to control several diseases are currently prospected and studied particularly the use of chemical inducers. These plant defence inducers showed successful results in controlling some plant diseases such as *Fusarium* wilt in chickpea (Sarwar *et al*., 2005), chocolate spot in faba bean (Hassan *et al*., 2006), *Fusarium* root rot and wilt in pepper (Abdel-Monaim *et al*., 2010). These chemical inducers are supposed to be much more environmentally safe than synthetic fungicides and cost less. They reduce environmental pollution and some of them (salicylic acid) create an induced systemic resistance in the host against several pathogens. Many other workers have used several chemical or natural compounds known to induce plant resistance including salicylic, citric, benzoic and oxalic acids (Achuo *et al*., 2004; Hassan *et al*.,

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2006; El-Hendawy *et al*., 2010). The aim of this work was to evaluate the efficacy of salicylic, oxalic, ascorbic and citric acids against chocolate spot disease *in vitro*, under glasshouse and field conditions and to assess the induction of phenolic compounds in leaves after being challenged with *B. fabae*.

Materials and Methods

Chemical inducers

Four chemical inducers (Salicylic Acid: SA at 2.1 mM, Citric Acid: CA at 0.5 mM, Oxalic Acid: OA at 2 mM and Ascorbic Acid: AA at 10 mM) produced by Sigma were used in this study. Inducer concentrations used were selected after preliminary tests conducted in glasshouse with different concentrations for each chemical. None of the concentrations tested produced any visible effects on faba bean plant growth. The most effective concentration to decrease chocolate spot severity was chosen for conducting this study. Aqueous solutions were prepared by dissolving the indicated amount of inducers in sterile distilled water and mixed with a stirrer for few minutes to ensure the acids were completely dissolved.

Plant material

Faba bean (*V. faba* var. *minor*) seeds of the susceptible cultivar to chocolate spot (cv. Badï) used in the experiments were obtained from the Field Crop Laboratory of the National Agricultural Research Institute of Tunisia (INRAT). The variety Badï was selected by INRAT and registered in the Tunisian Official Catalogue of Plant Varieties in 2003 and commercialized in Tunisia by the seed company COSEM.

Isolation, purification and identification of the causal pathogen

The isolate I_4 of *B. fabae* used in the trials was obtained from faba bean leaves showing typical symptoms of chocolate spot collected in Beja (Tunisia) region during the 2006-2007 growing season. I_4 was found to be the most virulent among 18 *B. fabae* and 4 *B. cinerea* isolates tested on 4 faba bean entries including cv. Badï (unpublished study).

The leaves were disinfected with 1% sodium hypochlorite for 2 min, rinsed twice in sterile distilled water, dried with filter paper, placed on Potato Dextrose Agar (PDA) medium and incubated at 20 °C for 7 days. Purified cultures were identified according to Morgan (1971). The most aggressive isolate I_4 was selected among 17 isolates on the base of pathogenicity test (unpublished data).

Inoculum preparation

Isolate I_4 was multiplied in Petri dishes (9 cm diameter) containing the Faba Leaf Dextrose Agar (FLDA) medium (200g of faba bean leaves, 20g dextrose, 30g sodium chloride and 20g agar in 1 litter of distilled water) and incubated at 20 °C in a cycle of 12h darkness and 12h visible light to induce sporulation. After 15 days of growth, an inoculum suspension was prepared by adding sterile distilled water. Spore suspensions were then adjusted to 5×10^5 spores/ml with the haemocytometer (Derckel *et al*., 1999).

Field trial

Two field trials were conducted at Morneg experimental station (Northern Tunisia, 36°38'15''N, 10°16'42''E, altitude 47 m) in the cropping seasons 2011-2012 and 2012-2013. Faba bean seeds (cv. Badï) were sown on 2nd December 2011 and 3rd December 2012. A split-plot randomized block design with three blocks was applied. In the main plots, two modalities were considered: application of *B. fabae* inoculum and application of tap water. In the subplots, 6 treatments were applied: the 4 inducers (SA, CA, AA, OA), the fungicide Carbendazim, C (Bavistin FL500) used at 5 g a.i/l and the sterile distilled water. Each plot consisted of 4 rows, 4 m long and spaced 0.5 m apart. Forty seeds were sown per 4 m row. Few days before faba bean reached the flowering stage (80 days after sowing), the chemical inducers and the fungicide were applied on the corresponding plots early in the morning using knapsack sprayer adjusted to 35 ml/m². The knapsack sprayer was well washed after each chemical treatment. Four hours later the main plots were either inoculated with *B. fabae* spores suspension at the concentration of 5×10^5

spores/ml or sprayed with tap water using another knapsack sprayer.

Plants were scored regularly 14, 32, 51, 74 and 86 days after inoculation using 0 to 9 scale on leaves (Ding *et al*., 1993), where 0 = no disease symptoms, 1 = a few lesions accounting for less than 5% of total leaf area; 3 = discrete spots less than 2 mm in diameter, accounting for 6-25% of leaf area; 5 = numerous scattered spots with a few linkages, diameter 3-5 mm, on 26-50% of leaf area with a little defoliation; 7 = large coalesced sporulating lesions covering 51-75% of leaf area, half the leaves dead or defoliated and 9 = complete destruction of the larger leaves, spot lesions covering more than 76% of leaf area, abundant sporulation, heavy defoliation and plants death. For disease symptoms on stem, a scale from 0 to 3 was used (William, 1975), where 0 = no visible infection; 1 = very small spots; 2 = some coalesced lesions; 3 = numerous coalescent lesions.

The disease scores were used to calculate the Mass Disease Index (MDI) on the leaves and the stem as reported by Tivoli *et al*. (2006).

Mass Disease Index on the leaves:

$MDI_L (\%) = [\sum^{9}_{i=0} (n \times i) / N \times 9] \times 100$

Mass Disease Index on the stem:

$MDI_S (\%) = [\sum^{3}_{i=0} (n \times i) / N \times 3] \times 100$

Where: **n** = number of plants scored as **i**

N = total number of plants

To evaluate the symptoms progress, Area Under the Disease Progress Curve (AUDPC) was calculated according to the following formula (Steffenson and Webster, 1992):

$$AUDPC = \sum_{i=1}^{n} [(Y_{i+1} + Y_i) \times 0.5] [T_{i+1} - T_i]$$

Y_i = infection index on the leaves MDI_L or on the stem MDI_S at the i^{th} observation

T_i = time (days) at the i^{th} observation

n = total number of observations

Glasshouse Trial

In order to evaluate the efficacy of chemical inducers in reducing chocolate spot disease under controlled conditions, 6 lots (each lot corresponds to one treatment) of 10 pots each filled with compost were used. Four seeds of cv. Badï were sown in each pot (25 cm diameter) and 10 pots were used for each treatment. Faba bean plants were sprayed by the different treatment as described above at 4-leaf stage. After 24 hours, the 10 pots of each treatment were divided in 2 sets: one sprayed with spore suspension of *B. fabae* (I_4) adjusted to a concentration of 5×10^5 spores/ml, and the second was sprayed with sterile distilled water. All pots were then covered with plastic bags for 48 hours to ensure a high relative humidity. The experiment was carried out according a completely randomised split plot design with 5 replications in glasshouse at the National Institute for Agricultural Research of Tunisia (INRAT) in Ariana. The glasshouse temperature during the experiment was maintained at 20 ± 3 °C.

Chocolate spot disease was scored five times (2, 4, 6, 10 and 14 days after inoculation) on leaves according to Ding *et al*. (1993) and on stem according to William (1975) scales. MDI_L, MDI_S and AUDPC were then calculated according the formula mentioned previously.

Effect of chemical inducers on the linear growth of *B. fabae*

The effect of chemical inducers on linear growth of the aggressive isolate I_4 of *B. fabae* was determined in Petri dishes. These chemical inducers were incorporated in PDA medium at concentration of 2.1 mM for SA, 0.5 mM for CA, 2 mM for OA and 10 mM for AA by adding the fixed amount of each substance aseptically to the melted PDA medium just before solidification. Disc (5 mm diameter) of *B. fabae* culture was put in the middle of the Petri dish containing 20 ml of PDA. For the control, the Petri dishes contained only the PDA medium. Three Petri dishes for each treatment were used as replicates. The Petri dishes were incubated at 20 °C for 6 days. The linear growth of *B. fabae* was measured in cm after 2, 3, 4, 5 and 6 days of incubation to determine the most effective chemical against the pathogen. The inhibition (I) percentage was calculated using the formula of Topps and Wain (1957):

$I (\%) = (A-B)/A \times 100$

where: A = Mean diameter of growth in the control.

B = Mean diameter of growth in treatment.

Total phenols

Total phenols were evaluated as an indicator for the effect of inducing resistance in *V. faba* L. The same protocol described for the previous experiment (glasshouse conditions) was repeated for determination of the total phenols. Six sampling were made (12, 24, 48, 72, 96 and 120 hours after inoculation) on leaves with the same isolate (I_4). One gram of the fresh foliar tissue was ground with a mortar and pestle (in an ice bath) in 2 ml cold acetone. After centrifugation (4500 g for 10 min at 4 °C) the supernatant was recovered and the pellet was subjected to two successive extractions with 2 ml of cold methanol. The supernatants were mixed and were subjected to total phenols determination with Folin Ciocalteu reagent (Nawar and Kuti, 2003). For each sampling, three replications were used. In a hemolysis tube containing 450 µl of sterile distilled water, 60 µl of the extract and 60 µl of Folin Ciocalteu reagent were added. After incubation for 5 min, 300 µl of saturated solution of sodium carbonate (Na_2CO_3) was added to the mixture and allowed to stand for 40 min. The absorbance was measured at 725 nm. The amount of total phenols was evaluated in equivalent of Chlorogenic acid (Sigma). The total phenols rate is expressed as µg Chlorogenic acid equivalents g^{-1} FW (µg/g FW).

Statistical analysis

Analysis of variance (ANOVA) of the data was performed using SAS (Statistical Analysis System, Version 9.2). The least significant difference (LSD) was calculated to separate the mean values if the effects were significant ($p = 0.05$).

For the glasshouse trial, all plants not inoculated with *B. fabae* were scored 0, therefore only treatments data inoculated with *B. fabae* were analysed following completely randomized model. For the mycelium growth, a completely randomized model was followed for the analysis. Data of total phenols were analysed following a factorial completely randomized design with 2 factors (chemicals and inoculation).

Results

Field experiment

ANOVA analysis of AUDPC and MDI 14, 32, 51, 74 and 84 days after inoculation revealed significant differences between treatments for the main plot (inoculation and non inoculation with *B. fabae*), for the subplots (chemical inducers, fungicide and control treatment) and their interaction for both seasons 2011-12 and 2012-13. According to MDI values and AUDPC, salicylic acid was the most effective treatment at the different scoring times. At 86 days after inoculation with *B. fabae*, it reduced disease severity in inoculated plots on leaves by 78% in 2012 and 82% in 2013 season and on stems by 57% in 2012 and 78% in 2013 season as compared to the control (Figs. 1 and 2). The other inducers (citric, ascorbic and oxalic acids) decreased the chocolate spot disease severity in the two seasons on the leaves and on the stem but at lesser degree than salicylic acid, resulting in almost similar control as the fungicide Carbendazim. Plants treated with citric acid showed the highest reduction of chocolate spot disease severity after salicylic acid for the 2 seasons on leaves (67% in 2012 and 72% in 2013) and on the stem (42% in 2012 and 82% in 2013).The lowest effect among the chemical inducers was that of oxalic acid (33% in 2012 and 61% in 2013) on leaves and (31% in 2012 and 72% in 2013) on stems.

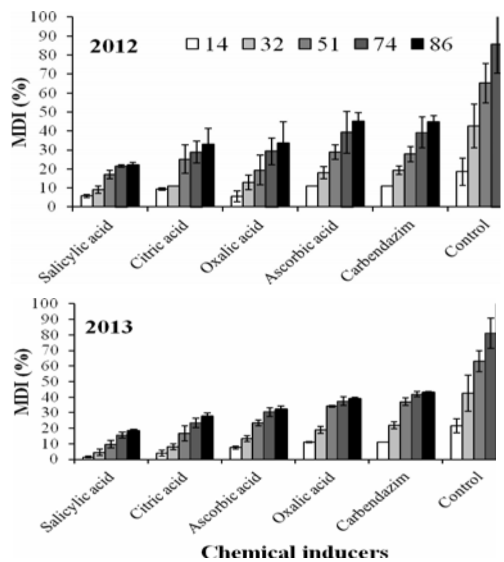

Figure 1 Effects of chemical inducers on chocolate spot disease on leaves (MDI) during the 2012 and 2013 growing seasons under field conditions at 14, 32, 51, 74 and 86 days after inoculation of *Botrytis fabae*. Vertical bars represent Standard Deviation.

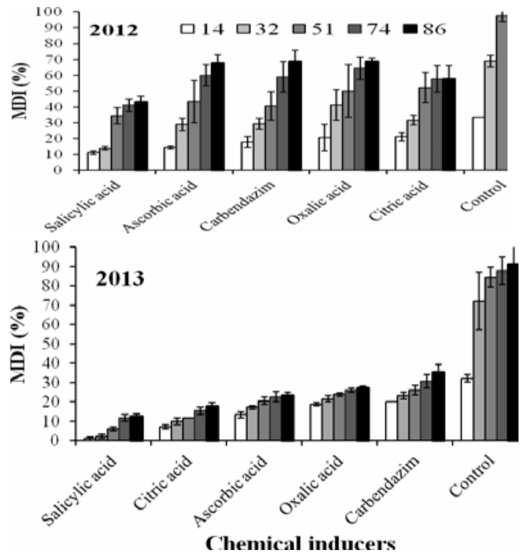

Figure 2 Effects of chemical inducers on chocolate spot disease on the stem (MDI) during the 2012 and 2013 growing seasons under field conditions at 14, 32, 51, 74 and 86 days after inoculation of *Botrytis fabae*. Vertical bars represent Standard Deviation.

Fig. (3) shows that salicylic acid was the best inducer to reduce the disease severity relative to the control with an AUDPC values equal to 923 and 594 on leaves for the 2012 and 2013 seasons respectively, and on stem, 1750 and 404 for the two years 2012 and 2013 respectively. For this treatment, AUDPC values on leaves were reduced by around 3 and 5 times as compared to the control in 2012 and 2013 seasons, respectively (Fig. 3).Treatment with the fungicide Carbendazim was found less effective in reducing disease severity under field conditions. In fact, salicylic acid controlled *B. fabae* significantly better than the fungicide Carbenbazim on leaves and stem in both seasons. The AUDPC values on leaves were reduced by 76% in 2012 and 84% in 2013 (Fig. 3). The efficiency of the treatments with citric, ascorbic and oxalic acids was variable (Fig. 3).For example the citric acid (958 and 745) reduced the disease severity better than oxalic (1729 and 1420) and ascorbic (1304 and 1183) acids in 2013 season on leaf and stem respectively. In contrast, oxalic acid (1219) in 2012 was better than citric acid (1295) on leaves but on stem ascorbic acid (2600) was more effective than citric acid (2716).

Glasshouse experiment

Analysis of variance of MDI values measured at 2, 4, 6, 10 and 14 days after inoculation showed high significant differences ($P < 0.0001$) between treatments. Plants treated with the chemical inducers and the fungicide Carbendazim reduced significantly chocolate spot disease severity as compared to the control (Fig. 4). The highest reduction was observed with salicylic acid treatment on the leaves (71%) (Fig. 4a) and on the stem (54%) (Fig. 4b).This inducer slowed down the disease development immediately after infection and later on, and presented low MDI levels. Oxalic acid, citric acid, Carbendazim and ascorbic acid showed disease reduction on the leaves, 58, 54, 40 and 37% respectively, 2 weeks after inoculation.. The AUDPC values on leaves showed also high significant differences between treatments confirming the differences observed for MDI values recorded across time (Fig. 4a and 5a). All the inducers and the fungicide Carbendazim had reduced considerably the disease development as compared to the control. According to AUDPC values, salicylic acid was the most effective in controlling chocolate spot disease showing significantly better control than the fungicide Carbendazim and the other inducers. However on stem, oxalic and ascorbic acids apparently losttheir efficacy one week after inoculation and had similar behaviour as the control under glasshouse conditions (Fig. 4b). Disease severity was reduced by application of citric acid and fungicide by 29 and 16% at 14 days after inoculation, respectively. According to AUDPC, salicylic acid followed by citric acid gave the highest control of the disease on the stem (65% and 47% disease reduction, respectively) (Fig. 5b). On both leaves and stem, salicylic acid was the most efficient in controlling *B. fabae* on faba bean.

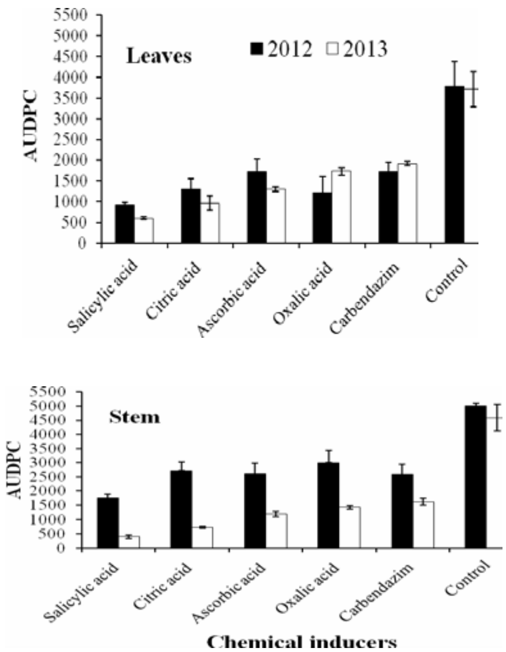

Figure 3 Classification of 6 treatments according to area under disease progress curve (AUDPC) values of *Botrytis fabae* determined under field conditions during the 2012 and 2013 growing seasons. Error bars represent Standard Deviation.

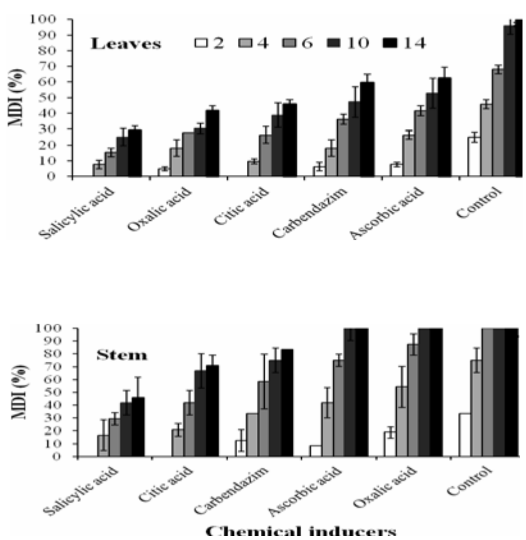

Figure 4 Effects of chemical inducers on chocolate spot disease (MDI) under greenhouse conditions at 2, 4, 6, 10 and 14 days after inoculation of *Botrytis fabae*.Vertical bars represent Standard Deviation.

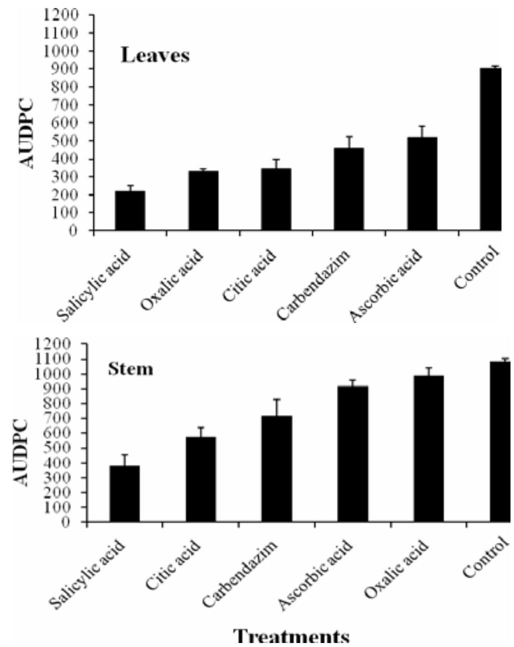

Figure 5 Classification of 6 treatments according to area under disease progress curve (AUDPC) values of *Botrytis fabae* determined under glasshouse conditions on the leaves and stem. Error bars represent Standard Deviation.

Effect of chemical inducers on the linear growth of *B. fabae*

The data presented in Table 1 show that all chemical inducers decreased significantly the mycelial growth of *B. fabae* compared to the control with salicylic acid the most effective inhibitor on the linear growth. For this inducer, the inhibition reached 41, 50, 47, 46 and 48% after 2, 3, 4, 5 and 6 days of incubation, respectively. Citric acid was found less effective, inducing 10% inhibition of fungal growth after 6 days of incubation. Ascorbic and oxalic acids were found to be moderately effective, with inhibition of 33 and 39% respectively after 6 days of incubation.

Total phenols

ANOVA showed significant to highly significant variation in the total phenols rate across time between the different treatments with the exception for 12 hours after inoculation, there was no significant effect between inoculated with *B.*

fabae and uninoculated leaves at that time. Interactions between the two factors (chemicals and inoculation) were not significant for all sampling period. After inoculation by *B. fabae*, the total phenols were progressively increased (Table 2). Furthermore, the treatments with salicylic, ascorbic, citric and oxalic acids induced an increase in the total phenols in the healthy and infected leaves. The highest increment of total phenols was observed in treatment by salicylic acid in inoculated and uninoculated leaves 12, 24, 48, 72, 96 and 120 hours after inoculation followed by citric and oxalic acids, Carbendazim and ascorbic acid.

Table 1 Effect of chemical inducers on the linear growth and inhibition of *Botrytis fabae*.

Treatments	Days of incubation									
	2		3		4		5		6	
	Colony diameter (cm)	I (%)	Colony diameter (cm)	I (%)	Colony diameter (cm)	I (%)	Colony diameter (cm)	I (%)	Colony diameter (cm)	I (%)
SA	1.37 ± 0.27	42	2.10 ± 0.17	50	3.03 ± 0.14	47	3.83 ± 0.28	46	4.70 ± 0.25	48
CA	2.07 ± 0.02	13	3.23 ± 0.08	23	4.43 ± 0.18	22	6.10 ± 0.05	14	8.07 ± 0.08	10
AA	2.10 ± 0.05	11	2.90 ± 0.05	30	4.03 ± 0.08	29	5.30 ± 0.34	25	6.00 ± 0.20	33
OA	2.00 ± 0.05	16	3.00 ± 0.05	28	4.00 ± 0.05	30	4.70 ± 0.40	34	5.50 ± 0.30	39
Control	2.37 ± 0.11	0	4.17 ± 0.11	0	5.70 ± 0.15	0	7.10 ± 0.05	0	9.00 ± 0.00	0
LSD at P ≤ 0.05	0.47	-	0.31	-	0.39	-	0.95	-	0.72	-

SA: salicylic acid, CA: citric acid, AA: ascorbic acid, OA: oxalic acid, I: inhibition.
Each value represents the mean of 3 replicates ± standard error.

Table 2. Rate of total phenols (µg/g FW) of different treatments by chemical inducers and fungicide in inoculated with *Botrytis fabae* (BF) and uninoculated (Uninoc) faba bean leaves.

Treatments	Hours after inoculation											
	12		24		48		72		96		120	
	Uninoc	BF	Uninoc	BF	Uninoc	BF	Uninoc	BF	Uninoc	BF	Uninoc	BF
Untreated	2.44 ± 0.23	4.45 ± 0.66	3.03 ± 0.04	4.98 ± 0.62	3.73 ± 0.06	6.48 ± 0.65	6.06 ± 0.15	7.39 ± 0.59	6.25 ± 0.48	9.95 ± 0.25	4.58 ± 0.66	8.29 ± 0.18
SA	7.74 ± 1.56	8.13 ± 3.15	9.30 ± 0.47	11.29 ± 2.48	11.00 ± 0.52	12.28 ± 1.94	12.31 ± 0.28	13.39 ± 1.89	13.77 ± 0.37	14.46 ± 1.62	13.22 ± 0.70	12.73 ± 1.23
CA	5.84 ± 0.17	5.8 ± 0.50	6.70 ± 0.31	7.94 ± 1.92	8.70 ± 0.19	9.9 ± 0.55	9.33 ± 0.19	10.33 ± 0.80	9.98 ± 0.45	11.05 ± 1.01	8.9 ± 0.17	10.12 ± 0.91
AA	3.24 ± 0.15	4.46 ± 0.02	3.85 ± 0.25	4.89 ± 0.34	4.50 ± 1.12	5.86 ± 0.07	5.90 ± 0.11	6.83 ± 0.22	6.20 ± 0.15	6.81 ± 0.43	5.87 ± 0.08	6.25 ± 0.22
OA	4.00 ± 0.22	5.10 ± 0.45	4.42 ± 0.49	5.85 ± 0.15	5.52 ± 0.98	6.90 ± 0.51	6.34 ± 0.24	7.83 ± 0.78	6.60 ± 0.22	8.02 ± 0.74	6.27 ± 0.04	7.55 ± 1.00
C	3.28 ± 0.24	4.21 ± 0.36	3.88 ± 0.20	4.51 ± 0.49	4.20 ± 0.36	4.79 ± 0.42	4.71 ± 0.63	6.99 ± 0.50	6.06 ± 0.28	8.70 ± 1.64	5.53 ± 0.18	6.84 ± 1.03
LSD at P ≤ 0.05	1.83		1.64		1.40		1.25		1.40		1.19	

SA: salicylic acid, CA: citric acid, AA: ascorbic acid, OA: oxalic acid, C: Carbendazim.

Discussion

The current trendin plant disease control is to use chemical inducers able to stimulate the innate defense mechanisms of the host plant and to create induced systemic resistance against several diseases. This induction of plant defense is mediated through various physiological, biochemical and molecular mechanisms (Idrees *et al*., 2011). In this regard, the use of four chemical inducers (salicylic, citric, ascorbic and oxalic acids) were investigated compared to the fungicide (Carbendazim) to increase the resistance of infected faba bean plants to *B. fabae*. According to our results, under field and glasshouse conditions, all used inducers reduced significantly chocolate spot disease severity as compared to the control. In contrast, El-Hendawy *et al*. (2010) showed varying efficiency in reducing chocolate spot disease in greenhouse and field conditions. They found that salicylic acid in greenhouse and ascorbic acid in field conditions were the most efficient as compared to several inducers. In addition, Aldesuquy *et al*. (2015) showed that application of salicylic and shikimic acids decrease the severity of chocolate spot disease on faba bean. Salicylic acid seems to inhibit disease development through different mechanisms involving the inhibition of extracellular fungal enzymes (cellulases, pectinases, lactase, xylanase).

Hassan *et al*. (2006) found that 2.1 mM salicylic acid caused 69.4% disease reduction of chocolate spot caused by *B. fabae*. The highest protection against chocolate spot disease in our experiments was also obtained by salicylic acid (2.1 mM). In fact, this treatment showed a high reduction of disease severity on the leaves and on the stem for two subsequent seasons (2012 and 2013) under field conditions and a high level of protection for faba bean plants against *B. fabae* under glasshouse conditions. Salicylic acid-induced pathway is characterized by the production of a cascade of pathogenesis related proteins. Furthermore, Saikia *et al*. (2003) showed that the exogenous application of salicylic acid was able to reduce the disease severity of *Fusarium* wilt of chickpea and stimulate systemic resistance. Sarwar *et al*. (2005, 2010) reported that seed treatment by salicylic acid and Bion showed a reduction in the wilt disease and induced systemic resistance in chickpea against *Fusarium oxysorum* f.sp. *ciceris* under growth room. However, the reduction of disease incidence in chickpea may be associated with induction of phytoalexins (Kuc, 2006) and pathogenesis-related proteins, chitinase and β-1,3-glucanase. In addition, this inducer caused 56% disease reduction of chickpea blight caused by *Ascochytar abiei* (Ghazanfar *et al*., 2011). Similar results were observed with Hadi and Balali (2010) for potato plants infected by *Rhizoctonia solani*. Under greenhouse conditions, the application of 2 mM of salicylic acid as foliar spray reduced the leaf blight of onion (40%) caused by *Stemphylium vesicarium* (Abo-Elyousr *et al*., 2009). Overall in plants, the application of the exogenous salicylic acid operates various physiological, biochemical and molecular processes (War *et al*., 2011) which play a key role in defense response and in inducing particular enzymes (Chen *et al*., 2006).

The high use of fungicides for decreasing infection may be attributed to their toxicity against the pathogen, whereas for salicylic acid, the effect is positive and may be due to its action as plant defense activator (Ata *et al*., 2008). For other inducers (citric, ascorbic and oxalic acids) the difference in the efficiency order to control chocolate spot disease observed under different conditions may be attributed to the differential mode of action. The inducers are well-known antifungal, antiviral and antibacterial compounds occurring in plants (Hayat and Ahmad, 2007).In this study, salicylic acid was the most effective inhibitor for the linear growth of *B. fabae*. These findings are in agreement with those of Shabana *et al*. (2008) who found that *in vitro*, salicylic and benzoic acids were the most effective inhibitors for the growth of *Bipolaris oryzae*. Several other workers have demonstrated the inhibitory power of this inducer such as Shahda (2000) on *F.oxysporum, F. solani and Rhizoctonia solani* isolated from tomato plants and Aldesuquy *et al*. (2015) on faba bean against chocolate spot disease. Cowan *et al*. (1999) attributed the phenolics toxicity observed on microorganismsto enzyme inhibition by the oxidized compounds most probably through reaction withsulfhydryl groups or through more nonspecific interactions with proteins.The position(s) and the number of hydroxyl groups on the phenol ring influenceitstoxicity to microorganisms. Increased hydroxylation results in increased toxicity. In fact, Scalbert *et al*. (1991) found that highly oxidized phenols produced higher inhibitory effect on pathogen.

Our results indicated that inoculation with *B. fabae* after treating plants by chemical inducers led to a significant increase in the total phenols. Malik and Singh (1980) showed that the phenols offered resistance to diseases and pests in plants. The present study revealed the role of the four inducers in phenols accumulation. high production of phenols in healthy and infected plants as a result of salicylic acid treatment suggests that phenolic compounds are implicated in the disease resistance. These results are in concordance with the finding of El-Hendawy *et al*. (2010) who showed maximum accumulation of total phenols after faba bean plants were treated with salicylic acid either by the foliar spray or by seed soaking compared with untreated control. Also, these findings are in agreement with Abo-Elyousr *et al*. (2009). Generally, the phenolic compounds show biological activity against a wide range of pathogens and are considered as bio-markers for the degree of plant resistance/tolerance (Vogelsang *et al*., 1994). Chérif *et al*. (2007) showed that phenolic compounds such as phytoalexins produced in response to infection by the pathogen constitute an active defense response.

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تأثیر برخی از القاکننده‌های شیمیایی بر بیماری لکه شکلاتی باقلا در تونس

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چکیده: گونه *Botrytis faba* یکی از بیمارگرهای قارچی مهم می‌باشد که به برگ‌ها و ساقه گیاه باقلا *Vicia faba* L. حمله می‌کند و خسارت زیادی را وارد می‌نماید. این تحقیق به‌منظور ارزیابی اثر چهار القاکننده شیمیایی (اسید سالیسیلیک، اسید سیتریک، اسید آسکوربیک و اسید اگزالیک) و یک قارچکش (کاربندازیم) بر *B. faba* در شرایط گلخانه و مزرعه انجام شد. در آزمایش‌های مزرعه‌ای و گلخانه‌ای انجام شده در طی دو سال، گیاهان تیمار شده با اسید سالیسیلیک در مقایسه با شاهد و تیمار قارچکش کاهش قابل‌توجهی در شدت بیماری روی برگ و ساقه نشان دادند. کاربرد اسید سالیسیلیک بسیار مؤثر بود و بیماری را بیش‌تر از کاربندازیم که حفاظت نسبی ایجاد می‌کند، کنترل نمود. بازدارندگی رشد قارچ در شرایط درون شیشه‌ای ارزیابی شد. اسید سالیسیلیک بهترین بازدارنده رشد (۴۸٪) قارچ شش روز بعد از نگه‌داری بود و اسید اگزالیک (۳۹٪)، اسید آسکوربیک (۳۳٪) و اسید سیتریک (۱۰٪) به‌ترتیب بعد از آن قرار گرفتند. افزایش چشم‌گیری در میزان فنل کل در برگ‌های سالم و آلوده گیاه باقلا تیمار شده با اسید سالیسیلیک، ۱۲، ۲۴، ۴۸، ۷۲، ۹۶ و ۱۲۰ ساعت پس از مایه‌زنی مشاهده شد. نتایج امیدبخش تحقیق حاضر در کنترل بیماری قارچی مخرب باقلا در تونس و مناطق دیگر، تأثیر مهمی در توسعه و تولید محصول خواهد داشت.

واژگان کلیدی: *Botrytis faba*، القاکننده‌های شیمیایی، فنل کل، *Vicia faba*