Egyptian Journal of Phytopathology, Vol. 50, No. 2, pp 151-159 (2022)
DOI 10.21608/ejp.2022.179094.1078

ORIGINAL PAPER

Effect of Frankincense on Controlling Chocolate Spot of Faba Bean

El-Nagar, T.S.*[1]; EL-Kot, G.A.[2]; Sarhan, E.A.D.[1]; Maswada, H.F.[3] and Elzaawely, A.A.[3]

Received: 06 December 2022 / **Accepted**: 30 December 2022 / **Published online**: 31 December 2022.

ABSTRACT

The effect of the natural substance Frankincense (*Boswellia carterii* Flueck.) on controlling chocolate spot disease of faba bean was studied in this work. Natural substance frankincense was used at different levels. Disease severity %, fresh and dry weights (g) of faba bean shoots, and enzymatic activity were estimated under greenhouse (artificial inoculation). Results indicated that Frankincense at 3 g/L had significantly decreased chocolate spot disease on faba bean causal organism under greenhouse conditions. Also, seed germination %, fresh and dry weights of shoot, polyphenol oxidase and peroxidase activity were significantly increased. On the other hand, under field conditions, the obtained results cleared that the natural substance frankincense at a high level (3 g/L) concentration showed the highest effect on decreasing chocolate spot disease severity %, as well as increasing germination percentage and also gave the highest effect on increasing yield per plot (kg), weight of 100 seeds, protein, and total chlorophyll.

Keywords: Frankincense, *Boswellia carterii*, Faba bean, *Vicia fabae*, chocolate spot, *Botrytis fabae*

***Correspondence: El-Nagar, T.S.**
E-mail: tameregypt743@gmail.com
Tamer S. El-Nagar
Ehab. A.D. Sarhan
https://orcid.org/0000-0002-9364-3966
1- Plant Pathology Research Institute, Agricultural Research Center, 12619, Giza, Egypt.
Gabr A. EL-Kot
https://orcid.org/0000-0001-6141-837X
2- Department of Agricultural Botany, Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh, Egypt.
Hanafey F. Maswada
https://orcid.org/0000-0003-1125-7314
Abdelnaser A. Elzaawely
https://orcid.org/0000-0001-9388-587X
3- Department of Agricultural Botany, Faculty of Agriculture, Tanta University, Tanta, Egypt.

INTRODUCTION

Faba bean (*Vicia fabae* L.) a nutritious leguminous cool tolerant crop, is widely cultivated throughout the world with a good protein source. It contains minerals (iron, zinc, calcium) and vitamins (B1, B2, and C). Also, it is used as a fodder and forage crop, it is cultivated mainly for protein, with about 18.5 to 37.8% (Dhull *et al*., 2022). Moreover, like other legumes, faba bean also plays a significant role in restoring soil fertility by fixing nitrogen and is a suitable rotation crop for cereals and other crops (Ghoneem *et al*., 2012 and Teshome and Tagegn, 2013).

The world production of faba beans was 5.43 million metric tons in 2019, (FAO, 2020). Cultivated area of the crop in Egypt during the 2019 season was estimated at about 75734 feddan, total production was about 101000 tons with the average of 1.48 tons/feddan of dry seeds (CAPMS, 2021) One of the most important factors affect the production of faba bean plants attack by various diseases. The major disease of this important crop in Egypt is forage disease (chocolate spot disease) caused by *Botrytis fabae* Sardiña that affects directly protein production and decreasing of yield production by more than 50% (Abd El-Hai *et al*., 2011 and Metwaly, 2014.), especially in the north and middle parts of the Nile Delta in Egypt.

This work's main objective was to find reasonable means of controlling this disease with natural extracts. Although several publications are available describing the use of biotic and abiotic induced resistance against plant diseases, however, there is little data on the use of natural plant extract frankincense. Studies showed that the ingestion of a defatted alcoholic extract of Boswell decreased polymorph nuclear leukocyte infiltration and migration, decreased primary antibody synthesis (Sharma and Singh, 1989), and caused almost total inhibition of the classical complement pathway (Wagner, 1989) and alternate complement system (Knaus and Wagner, 1996). Boswellia gum has been used to treat diabetes, skin and blood diseases, fever, cardiovascular disorders, neurological disorders, dysentery, and diseases of the tests (Adrian, 1996). β-boswellic

acid and its derivatives have anti-tumor and anti-hyperlipidemia activities (Huan *et al*., 2000). The gum resin extract from Boswellia was recently shown to have positive therapeutic effects on inflammatory bowel disease. However, the mechanisms and constituents responsible for these effects are poorly understood (Krielgstein, 2001). Moreover, a clinical study reported that Boswellia extract appears superior to meclizine in terms of a benefit-risk evaluation (Gehardt *et al*., 2001). Frankincense triterpenoids showed Anti-herpes activity and Immunomodulatory effects (Badria *et al*. 2003 and Botros *et al.* 2003). Frankincense is an oleo gum resin obtained through a deep longitudinal incision in the trunk of the Boswellia tree. Nine compounds with immune stimulant and antiviral activity were isolated from the oleogum resin of Frankincense (*Boswellia carterii* Flueck.) The frankincense essential oil was found to contain monoterpenes, sesquiterpenes, and diterpenes (Both isolated oil and resins exhibited strong immune stimulant activity).

Moreover, more than 300 volatiles in Frankincense have been reported in the literature. In particular, a broad diversity has been found in the qualitative and quantitative composition of the volatiles concerning different varieties of *Boswellia* Michaela *et al.* (2009). The main component of frankincense is oil (60%). It contains mono-penes and diterpenes as well as ethyl acetate, acetyl acetate, and methyl anisole. The highest biological activity among terpenes is characteristic of 11-keto-ß-acetyl-beta-boswellic acid, acetyl-11-keto-ß-boswellic acid, and acetyl-α-bowelled acid. Contemporary studies have shown that resin has analgesic, tranquilizing and anti-bacterial effects (Ali *et al*., 2016).

MATERIALS AND METHODS

Source of faba bean seeds:

Faba bean seeds cv. Giza 40 was obtained from Legume Crop Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt.

Isolation and identification of fungal pathogen:

Samples of naturally infected faba bean leaves were collected from fields located at different districts of Kafrelsheikh Governorate, Egypt, (Elriyad, Qillin, Dosouq, Biyala, Sakha, ELhamoul, and Sidi Salem). The aggressive isolate was obtained from the Elriyad county and was used in these studies. Infected leaves showing symptoms of chocolate spot disease were collected, cut into small pieces and surface sterilized using 3% sodium hypochlorite for 2 min, washed several times in sterilized distilled water, then dried between two layers of sterilized filter paper to remove excess distilled water, pieces each, with single lesion of the concerned disease was plated on faba bean leaf extract agar medium "FBLA" (extracts of 250 g faba bean leaves + 30 g sucrose + 20 g sodium chloride and 20 g agar in one-liter distilled water) as described by Hanouike and Hasanain (1986). Four pieces were plated in each Petri dish and incubated at 20 °C for 12 days. The isolated fungus was purified using the hyphal tip method, as described by Sinclair and Dhingra (2019).

Evaluation of the isolated isolates of *Botrytes fabae*:

Botrytes fabae isolates, collected from different regions, were grown on "FBLA" medium poured into sterilized Petri dishes to obtain a large number of spores (Haggag *et al.*, 2006). The plates were inoculated with discs (5 mm in diameter) of the seven isolates from Kafrelsheikh and incubated for 12 days at 20 °C, with a photoperiod of 12 h light/12 h dark, five replicates for each isolate. After incubation, 10 ml aliquots of sterilized water were added to each culture, and a fine brush was used to fragment the spores and suspend them in the water. A concentration of $2.5 \times l0^5$ conidia/ml was prepared for each isolate. Faba bean seeds cultivar Giza 40 was sown in pots (30 cm in diameter), with five seeds per pot (5 replicates for each cultivar). Forty-five days after sowing, the grown plants were sprayed with the spore suspension of the desired isolate and covered with polyethylene bags for 24 h to maintain high relative humidity (Abou-Zeid, 1985). Plants sprayed with distilled water were used as a control. The inoculated and uninoculated plants were maintained for 48 h at 20 °C under greenhouse conditions. The infection type (disease severity) and severity of leaf damage (area) after 48 h were assessed using a scale (0 to 9) that depended on the extent of the lesions as described by Ding *et al*. (1993) and Table (1).

$$\textbf{Disease severity \%} = \frac{\mathbf{n \times v}}{\mathbf{9n}} \times \mathbf{100}$$

Where:

n = number of plants in each category.

v = numerical values of symptoms category.

N = total number of plants.

9 = maximum of numerical values of symptoms categories.

Table (1): The infection type scale of faba bean chocolate spot disease.

Disease rating	Description
0	No visible infection on leaves
1	A few dots like accounting for less than 5% of the total leaf area
3	Discrete spots less than 2mm in diameter account for 6-25 % of leaf area
4	Some coalesced lesions on infected leaf areas in large than 25 %
5	Numerous scattered spots with a few linkages diameter 3-5 mm, on 26-50% of leaf area with a little defoliation
6	Confluent spot lesions account for 51-75 % of leaf area mild sporulation. Half the leaves are dead or defoliated
7	Destruction of the larger leaves spot lesions covering more than 76% of leaf area abundant sporulation, heavy defoliation and plant darkened and dead
8	Whole plant is dead except the apex
9	Death of the whole plant

Method of processing the used Frankincense:

Each concentration of Frankincense (*Boswellia carterii* Flueck) (1, 2, and 3g/l) was added to a liter of water to obtain 1 & 2 and 3 g/L, respectively, and then Triton B1965 was added -50 ml/L water to place on the flame until it was completely dissolved in the water, one day before the spray treatment.

Study the effects of Frankincense on chocolate spot disease under greenhouse conditions:

The experiment was carried out at the Experimental Farm of Sakha Agricultural Research Station. Faba bean seeds (Giza 40) were sown in pots (30 cm in diameter and 25 cm in depth) containing 3.5 kg of sterilized loamy clay soil. Five seeds were sown per pot. All cultural practices were done as recommended. After 50 days from sowing, the plants were sprayed with a spore suspension of *Botrytis fabae* (2.5×10^5 spore/ml). All plants were covered with polyethylene bags for 24 h to maintain high humidity and then kept under greenhouse conditions.

On the other hand, the natural substance frankincense was sprayed as (1 & 2 and 3 g/L) at five days after artificial inoculation by the pathogen. Three pots were used as replicates for each treatment. Also, pots containing untreated healthy plants were used as a control group. At the end of the experiment, the following parameters were accounted:

Disease severity:

As described before disease severity was determined according to Dinge *et al.* (1993)

Some chemical composition analysis:

Grain protein content:

The micro-Kjeldahl method was used to determine the total nitrogen in the seed and multiplied by 6.25 to obtain the percentage to crude according to AOAC (1990).

Determination of Chlorophyll (SPAD value):

At the flowering stage, after 75 days from sowing, the Chlorophyll content was measured by SPAD - meter Model L13000L (Ling *et al*., 2011)

Enzymes activity:

Two days after the last spray by Frankincense, the treated faba bean plants were sampled and tested for the activity of peroxidase (PO), catalase (CAT) and polyphenol oxidase (PPO) as indicators of induced resistance.

Peroxidase activity:

A sample of 3 ml of the reaction mixture containing 100 mM sodium phosphate buffer (pH 5.8), 7.2 mM guaiacol, 11.8 mM H_2O_2 and 100 µl enzyme extract were used for the assay. With the addition of H_2O_2, the reaction was initiated, and the change in the absorbance was measured at 470 nm. POD activity was assayed according to Kato and Shimizu (1987) and was expressed as unit/mg protein/min.

Polyphenol oxidase activity:

The reaction mixture consisted of 200 µl of the enzyme extract and 1.5 ml of 100 mM sodium phosphate buffer (pH = 6.5). For the reaction, 200 µl of 100 mM catechol were added. The change in the absorbance was measured at 490 nm, and PPO activity was expressed as unit/mg protein/min.

Polyphenol oxidase activity:

PPO activity was determined according to Mayer *et al.* (1965) the absorbance was measured at 490 nm, activity was expressed as unit/mg protein/min.

Catalase activity:

A sample of 3 ml of the reaction mixture containing 100 mM sodium phosphate buffer

(pH 7), 2 mM H_2O_2 and 100 µl enzyme extract were prepared for the assay. The CAT activity was expressed as changes in absorbance of the reaction mixture at 240 nm min^{-1} g^{-1} fresh tissue unit/mg protein/min. The activity of CAT was assayed according to Kato and Shimizu (1987)

Yield components:

At physiological maturity (155 days), the plants were harvested by hand and left to dry for 5 days under natural conditions. Yield components, fresh and dry weight of shoot, were estimated for all plants. Seed yield per plant and weight of 100 seeds were also recorded.

Field experiment:

Effect of the natural substance Frankincense on controlling chocolate spot under field conditions during 2018/19 and 2019/20 growing seasons:

A field experiment was carried out at the Experimental Farm of Sakha Agricultural Research Station, Kafrelsheikh Governorate, to study the role of the Frankincense extract in controlling the chocolate spot disease of faba bean 45 days after planting faba bean c.v. Giza 40, the most susceptible to the studied disease. Frankincense extract was used as 1, 2 and 3g/l for three sprays with two weeks intervals. A complete block random design with three replicates was applied; The plot area was 10.5 m^2 (3 × 3.5 m). The recommended agricultural practices were done at the suitable time**.** Control plants were similarly sprayed with sterile distilled water. The disease severity% to Chocolate spot was estimated after 80 days from sowing according to the scale reported by Dinge *et al*. (1993)**.** At the end of this experiment, the following plant and yield parameters were accounted:

Disease severity, some chemical composition analysis, *i.e.,* Grain protein content, Determination of Chlorophyll (SPAD value) and Yield components.

Statistical analysis:

Analyses were performed with IRRISATE statistical software program. ANOVA was used to test differences among treatments prior to applying multiple comparison techniques, according to Duncan (1955).

RESULTS

Isolation of the causal fungi:

The isolation from samples of naturally infected faba bean (*Vicia faba* L.) leaves collected from different fields in seven districts locations, *i.e*., (Elriyad, Qillin, Dosouq, Biyala, Sakha, ELhamoul and Sidi Salem) of Kafrelsheikh Governorate, resulted in seven different fungal isolates which were identified as *Botrytis fabae* Sardiña according to their cultural and morphological characters as mentioned by **Hanouike and Hasanain (1986**). *Botrytis fabae* isolates and the location of each isolate were classified as isolate No. 1 (Elriyad), Isolate No.2 (Qillin), Isolate No. 3 (Dosouq), Isolate No. 4 (Biyala), Isolate No. 5 (Sakha), Isolate No. 6 (ELhamoul) and Isolate No. 7 (Sidi Salem).

Pathogenicity test of *B. fabae* Isolates:

Pathogenicity test of the seven isolates of *B. fabae* isolated from different locations was carried out on the susceptible faba bean cultivar (Giza 40) during the 2017-2018 growing season.

Data presented in Table (2) show that the highest disease severity was due to isolate No. 1 (94.47%). On the other hand, the other tested isolates differed in their reactions since isolates Nos. 6 and 7 caused the lowest disease severity % (26.28 and 14.94%, respectively).

Table (2): Pathogenicity test of seven isolates of *B. fabae* on the basis of susceptible Faba bean (Giza 40).

Isolate No.	Chocolate spot	
	Disease incidence %	Disease severity %
No. 1 (Elriyad)	95 a	94.47 a
No. 2 (Qillin)	89 b	74.36 b
No. 3 (Dosouq)	79 c	51.31c
No. 4 (Biyala)	75 c	38.13 d
No. 5 (Sakha)	77 c	34.02 d
No. 6 (ELhamoul)	76 c	26.28 e
No. 7 (Sidi Salem)	68 d	14.94 f
L.S.D. at 0.05	5.8	6.62

In the same column, means followed by the same letter are not significantly different at 0.05 level of probability according to Duncan's multiple range test (Duncan, 1955).

Greenhouse experiment:

Effect of natural substance Frankincense on chocolate spot severity under greenhouse conditions:

Data presented in Table (3) show that the application of the natural substance Frankincense significantly decreased the disease severity % of *B. fabae* under greenhouse conditions, compared to the untreated control treatment, increasing the concentration of the Frankincense continued to more decrease of the disease severity, the 3 gm/L concentration was the most effective being 15 and 13.34% disease incidence and severity by 75.67% reduction of severity comparing to the untreated control 88 and 54.83% incidence and severity respectively.

Table (3): Effect of natural extract Frankincense on chocolate spot severity on the susceptible Faba bean cultivar (Giza 40) under greenhouse conditions.

Treatment	Conc. (g/L)	Disease incidence %	Disease severity %	Reduction %
Frankincense	1	38 b	31.86 b	41.90
	2	21 c	18.03 c	67.12
	3	15 d	13.34 d	75.67
Dithane M-45	2.5	12 e	9.24 e	83.16
control	-	88 a	54.83 a	-
L.S.D. at 0.05	-	2.6	1.49	

In the same column, means followed by the same letter are not significantly different at 0.05 level of probability according to Duncan's multiple range test (Duncan, 1955).

Further, the application of the natural substance Frankincense significantly increased fresh weight (g), dry weight (g) and leaf area of the treated plants compared to the untreated control treatment (Table 4), as well as significantly increased both amounts of photosynthetic pigments (Chlorophyll) and Protein content %, meanwhile increasing concentration of the Frankincense increased the Chlorophyll, (Ch1). Protein content % values, the 3 g/L concentration was the most effective (Table 5).

Table (4): Effect of the natural substance Frankincense on Fresh, Dry weights (g) Leaf area of faba bean susceptible cultivar (Giza40) under greenhouse conditions.

Treatments	Conc. (g/L)	Shoot		
		Fresh weight (g)	Dry weight (g)	Leaf area
Frankincense	1	147.53 d	122.29 c	181.30 d
	2	154.70 c	125.92 bc	213.37 c
	3	163.64 b	127.60 b	239.98 b
Dithane M-45	2.5	176.18 a	145.02 a	286.70 a
control	-	140.43 e	87.18 d	159.38 e
L.S.D. at 0.05	-	5.73	1.77	9.69

Table (5): Effect of the natural substance Frankincense on amounts of photosynthetic pigments (Chlorophyll) and Protein content % of faba bean susceptible cultivar (Giza40) under greenhouse conditions.

Treatments	Conc. (g/L)	Chemical components	
		Chlorophyll (Chl) content	Protein content %
Frankincense	1	52.02 c	22.30 c
	2	54.74 bc	24.62 bc
	3	56.81 ab	27.04 b
Dithane M-45	2.5	60.16 a	30.72 a
control	-	38.15 d	19.45 d
L.S.D. at 0.05	-	3.70	2.47

Effect of faba bean treatments with the natural substance Frankincense on the activity of oxidative enzymes:

Activities of PO, PPO and CAT enzymes of faba bean plants were evaluated with Frankincense in the presence of *B. fabae* (Table 6). Results show that the natural substance Frankincense was effective in increasing enzyme activities. The highest increase of the three tested enzymes activities as compared to the untreated control was achieved with 3 g/L concentration, being 0.862, 0.095 and 4.582 unit/mg protein/min by 159.88, 170.00 and 262.17% increase over the untreated control for the three tested enzymes, respectively, meanwhile increasing concentration of the Frankincense increased the activities of the enzymes.

Field experiment:

Effect of natural substance Frankincense on control of chocolate spot disease during the 2018/19 and 2019/20 growing seasons:

Data presented in Tables (7,8 and 9) indicate that Frankincense at the high level (3 g/L) was the most effective in decreasing disease severity of chocolate spot (12.00), increasing yield per plot, weight of 100 seeds, 1.531 Kg, 95.10 g, respectively), leaf area 418.63, chlorophyll 43.84, protein content 27.25, respectively), compared to control treatment. The Frankincense moderate level (2 g/L) had an intermediate effect on the tested characters. While the Frankincense at the lowest level (1g/l) showed the lowest effect on decreasing disease severity % of chocolate spot (21.48%), and the lowest effect on increasing yield per plot, weight of 100 seeds (1.228 and 88.76 g, respectively), leaf area, Protein content and Chlorophyll. (263.21, 23.28 and 33.99, respectively) compared to control treatment. Regarding Frankincense at the tested three levels during 2018/19, data in 2019/20 season showed the same trend as in 2018/19 season.

Table (6): Effect of the natural substance Frankincense on enzyme activity on susceptible cultivar (Giza40) under greenhouse conditions.

Treatments	Conc. (g/L)	Peroxidase (unit/mg protein/min)		Polyphenol-oxidase (unit/mg protein/min)		Catalase (unit/mg protein/min)	
		activity	Increase over control %	activity	Increase over control %	activity	Increase over control %
Frankincense	1	0.408 c	22.93	0.075 c	112.86	2.429 d	92.02
	2 l	0.682 b	105.58	0.085 b	141.43	3.803 c	200.63
	3 l	0.862 a	159.88	0.095 a	170.00	4.582 b	262.17
Dithane M-45	2.5	0.953 a	187.48	0.056 d	58.57	5.243 a	314.43
Control	-	0.332 c	-	0.035 e	-	1.265 e	-
L.S.D. at 0.05	-	0.110		0.004		0.072	

Frankincense **(**unit/mg protein/min**)** is the change in absorbance at Δ470 nm. In the same column, means followed by the same letter are not significantly different at 0.05 level of probability according to Duncan's multiple range test (Duncan, 1955).

Table (7): Effect of the natural substance Frankincense on control of chocolate spot, disease severity % on the susceptible faba bean cultivar (Giza40) under field conditions during 2018/19 and 2019/20 growing seasons.

Treatment	conc.	Disease incidence%			Efficiency%			Disease severity%			Efficiency%		
		2018/19	2019/20	Mean	2018/19	2019/20	Mean	2018/19	2019/20	Mean	2018/19	2019/20	Mean
Frankincense	1 gm/L	31.67	29.67	30.67	66.07	69.42	67.74	21.48	20.92	21.20	64.11	65.47	64.79
	2 gm/L	22.33	19.67	21.00	76.07	79.73	77.90	17.62	16.25	16.94	70.56	73.17	71.87
	3 gm/L	16.67	15.67	16.17	82.14	83.85	83.00	12.00	11.92	11.96	79.96	80.33	80.14
Dithane M-45	2.5 g/L	12.67	10.33	11.50	86.43	89.35	87.89	9.58	9.88	9.73	84.00	83.70	83.85
Control	-	93.33	97.00	95.17				59.86	60.59	60.22			
L.S.D at 0.05													
Treatments (T)			1.98						2.51				
Season (S)			1.25						1.59				
(T × S)			2.80						3.56				

Table (8): Effect of natural substance Frankincense on chemical components on the susceptible faba bean cultivar (Giza40) under field conditions during the 2018/19 and 2019/20 growing seasons.

Treatment	Conc. (g/L)	Chemical components					
		Chlorophyll (Chl)			Protein content %		
		2018/19	2019/20	Mean	2018/19	2019/20	Mean
Frankincense	1	33.99	36.92	35.45	23.28	22.66	22.97
	2	38.39	39.47	38.93	25.54	25.98	25.76
	3	43.84	44.77	44.30	27.25	27.72	27.48
Dithane M-45	2.5	50.03	49.87	49.95	31.29	28.84	30.07
Control	-	26.54	23.11	24.82	18.88	18.39	18.63
L.S.D at 0.05							
Treatments (T)			2.08			2.47	
Season (S)			1.27			1.56	
(T × S)	-		2.84			3.50	

Table (9): Effect of natural substance Frankincense on yield components on the susceptible cultivar (Giza40) under field conditions during the 2018/19 and 2019/20 seasons.

Treatments	Conc. (g/L)	Leaf area			100 seeds weight (g)			Yield/plot (kg)		
		2018/19	2019/20	Mean	2018/19	2019/20	Mean	2018/19	2019/20	Mean
Frankincense	1	263.21	273.62	268.42	88.76	87.70	88.23	1.228	1.240	1234.00
	2	311.96	355.14	333.55	92.07	91.65	91.86	1.343	1.350	1346.66
	3	418.63	432.70	425.66	95.10	96.06	95.58	1.531	1.544	1537.66
Dithane M-45	2.5	492.21	486.99	489.60	96.84	97.22	97.03	1.664	1.669	1667.00
Control	-	221.67	208.92	215.29	62.87	60.40	61.63	966	987	976.66
L.S.D at 0.05										
Treatments (T)			16.83			1.45			14.90	
Season (S)			10.64			0.91			9.42	
(T × S)	-		23.80			2.05			21.08	

DISCUSSION

The results obtained from the effect of the natural substance frankincense on chocolate spot disease indicate that spraying with frankincense extract at a concentration of 3g/l effectively reduced the incidence of chocolate spots disease and improved the yield characters of faba bean crop, increased activity of peroxidase, Polyphenol-oxidase and catalase enzymes. These results are similar to those reported by Badria (2015) and Ali *et al.* (2016). They showed that nine compounds with immune stimulant and antiviral activity were isolated from the oleo-gum-resin of frankincense (*Boswellia carterii* Flueck). The frankincense essential oil (3%) was found to contain monoterpenes (13.1 %), sesquiterpenes (1 %), and diterpenes (42.5 %). Both isolated oil and resins exhibited a strong immune stimulant activity. They added that resin indeed has analgesic, tranquilizing and anti-bacterial effects. Regarding the enzyme activity, the obtained results showed that increasing the activity of peroxides, polyphenol-oxidase and catalase led to decrease of disease incidence and increased yield characters compared with the control treatment. The Frankincense at moderate level (2 g/L) had an intermediate effect on the tested characters. While the Frankincense at the lowest level (1 g/L) showed the lowest effect on decreasing the severity of chocolate spot disease (21.20 %), and the lowest effect on increasing yield per plot, the weight of 100 seeds, leaf area and chlorophyll. This inducer of resistance (Frankincense) was effective in reducing the incidence of chocolate spot disease and increase of enzymes activity related to disease resistance, and these were the same as those reported by Zyton and Hassan (2017), who found that the highest activities of defense related enzymes, *i.e.*, peroxidase, polyphenol-oxidase, and catalase were recorded in treated bean plants with organic acids (Fulvic, citric, salicylic acids and BTH) as well as reduced the disease incidence. This reduction was gradually increased by increasing the concentration of the tested chemicals inducers substances, and also agreed with the results recorded by Aldesuquy *et al*. (2015) who obtained an additional increase in peroxidase, polyphenol oxidase and phenylalanine ammonia lyase in infected faba bean plants sprayed with phenolic compounds (shikimic acid and salicylic acid) than that sprayed with fungicide, and was in the same line with the finding reported by Ismail and Afifi (2019) who revealed that spraying by tested chemical inducers led to a marked increase in the activity of peroxidase, polyphenol-oxidase, catalase enzymes and seeds in faba bean.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest

REFERENCES

Abd El-Hai, K.M.; El-Ghamry, A.M. and Ghoneem, K.M. 2011. Induction of Faba bean resistance to Chocolate Spot and rust diseases and improving the productivity using amino and humic acids. Egypt. J. Phytopathol., 39(2): 43-58.

Abou-Zeid, N.M. 1985. Contribution a L'amelioration de la resistance de *Vicia faba* L. au *Botrytis fabae*. These de Doctor es science University de Rennes. France.

Adrian, A.L. 1996. *Boswellia serrata*. A unique Ayuvredic Anti-rheumatic. Issue of VRP's Nutritional News.

Ali, R.M.A. and Bożena, K. 2016. Frankincense – therapeutic properties Postepy. Hig. Med. Dowse., 70: 380-391.

Alan, O. and Ceren. H. 2007. Evaluation of heritability and correlation for seed yield and yield components in faba bean. J. Argon., 6: 484-487.

Aldesuquy, H.; Baka, Z. and Alazab, N. 2015. Shikimic and salicylic acids induced resistance in faba bean plants against chocolate spot disease. J. Plant Pathol. Microb., 6: 2.

AOAC (Association of Official Analytical Chemists) 1990. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th edition, Published by Association of Official Analytical Chemists, Arlington, Virginia, USA.

Badria, F.A. 2015. Frankincense (Heaven's Gift)-Chemistry, Biology, and Clinical Applications. Evidence-based Strategies in Herbal Medicine, Psychiatric Disorders and Emergency Medicine. 108 pp.

Badria, F.A.; Houssen, W.E.; El-Nashar, E.M. and Saaed, S.A .2003. Effect of Glycyrrhizin and *Boswellia carterii* extract on liver injury: Biochemical and Histopathological Evaluation, Biosci. Biotech. Res. Asia; 1(2): 93-96.

Botros, R.M.; Gala,T.M.; Houssen, B.F.A. and Mohammed, M.A. 2003.Chemistry and immunomodulatory Activity of Frankincense oil Z. Naturfor- schung, 58c: 230-238.

CAPMS (Central Agency for Public Mobilization and Statistics), 2021. Annual Bulletin of Statistical Crop Area and Plant Production, Issue February 2021. 94 pp.

Dhull, S.B.; Kidwai, M.K.; Noor, R.; Chawla, P. and Rose, P.K. 2022. A review of nutritional profile and processing of faba bean (*Vicia faba* L.). Legume Science, 4(3): e129.

Ding, G.; Xung, L.; Oifang, G.; Pingxi, L.; Dazaho, Y. and Ronghai, H. 1993. Evaluation and screening of Faba bean germplasm in China. FABIS Newsletter, 32: 8-10.

Duncan, M.B. 1955. Multiple range and multiple F-test Biometrics,11:1-42.

FAO (Food and Agriculture Organization). (2020). Crop Production and Trade Data. http://www.fao.org/faostat/en/#data (accessed December 30, 2020).

Gerhardt, H.; Seifert, F.; Buvari, P.; Vogelsang, H. and Repges, R. 2001. Therapy of active Crohn disease with *Boswellia serrata* extract H15Z. Gastroenterol, 39(1): 11-7.

Ghoneem, K.M.; Saber, W.I.A and El-Baz, S.M. 2012. Influence of essential oils of some medicinal and aromatic plants on growth and control of *Botrytis fabae* the causal of faba bean chocolate spot. Egypt. J. Phytopathol., 40(1): 101-117.

Hanouike, S.B. and Hasanain, M.A. 1986. Inhibition of *Botrytis fabae* in the philosopher of *Vicia faba* leaves. FABIS Newsletter, 16: 41-44.

Huan M.T.; Badmaev V.; Ding Y.; Liu Y.; Xie, J.G. and Ho, C.T. 2000. Antitumor and anti-carcinogenic activities of triter period, beta-boswellic acid. In. Bio. Factor., 13(1-4): 225-30.

Haggag, W.M.; Kansoh, A.L. and Aly, A.M. 2006. Proteases from *Talaromyces flavus* and *Trichoderma harzianum*. Purification, characterization and antifungal activity against brown spot disease on faba bean. Plant Pathol. Bull., 15:231-239.

Ismail, A.M. and Afifi, M.I.M. 2019. Efficacy of some biotic and abiotic factors in controlling common bean rust disease caused by *Uromyces appendiculatus.* Egypt. J. Phytopathol., 47(1): 313-329.

Kato, M. and Shimizu, S. 1987. Chlorophyll metabolism in higher plants. VII. Chlorophyll degradation in senescing tobacco leaves: phenolic-dependent peroxidative degradation. Canad., J. Bot., 65: 729-735.

Knaus, U. and Wagner, H. 1996. Effects of boswellic acid of *Boswellia serrata* and other triterpenic acids on the complement system. Phytomedicine, 3(1): 77-80.

Krieglstein, CF.; Anthoni,C.; Rijcken, E.J.; Laukotter, M.; Spiegel, H.U.; Boden, S.E.; Schweiz. S.; Safayhi, H.; Senninger, N. and Schurmann, G. 2001. Acetyl-11-Keto-beta-boswellic acid, a constituent of a herbal medicine from *Boswellia serrata* resin, attenuates experimental ileitis. Int. J. Colorectal. Dis., 16(2): 88-95.

Ling, Q.; Huang, W. and Jarvis, P. 2011. Use of a SPAD-502 meter to measure leaf chlorophyll concentration in *Arabidopsis thaliana*. Photosynth Res., 107: 209-214.

Mayer, A.M.; Harel, E. and Shaul, R.B. 1965. Assay of catechol oxidase: a critical comparison of methods. Phytochem., 5: 783-789.

Metwaly, H.A. 2014. Control of chocolate spot disease by non-traditional methods on faba bean plants. Egypt. J. Phytopathol., 42(1): 143-158.

Michaela, M.; Buettner, A. and and Eva, K. 2009. The volatile constituents of Frankincense – a review. Flavour Fragr. J., 24(6): 279-300.

Sharma, M.L., Bani, S.; Singh, G.B. 1989. Antiarthritic activity of boswellic acid in bovine serum albumin (BSA)-induced arthritis. Int. J. Immunopharmacol., 11(6): 647-652.

Sinclair, J.B. and Dhingra, O.D. 2019. Basic Plant Pathology Methods, 2nd Ed. CRC Press, Boca Raton, 448 pp.

Teshome, E. and Tagegn, A. 2013. Integrated management of chocolate spot (*Botrytis fabae* Sard.) of faba bean (*Vicia faba* L.) at highlands of Bale, Southeastern Ethiopia. Res. J. Agric. Environ. Manage., 2(1): 11-14.

Wagner, H. 1989. Search for new plant constituents with potential antichloristic and anti-allergic activity. Planta Medica., 55:235-241.

Zyton, M.A. and Hassan, E.O. 2017. Effect of the combination between bio agents and benzothiadiazole (BTH) on management of *Uromyces pisi* the causal of pea rust. Am. J. Life Sci., 5(3-1): 15-23.