University of Sadat City,
Environmental Studies and Research Institute
6Th International Conference

"New Horizons Towards Sustainable Development"

Journal of Environmental Studies and Researches (2021), 11(4C):369-378

Induce Systimic Resistance Of Chocolat Spot Diseasses In Faba Bean

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Abstract

Two field experiments were conducted field experiment were designed in a split plot design with three replicates in Al- sadat city location during the 2019/2020 and 2020/2021 growing seasons where the faba beans cultivars 429 were placed in the main plots and the three treatments and the control were placed in the sub-plots. Efficay oof chemical inducers, *i.e.* Ascorbic cid, salicylic acids and Houmic acids as foliar spray against of chocolate spot disease severity of faba bean **(***Vicia faba* L.**)** cv. Giza 429 was evaluated. All treatments recorded the highest efficacy percentage for reducing severity of chocolate spot disease which caused by *Botrytis fabae* and/or *B. cinerea* compared to control during the 2019/2020 and 2020/2021 growing seasons. Also, all treatments increased faba bean yield, pods number/plant, seeds number/pod, 100-seed weight, seed yield/ plot, seed yield/ fed., peroxidase and polyphenol oxidase activity as compared to non-treated plants.

Ascorbic acid is the most superior positive resistance effect, increasing the yield and enzyme activity content of faba bean among other inducers. In the contry, Houmic acids expressed the lowest effective one during the two successive seasons 2019/2020 and 2020/2021 in comparison with control treatment.

Key words: Faba bean, Choclot spot disease and Induced resistance

Introduction

Faba bean is the first legume crop in Egypt, as they are a daily meal for most of the Egyptian people, and it is the best vegetarian alternative to animal protein. Dry beans content about 11% water, 58% carbohydrates, 26% protein, and 2% fat. Also, faba bean seeds rich of manganese, phosphorus, magnesium, and iron as well as moderate to rich content of vitamins B. There is a big gap between the production and consumption of faba beans in Egypt. This gap has increased significantly in the last decade for several reasons, the most important of which is the large decline in cultivated areas due to the spread of broom rape or the sugar beet cultivation, which is one of the contract crops that occupied large areas that were previously cultivated with beans. As well as the reluctance of many farmers to cultivate faba bean as a result of the decrease in production due to the spread of fungal diseases such as chocolate spot **(Abdel- Wahhab, *et al.*, 2020)**. In order for the farmers to come back to cultivate the faba beans, it was necessary to solve these problems. The solution often begins with improving faba bean cultivars, developing their productivity, and

their tolerance to fungal and insect diseases. In Egypt, there is a great genetic diversity in the faba bean varieties, which allowed farmers great freedom to choose the suitable variety from them. Many previous studies confirmed the existence of this genetic diversity of the Egyptian faba bean varieties, which led to a marked difference in the growth and yield of these varieties as well as their tolerance to disease infections **(El-Sayed *et al.* (2011)**, **Abido and Seadh, (2014)**, **El-Shafey *et al.*, (2016)**, **Abdel-Baky *et al.*, (2019)** and **Kandil *et al.* (2019))**.

Faba bean is liable to be attacked by many foliar diseases as chocolate spot (*Botrytis fabae and B. cinerea*), rust (*Uromyces fabae*), Ascochyta blight (*Ascochyta fabae*), leaf spots (*Cercospora zonata and Alternaria alternata*), downy mildew *(Peronospora viciae*) and root-rots as well as viral diseases, which are responsible to cause considerable losses in the yield and its components.

Foliar diseases are the most common diseases especially in Delta region due to the high humidity, rain fall and favourable temperature which are prevailing during the season. Therefore, chocolate spot disease of faba bean caused by *B. fabae* and *B. cinerea* is considered the most important disease in Egypt, which causes serious damage to the crop.where the yield losses could be more than 50% of the crop according to (**Hussein, 1963 and Mohamed, 1982**).

In the last ten years two severe epidemics of chocolate spot disease were recorded during 1987/88 and 1990/91 growing seasons in Egypt, where the yield was reduced by 50% in both seasons **(Nassib *et al.* 1991)**. In low infected seasons, when the environmental condition is not optimum, yield losses ranged from 5 to 15% **(Mansour and Amer, 1976)**.

Because of hazards of pesticides in general, and fungicides in specific, on public health and environmental balance, a relatively recent direction of pest control management was introduced. The induced resistance is a promising modern approach on of non chemical methods with a broad spectrum in plant disease control It could be induced in plants by applying chemical elicitors **(Reglinski *et al.*, 2001**). The weather conditions in Egypt are very favorable to the spread of many fungal diseases such as chocolate spot. Choosing the resistant variety and taking care of the irrigation and fertilization processes are good things that limit the spread of fungal diseases in the faba bean. But with the increase in the severity of the infection and in order not to cause significant losses in the yield, it becomes necessary to chemical control of these diseases by using specialized fungicides. The use of chemical insecticides and fungicides in controlling pests caused air and water pollution make environmental hazards **(Zaker, 2016)**. Moreover it has recently been reported that *B. fabae* and *B. cinerea* become more resistance to those chemicals pesticides **(Maggie *et al.* 2006)** as a result of the previous dangerous of chemical pesticides it is very important to search for new environmental friendly alternatives for the control of these pests. In recent times studies have tended to use plant extracts as safe alternatives to chemical pesticides. Certain chemical inducers.Ascorbic acid, salicylic acids and Houmic acids revealed the most superior positive resistance effect among other inducers. One of the most important used to combat fungal diseases. In additional to

increasing the yield, enzyme and fenole activites in comparison with control treatment.

Material And Methods

Two field experiments were conducted field experiment were designed in a split plot design with three replicates in Al- sadat city location during the 2019/2020 and 2020/2021 growing seasons where the faba beans cultivars 429 were placed in the main plots and the three treatments and the control were placed in the sub-plots. The area of experimental plot was 9 m^2 and composed of 3 rows (3m×25cm) with about 50 cm apart. Each row was planted with 60 seeds of faba bean (Giza-429 cv.) as winter crop **(**15st October 2019/2020 and 2020/2021 growing seasons**)**.

During the experiment, the effect of spraying chemical inducers, *i.e.* (Ascorbic acid, salicylic acids at the rate of 200ppm. /100 Lit water/fed.) and Houmic acids (at the rate 2000ppm/100 lit water/fed.) are studied to resistance the severity of chocolate spot. The control plants were sprayed with sterilized water. The agricultural practices were applied as recommended for all treatments. In additional to, study the effect of these chemical inducers on some vegetative growth, yield and its components on faba beans cultivars Giza 429 during the successive seasons 2019/2020 andd 2020/2021 compared with control treatment.

All treatments were applied as a foliar spry with the recommended dose as mentioned above three times: the first one after 45 days from sowing at the beginning of the flowering stage, the second one was 60 days after sowing in the end of flowering stage and the third was 90 days after sowing in the end of pod filling stage.

Some of agro climatological data for the tree locations during 2019/2020 and 2020/2021 summer seasons are presented in Table (1). Also, soil physical and chemical properties of the experimental field of El-Al – Sadat city location are presented in Table (2).

Table (1): Some of agro-climatological data (environment factors) for El-Sadat city location during 2019/2020 and 2020/2021 growing seasons.

Months	2019/2020 growing season			2020/2021 growing season		
	Avareg of Temperature ^{o}C	Relative humidity %	Wind speed m/s	Avareg of Temperature ^{o}C	Relative humidity %	Wind speed m/s
October	24.65	53.85	2.71	25.48	52.61	2.63
November	20.37	53.56	2.26	18.86	61.82	2.37
December	14.47	64.01	2.89	15.73	60.94	2.16
June	11.95	67.47	2.93	14.18	60.12	2.55
February	13.32	64.82	2.41	14.21	60.23	2.28
March	16.54	52.75	2.95	15.67	57.88	2.70
April	18.98	53.99	2.66	20.99	36.92	3.22

Table (2): Soil physical and chemical properties of the experimental field in El-Sadat city location in 2019/2020 and 2020/2021 seasons.

Physical properties				Chemical properties					
Sand (%)	Silt (%)	Clay (%)	Texture	PH	EC (ds/m^{-1})	$CaCo_3$ (%)	Available (ppm)		
							N	P	K
73.53	19.14	7.33	Sand Loamy	7.69	1.83	5.00	11.10	6.85	29.50

Studied characters were:-

Chocolate spot infection development

Chocolate spot infection was observed during the 2nd week of March, 2019/20 and 2020/21 for the two seasons, respectively. The mean plant infection was calculate as an average of the whole plant. First score was at 15th March and every 2 weeks up to the fifth score in the both season.

Area under disease progress curve (AUDPC) was calculated using the adapted formula by **Pandey *et al*. (1989)** as follows:

$$\mathrm{AUDPC} = D\left[\frac{1}{2}(Y_1 + Y_K) + (Y_2 + Y_3 + \ldots + Y_K - 1)\right]$$

Yield components

At harvest, 10 plants from each sup plot were taken randomly to be recorded number of pods/plant, number of seeds/plant and 100-seed weight (g) while, seed yield (kg)/plot: and seed yield (ardab/fad) were recored on sub-plot baise.

Determination of Enzyme Activities:

The sample of one g of leaves (after 90 days from sowing) was homogenized in 2 ml of 0.1 M sodium phosphate buffer (SPB) pH 6.5 at 4°C. The filtrate was centrifuged at 20,000 rpm at 4°C for 15 min., the supernatant served as an enzyme extract for enzyme assay of polyphenoloxidase and peroxidase.

Peroxidase activity: Peroxidase activity was assayed colorimetrically according to the method described by **(Amako *et al.*, 1994)**. The increase in optical density at 430 nm against blank was continuously recorded every minute. Peroxidase enzyme activity was expressed as change in absorbance per min/g fresh leaves.

Polyphenol oxidase (PPO) activity: Polyphenol oxidase activity was estimated as described by **Mayer and Harel (1979)** with some modifications. The polyphenol oxidase activity was expressed as change in absorbance at 495 nm against blank per min g fresh leaves.

Statistical Analysis: Results were expressed as mean. The data were analyzed by using **Two-way** ANOVA followed by LSD test through SPSS 16 (version 4). The treatments means were compared using least significant difference (LSD) tested at significant levels of 5% and 1% respectively as described by **Gomez and Gomez (1984)**.

Results

Effects of different treatments on yield components of the faba bean cultivars Giza 429 that growing in EL-satat city location during 2019/2020 and 2020/2021 seasons.

Data in Tables 1,2,3 and 4 indicated that, pods number/plant, seeds number/pod, 100-seed weight, seed yield/plot, seed yield/fed and significantly affected used treatments and their interactions in both seasons.

1-1- Effect of used treatments.

The response of yield and its components that attributed to the availability of N, P and K by plants in this soil as shown in Table (1) Soil physical and chemical properties in the Table (2). The results in Table (3) showed that, faba bean plants that sprayed with different chemical inducers at the certin doses showed the highest effect in yield component compared with control treatment. Scorbic acid gave the highest effect one in pods number/plant and recorded (11.89 and 11.46), 100-seed weight (82.16and 79.51 g), seed yield/ plot (5.76 and 5.55 kg) and seed yield/fad (17.91 and 17.27 erdab) during the two successive seasons 2019/2020 and 2020/2021. While, faba bean plants that sprayed with salicylic acid had the highest seeds number/pod (3.36 and 3.30) in the first and second seasons, respectively. In contaray the humic acid showed the lowest effective.

Table (3): Effects of different treatments on faba seed yield and yield components traits in EL-Sadat city location during 2019/2020 (1st) and 2020/2021 (2nd) seasons.

Treatmentes	Number of pods/plant		Number of seeds/pod		100-seed weight (g)		Seed yield /plot (kg)		Seed yield/fed	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Salicylic acid	10.7	10.3	3.36	3.30	79.40	77.20	5.13	4.94	15.9	15.3
Scorbic acid	11.8	11.4	3.24	3.19	82.16	79.51	5.76	5.55	17.9	17.2
Humic acid	10.0 5	9.39	2.98	2.85	77.12	71.56	3.96	3.70	12.3 1	11.5 1
Control	7.95	7.36	2.72	2.55	69.41	64.90	2.74	2.54	8.54	7.91
LSD at 5%	**0.32**	**0.34**	**0.07**	**0.08**	**1.17**	**1.42**	**0.27**	**0.27**	**0.85**	**0.85**

Effects of different treatments on chocolate spot disease severty and defense enzymes activity of the faba bean cultivars Giza 429 that growing in El-Sadat city location during 2019/2020 and 2020/2021 seasons.

The severity of faba bean plants was estimated as Area Under Disease Progress Curve (AUDPC). The results presented in Table (4), confirmed that there was a significant difference between the three used treatments as well as their interaction in the severity of chocolate, the activity of peroxidase and polyphenol oxidase in both seasons compared with control treatment.

Table (4): Effects of different treatments on chocolate spot severity of the faba bean Giza 429 that growing in EL-Sadat city location during 2019/2020 and 2020/2021 seasons.

Treatmentes	AUDPC		Peroxidase activity		Polyphenol oxidase activity	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Huomic acid	340.07	525.67	0.56	0.54	0.59	0.62
Cilcylic acid	267.67	427.10	0.59	0.56	0.63	0.64
Scorpic acid	201.73	344.58	0.63	0.61	0.65	0.69
Control	345.06	527.05	0.54	0.52	0.55	0.60
LSD 5%	**23.07**	**30.22**	**0.01**	**0.01**	**0.02**	**0.01**

Effect of used treatments.

Respect to the used treatments the results in Table (4) indicated that, all used treatments significantly reduce chocolate spot infections compared to the control in both seasons. Faba bean plants that sprayed with Scorpic acid showed the lowest AUDPC (201.73 and 344.58), the highest peroxidase activity (0.63 and 0.61) and the highest polyphenol oxidase activity (0.65 and 0.69) in both seasons, respectively in comparison with control treatment.

The activity of polyphenol oxidase.

With respect to interaction between faba bean cultivars and the used treatments the results in Table (4) showed that the faba bean cultivars Giza 429 differ in their responses to the different treatments. The sprayed with scorpic acid showed the lowest chocolate spot disease severities and the highest peroxidase and poly phnol oxidase activity in both seasons. On the other hand, huomic acid under the control treatment showed the highest chocolate spot severities and the lowest peroxidase and poly phnol oxidase activity in both seasons.

Discussions

Faba bean yield is severely affected by many factors such as the environment in which it is grown, the cultivar grown and agricultural practices such as fertilization and control of fungal and insect diseases.

In this study sprayed under the treatment of three inducer chmical, *i.e.* cilcylic acid, scorpic acid and humic acid what ever the treatment of the scorpic acid showed the highest mean values for all yield traits as well as chocolate spot seveity. The current trendin plant disease control is to usechemical 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 contrast, **El-Hendawy *et al.* (2010**) showed varying efficiencinreducing chocolate spot disease in greenhouse and field conditions.Theyfoundthat salicylic acid in greenhouse and ascorbic acid in field conditions were the most efficient as compared to several inducers.

In addition, **Al-desuquy *et al*. (2015)** showed that application of salicylic acid 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). Salicylic acid-induced pathway is characterized by the production of a cascade of pathogenesis related proteins.

The response of yield and its components that attributed to the availability of N, P and K by plants in this soil as shown in Table (1) Soil physical and chemical properties in the Table (2) which positively effect on the vegetative growth parameters, yield component and disease severity as shown in Table 3 and 4 and plant is stayed without damage and in a good healthy (**Ahmed and Shaheen (2016)**).

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 werethe most effective inhibitors for the growth of Bipolarisoryzae. Several other workers have demonstrated the inhibitory power of this inducer such as **Shahda (2000)** on F.oxysporum, F. solani and Rhizoctoniasolaniisolated 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 interactionswith proteins.The position(s) and the number of hydroxyl groups on the phenol ringinfluenceitstoxicity 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. fabaeafter treating plants by chemical inducers led toasignificantincrease diseases and pests in plants. The present study revealed the role of the four inducersin phenols accumulation. High production of phenols in healthy and infected plantsas a result ofsalicylic acid treatment suggeststhat phenolic compounds are implicatedin the disease resistance.

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