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Influence of Essential Oils of some Medicinal and Aromatic Plants on Growth and Control of *Botrytis fabae* the Causal of Faba Bean Chocolate Spot

K.M. Ghoneem[*]; W.I.A. Saber[**] and Sahar M. El-Baz[***]

[*] *Seed Pathol. Res. Dept., Plant Pathol. Res. Inst., Agric. Res. Centre, Giza, Egypt.*

[**] *Microbiol. Dept., Soils, Water and Environ. Res., Inst., Agric. Res. Centre, Giza, Egypt.*

[***] *Mycol. and Plant Dis. Surv. Dept., Plant Pathol. Res. Inst., Agric. Res. Centre, Giza, Egypt.*

The aim of this study was to find an alternative to synthetic fungicides currently used in the control of faba bean chocolate spot fungal pathogen, *Botrytis fabae*. For this purpose, the effect of 12 essential oils (EOs) isolated from different medicinal and aromatic plant species against *B. fabae* has been studied *in vitro*. Complete inhibition of growth and spore formation of the pathogen by EOs of thyme and caraway however, was observed at 1.0 µl ml^{-1}. Oils of rue, garlic and cloves were inhibitory at relatively higher concentrations (2.5 µl ml^{-1}). Under field conditions, oils of thyme, rue caraway and garlic, gave pronounced protection of faba bean plants against invasion of chocolate spot pathogen. The maximum reduction of disease incidence (DI) and severity (DS) of chocolate spot along the growth period in both growing seasons (2010/2011 & 2011/2012) was observed by garlic, followed by caraway and cloves oils. All morphological traits (plant height and no. of branches & leaves/plant) and yield components (pods no./plant and weight of 100 seeds) significantly increased by the application of garlic oil, while caraway oil came in the second order. Also, application of the previous treatments presented significant increases in the photosynthetic pigments (chlorophyll a and b and carotenoids), polyphenol oxidase and total phenol in faba bean plants. This study has demonstrated that the EOs are potential and promising antifungal agents, which could be used as biofungicide in the protection of faba bean against *Botrytis fabae*.

Keywords: Aromatic Plants, *Botrytis fabae,* chocolate spot, essential oils, faba bean, medicinal and *Vicia faba*.

One of the most important food legumes is faba bean (*Vicia faba,* L.) that plays a vital role in the traditional diets of the Mediterranean, Indian, Chinese, English, Middle Eastern, African and South American people. Its seed is extremely rich in protein, complex carbohydrates, dietary fiber, choline, lecithin, minerals and secondary metabolites such as phenolics and levo dihydroxy phenylalanine (Randhir *et al*., 2002).

Chocolate spot of faba bean has been reported in many parts of the world, but is more prevalent in humid regions than in the arid climatic zones (Hanounik, 1986).

The disease caused mainly by *Botrytis fabae* Sard. and to some extent, *B. cinerea* Pers. ex. Fr. is the most destructive disease and the major cause of yield losses in almost all regions where crop is grown (Hanounik and Bisri, 1991; Harrison, 1988 and Koike, 1998). In Egypt, it causes an estimated 5-20% loss production annually, but losses as high as 60-80% has been reported under epiphytotic conditions (Noorka and El-Bramawy, 2011). The disease results in harmful effects on growth, most physiological activities and yield of the unprotected crop (Khaled *et al*., 1995). Defoliation and lodging, flower drop, stem collapse, tissue necrosis and finally plant death occur after warm (15-25ºC) humid conditions (over 70%) that extend for 4-5 days, favour disease development. In addition, inoculums density, water logging, high plant density and host physiology have been shown to be closely related with disease development (Hanounik and Bisri, 1991).

Essential oils are volatile complex compounds characterized by strong odour and are formed by medicinal and aromatic plants as secondary metabolites. In nature, essential oils play an important role in the protection of the plants as antimicrobial, insecticides and against herbivores by reducing their appetite for such plants (Jobling, 2000; Bakkali *et al*., 2008 and Combrinck *et al*., 2011). Because of the mode of extraction, mostly by distillation from aromatic plants, they contain a variety of volatile molecules such as terpenes and terpenoids, phenol-derived aromatic components and aliphatic components (Bakkali *et al*., 2008). The main reason for using essential oils as antifungal agents is their natural origin and low chance of pathogens developing resistance. Also, they may have a minimum adverse effect on physiological processes of plants and less environmental hazards compared to their synthetic alternatives, being plant products are easily convertible into save common organic materials (Gnanamanickam, 2002). Quite a lot of preliminary work has been done to demonstrate the potential of essential oils, *i.e*. red thyme (*Thymus zygis*), clove buds (*Eugenia caryophyllus*) and cinnamon leaf *(Cinnamomum zeyophllata*), against postharvest fungi *in vitro* such as *Botrytis cinerea* (Jobling, 2000). Many publications have documented the antimicrobial activity of essential oils of garlic and cumin (Hammad and Youssef, 1994), thyme, marjoram and lavender (Daferera *et al*., 2003; Arslan and Dervis, 2010; Soylu *et al*., 2010 and Combrinck *et al*., 2011), black cumin (Rahhal, 1997 and Farid *et al*., 2000), clove (Velluti *et al.,* 2003) and fennel, peppermint and caraway (Abo-El Seoud *et al.,* 2005) against different plant pathogens.

Chemical fungicides are commonly used successfully for controlling chocolate spot of faba bean (Khaled *et al*., 1995). However, their field application may not always be desirable. The persistent, injudicious use of chemicals was discouraged owing to their toxic effects on non-target organisms, the undesirable changes they inflict upon the environment (Arcury and Quandt, 2003) and due to development of resistant strains of pathogens against various chemical fungicides (Deising *et al*., 2008), that is why essential oils are used as bio-fungicidal compounds (Al-Askar and Rashad, 2010).

The objective of the performed research work was to investigate the antifungal activity of steam-distillation extracts of 12 essential oils *in vitro* on growth and sporulation of *B. fabae* as well as to evaluate the protective effects of the most effective ones against the fungal invasion of faba bean plants under field conditions. Additionally, to determine the response of faba bean plants in the form phenols, polyphenol oxidase and cholorophyll content as a probable defence mechanism.

Materials and Methods

Plant materials and extraction of essential oils (EOs):

Essential oils were extracted from different parts of several plants, i.e. seeds of *Carum carvi*, *Foeniculum vulgare*, *Pimpinella anisum*, *Nigella sativa* and *Eruca sativa*, fresh bulbs of *Allium sativum* and *Allium cepa*, fresh leaves at flowering stage of *Thymus vulgaris*, *Rute angustifolia* and *Origanum majorana*, flower spikes of *Lavandula angustifolia* and dried flower buds of *Syzygium aromaticum*. All samples were collected locally from different districts of Dakahlia, Faiyum and Minya governorates. Plant materials (200g) were placed in a 5-liter round-bottom distillation flask and 3 litres of double distilled water was added. The essential oils were obtained by steam distillation for 3h using Clevenger-type apparatus (Chialva *et al*., 1982 and Charles & Simon, 1990). The extracted essential oils in pure form were stored at 4°C in a clean amber glass bottle until used.

Isolation of Botrytis fabae:

Isolate of *B. fabae* was obtained from diseased leaves heavily infected by chocolate spot. Isolation was carried out on Potato Dextrose Agar (PDA). The fungus was grown for 3 to 6 days on PDA at 22°C under cool white fluorescence light with alternating cycle of 12h light /12h darkness. Stock cultures obtained from single spore were maintained on Potato Carrot Agar (PCA) and kept at 4°C and sub-cultured monthly.

Antifungal activity of the essential oils tested in vitro:

Antifungal activities of the twelve essential oils extracted were evaluated. Fifty ml of PD broth containing 5% Tween-20 in 250 ml Erlenmeyer flasks were amended with different concentrations of each essential oil (1.0, 2.5, 5.0 and 10.0 µl ml^{-1}) after autoclaving. Corkborer was used to prepare inoculum discs (5 mm diameter) of *Botrytis fabae*, from the periphery of an actively growing culture on PDA plates. The discs were used to inoculate flasks and then incubated in a dark at 26±2ºC for 7 days. Kocide 101 (2.5 g/l) as a fungicide (El-Ghamry *et al*., 2009) was used for comparison and untreated medium was used as control. Triplicate flasks were used for each treatment. At the end of incubation period, the mycelium was filtered and washed several times with distilled water, then dried in an oven at 80°C till constant weight. The antifungal activities of the essential oils tested were expressed as percentage inhibition of mycelial growth in comparison with the untreated medium, according to the formula: MGI= (A-B) / A x 100, whereas; MGI (%) is the percent of mycelial growth inhibition, A: dry weight of the pathogen when growing without EO and B: dry weight of the pathogen for each EO concentration. Sporulation (spore ml^{-1}) was determined using haemocytometer. The final culture pH of the medium as affected by different essential oil concentrations was determined after the growth period of *B. fabae* using pH-meter with glass electrode (HI 9321 microprocessor pH-meter).

In vivo experiments:

Field experiments were carried out at the experimental farm of Tag El-Ezz, Agric. Res. Station, Dakahlia, Egypt, during two successive seasons (2010/2011 and 2011/2012). The soil analysis was carried out in Mansoura Laboratory for Soil Fertility, Dakahlia, Egypt. However, soil is clayey in texture containing 54.1% clay, 36.5% silt and 9.4% sand. The EC in soil paste = 6.04 dSm^{-1}, pH in water suspension (1:2.5) = 8.0, E.S.P = 8.16%, organic matter = 1.67% and $CaCO_3$ = 2.24%. Available N, P and K were 45.64, 11.21 and 283 mg. kg^{-1}, respectively.

Performance of the most effective essential oils, *i.e.* thymes, rue, garlic, caraway and rocket, were *in vitro* investigated at concentration of 2.5 µl ml^{-1} as foliar spraying on faba bean susceptible cultivar Giza 2 against chocolate spot. Seeds were obtained from The Central Administration for Certification, Ministry of Agriculture, Egypt. The sowing (November10^{th}, for both seasons) was done in plots, each of 3.5×1.5. The plots were prepared and all recommended agricultural practices for *Vicia faba* were carried as usual. Complete randomized plot design was allocated. The plants developed from each assigned treatment were sprayed twice, 35 and 55 days after sowing with the defined five essential oils. Plants sprayed with tap water only served as control.

Disease assessment:

At 50 and 70 days from sowing (15 days after each spray), plants were rated for disease incidence (DI); as percentage of infected leaves and disease severity (DS) according to the scale of Bernier *et al*. (1993) following the formula:

$$DS\ (\%) = \frac{\sum (NPC \times CR)}{NIP \times MSC} \times 100$$

Whereas: NPC: number of plants in each class rate, CR: class rate, NIP: number of infected plants and MSC: maximum severity class rate.

Performance and Physiological activities of faba bean plants:

After 70 days from sowing, both of growth parameters per plant (height, numbers of branches and leaves) and nodulation status in terms of counts of nodules per plant were examined in samples of faba bean, in both seasons. At the same growth period in the second season only, the following physiological activities were tested; 1) Photosynthetic pigments (chlorophyll [Chl] a, b and total Chl and carotenoids) were determined in the blade of the third leaf of plant tip; terminal leaflet (Mackinney, 1941), 2) Total phenolic compounds were determined in fresh shoot using the folin-ciocalteau reagent (Malick and Singh, 1980) and 3) Polyphenol oxidase was assayed colorimitrically using catichol as substrate (Matta and Dimond, 1963), the activity was expressed as the change in the absorbance of the reaction mixture every 50 sec for 5 min period at 495 nm. At harvest, pods number $plant^{-1}$ and seeds number pod^{-1} and seed index (weight of 100 seeds) were recorded. All the statistical analysis were carried out using the statistical analysis software; CoStat v6.4.

Results

Antifungal activity of essential oils tested in vitro:

The antifungal activities of the twelve plant essential oils tested against growth and sporulation of *B. fabae* were evaluated. However, thyme and caraway oils completely inhibited the mycelial growth (Table 1) and sporulation (Table 2) of *B. fabae* at concentration of 1.0 µl ml^{-1}. Whereas, oils of rue, garlic, rocket at 2.5 µl ml^{-1} and marjoram at 5.0 µl ml^{-1} concentrations exhibited fungicidal activity against growth and sporulation of chocolate spot fungus. At 10.0 µl ml^{-1}, onion oil showed a strong inhibition rates on mycelial growth and prevented spore formation of *B. fabae*. On the other hand, lavender, fennel and anise essential oils had the lowest inhibition rates (<70%) on mycelium growth of *B. fabae* (Table 1).

Table 1. Mycelial growth (µg ml^{-1}) of *B. fabae* as affected by four essential oil concentrations

Essential oil		Oil concentration (µl ml^{-1})*			
Scientific name	English name	1.0	2.5	5.0	10.0
Thymus vulgaris	Thyme	0.000 o	0.0 o	0.000 o	0.000 o
Rute angustifolia	Rue	0.150 h-k	0.0 o	0.000 o	0.000 o
Allium sativum	Garlic	0.133 i-k	0.0 o	0.000 o	0.000 o
Carum carvi	Caraway	0.000 o	0.0 o	0.000 o	0.000 o
Syzygium aromaticum	Clove	0.066 l-n	0.0 o	0.000 o	0.000 o
Eruca sativa	Rocket	0.273 b	0.21 c-g	0.176 e-i	0.006 o
Foeniculum vulgare	Fennel	0.25 bc	0.183 d-i	0.116 j-l	0.113 j-l
Pimpinella anisum	Anise	0.23 b-e	0.193 d-h	0.166 f-j	0.057 m-o
Nigella sativa	Black cumin	0.15 h-k	0.233 b-d	0.283 b	0.193 d-h
Allium cepa	Onion	0.25 bc	0.153 h-k	0.156 g-j	0.020 no
Lavandula angustifolia	Lavender	0.183 d-i	0.216 c-f	0.250 bc	0.116 j-l
Origanum majorana	marjoram	0.10 k-m	0.356 a	0.000 o	0.000 o
Control A (Kocide 101)		0.000 o	0.000 o	0.000 o	0.000 o
Control B (without treatment)		0.183 d-i			

* Numbers followed by the same letter(s) are not significantly differed at $P \leq 0.05$ (Tuckey test).

With respect to the final pH of the growth medium, no specific trend could be observed either for the different oils or the different concentrations in spite of the significant variation among the treatments (Table 3). However, at all concentrations of the different oils, there was an obvious variation in the final culture pH. Analysis of correlation coefficient (r) (Table 4) between every pairs of the tested parameters of *B. fabae* reveals positive correlation between growth and sporulation in which r = 0.426287 at $P \leq 0.05$. On the other hand, the correlation was negative between final culture pH from one side and growth (r = -0.731, $P \leq 0.05$) and sporulation (r = -0.402, $P \leq 0.05$) from the other side.

Table 2. Sporulation (spore ml^{-1}) of *B. fabae* as affected by four essential oil concentrations

Essential oil	Oil concentration (µl ml^{-1}) *			
	1.0	2.5	5.0	10.0
Thyme	0.000 k	0.000 k	0.000 k	0.000 k
Rue	16.866 e	0.000 k	0.000 k	0.000 k
Garlic	2.906 h-k	0.000 k	0.000 k	0.000 k
Caraway	0.000 k	0.000 k	0.000 k	0.000 k
Clove	0.546 jk	0.000 k	0.000 k	0.000 k
Rocket	11.90 f	16.866 e	6.280 g	0.000 k
Fennel	12.95 f	3.940 gh	3.233 h-j	0.966 i-k
Anise	38.736 b	23.660 c	12.330 f	0.000 k
Black cumin	1.466 h-k	1.333 h-k	1.100 h-k	0.326 jk
Onion	1.900 h-k	1.200 h-k	0.533 jk	0.000 k
Lavender	1.450 h-k	1.333 h-k	1.066 h-k	0.133 k
marjoram	2.400 h-k	3.800 g-i	0.000 k	0.000 k
Control A (Kocide 101)	0.000 k	0.000 k	0.000 k	0.000 k
Control B (without treatment)	52.413a			

* Numbers followed by the same letter(s) are not significantly differed at $P \leq 0.05$ (Tuckey test).

Table 3. Final pH of *B. fabae* culture growth as affected by four essential oil concentrations

Essential oil	Oil concentration (µl ml^{-1}) *			
	1.0	2.5	5.0	10.0
Thyme	6.87 a-c	6.71 a-e	6.61 b-e	6.65 b-e
Rue	6.26 e-h	6.67 b-e	6.74 a-e	6.74 a-e
Garlic	5.85 h-l	6.34 d-g	6.25 e-h	6.25 e-h
Caraway	6.88 a-c	6.87 a-c	6.83 a-d	6.83 a-d
Clove	6.95 ab	6.86 a-c	7.18 a	7.18 a
Rocket	5.39 k-n	5.48 i-n	5.38 l-n	6.40 c-f
Fennel	5.90 g-j	6.80 a-d	6.62 b-e	6.79 a-d
Anise	5.72 i-n	5.95 f-i	4.90 o	6.40 c-f
Black cumin	5.54 i-n	5.27 no	5.33 mn	5.73 i-n
Onion	5.73 i-n	5.81 h-m	5.60 i-n	6.50 b-e
Lavender	5.69i-n	5.66 i-n	5.97 f-i	5.66 i-n
marjoram	6.42 c-f	5.88 g-k	6.52 b-e	6.55 b-e
Control A (Kocide 101)	6.79 a-d	6.79 a-d	6.78 a-d	6.79 a-d
Control B (without treatment)	5.44 j-n			

* As mentioned in footnote of Table (2).

Table 4. Correlation coefficients between every pair of growth, sporulation and final culture pH of *B. fabae*

Parameter	Growth	Sporulation
Sporulation	0.426*	
Final pH	-0.731*	-0.402*

* Significant at $P \leq 0.05$.

Field evaluation of essential oils on:
Incidence (DI) and severity (DS) of chocolate spot:

Data in (Table 5) show that, most of the tested oils (thyme, rue, garlic, caraway and rocket) reduced DI and DS along the tested period in both growing seasons. In the second season, much more reduction in the disease parameters by the tested oils was recorded. Among the tested oils, garlic followed by caraway and rocket were found to be the best treatments in reducing DI and DS. The fungicide Kocide 101 application showed the highest reduction in disease parameters in comparison with all other treatments.

Table 5. Efficacy of essential oils spraying on faba bean chocolate spot development under field conditions

Essential oil	After 50 days				After 70 days			
	DI		DS		DI		DS	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Thyme	46.14 a*	31.35 ab	12.31 ab	9.21 b	50.14 a	36.85 bc	19.31 b	17.21 b
Rue	32.59 b	29.65 bc	11.40 bc	9.57 b	40.60 b	39.65 b	18.40 b	16.87 b
Garlic	21.89 cd	22.36 c	7.16 de	6.56 cd	26.90 c	29.30 4c	13.97 c	12.88 c
Caraway	28.85 bc	28.85 bc	7.67 de	8.45 bc	32.85 bc	37.35 bc	14.67 c	13.37 c
Clove	28.98bc	26.62 bc	8.36 cd	7.98 b-d	34.98 bc	32.62 bc	15.36 c	14.32 c
Cont.A**	15.93 d	12.34 d	4.80 e	5.70 cd	12.93 d	18.34 d	7.46 d	7.44 d
Cont.B	47.80 a	38.32 a	15.23 a	14.58 a	57.30 a	50.32 a	29.36 a	25.26 a

* Different letters within a column indicates significant difference at $P \leq 0.05$ (Duncan test).
** Cont. A: Kocide 101 and Cont. B: water.

Growth and yield attributes:

Data presented in Table (6) reveal pronounced improvements in both growth and yield attributes of faba bean. With respect to vegetative growth, garlic oil is the only treatment that did not show any significant differences with the recommended fungicide (Kocide 101), caraway oil came in the second order. Data of the yield attributes of faba bean in both seasons (Table 7) show that garlic oil was the best treatment in inducing number of pods per plant, weight of seeds per plant and weight of 100-seed. The same trend was observed in case of caraway oil. The other oils came in the next order but they were found to be superior in their effects in relation to control treatment (water only).

Physiological characters of faba bean:

The photosynthetic pigments of 70-days old plants showed different responses to the tested oils as shown in Fig (1). No specific trend could be noticed, as spraying with thyme oil recorded the highest content of Chl a (0.949 mg g^{-1}) and carotenoids (0.368 mg g^{-1}), garlic recorded the maximum content of Chl b (0.584 mg g^{-1}) and total Chl (1.470 mg g^{-1}). However, the variation in chlorophyll content between the tested oils and the fungicide (Kocide 101) did not reach the level of significance, but it did in the case of carotenoids.

Table 6. Efficacy of essential oil spraying on vegetative growth of faba bean after 70 days from sowing

Essential oil	Plant height		Branches No. plant^{-1}		Leaves No. plant^{-1}		Nodulation No. plant^{-1}	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Thyme	84.4 b*	87.9 c	6.3 d	6.7 a	79.0 c	85.0 c	254.7 bc	320.0 c
Rue	85.4 b	88.3 c	6.7 cd	6.3 a	81.3 bc	89.0 bc	375.0 a	405.0 a
Garlic	98.9 a	100.2 a	7.7 a	7.3 a	89.0 a	93.0 ab	267.0 b	360.0 b
Caraway	90.3 b	98.7 a	7.3 ab	7.0 a	86.3 ab	90.0 bc	189.3 d	210.0 e
Clove	89.1 b	91.5b c	7.0 bc	6.7 a	85.3 ab	88.3 bc	87.0 e	180.0 e
Cont. A **	90.4 b	95.5 ab	7.3 ab	7.0 a	90.0 a	96.0 a	249.7 c	385.0 ab
Cont. B	84.0 b	86.2 c	5.7 e	4.7 b	64.3 d	76.0 d	192.0 d	277.3 d

* Different letters within a column indicates significant difference at $P \leq 0.05$ (Duncan test).
** Cont. A: Kocide 101 and Cont. B: water.

Table 7. Efficacy of essential oil spraying on faba bean yield and its attributes

Essential oil	Yield attributes *					
	Pods No. plant^{-1}		Seeds weight plant^{-1}		Seed index (100 seeds wt.)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Thyme	57.67 b	55.60 c	95.82 d	99.00 d	56.50 cd	60.46 bc
Rue	60.33 b	56.33 c	106.20 cd	102.75 cd	59.13 bc	61.56 bc
Garlic	69.33 a	68.33 ab	140.75 ab	130.39 ab	68.23 a	64.70 ab
Caraway	68.00 a	66.33 ab	124.50 bc	121.70 b	61.16 b	63.82 bc
Clove	63.66 ab	64.33 b	115.24 cd	117.04 bc	61.13 b	61.56 bc
Cont. A **	71.00 a	70.33 a	149.03 a	144.97 a	70.67 a	69.47 a
Cont. B	46.33 c	41.66 d	74.64 e	71.34 e	54.23 d	58.94 c

* & ** As described in footnote of Table (6).

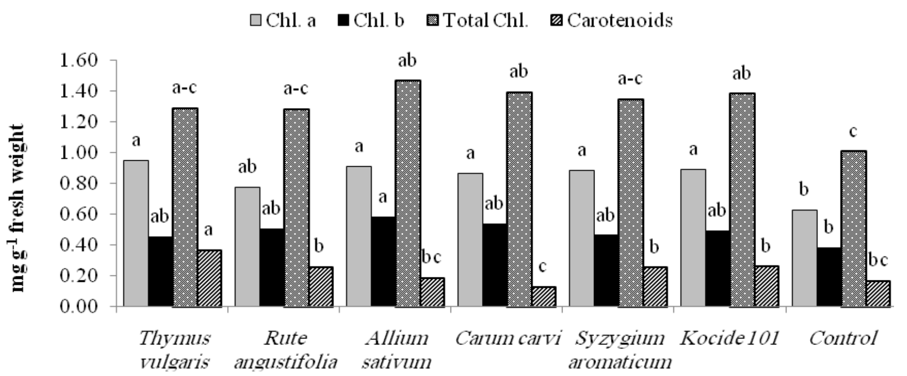

Fig. 1. Photosynthetic pigments of faba bean plants as response to spraying with essential oils after 70 days from sowing. For each type of column, different letters indicates significant difference at *P* ≤ 0.05 (Duncan test).

The effect of the tested oils on self-defence compounds of faba bean plants was tested by means of measuring the total phenols and polyphenol oxidase activity (Fig 2). Thyme oil was the most inductor one of both tested criteria. Generally, the total phenols of oils treated faba bean was better and recorded higher phenol content than those of Kocide 101 treated plants. On the other side, polyphenol oxidase activity recovered from garlic, caraway and rocket treated plants did not record any significant differences in comparison with Kocide 101, but reached to the significance level in the case of thyme and rue.

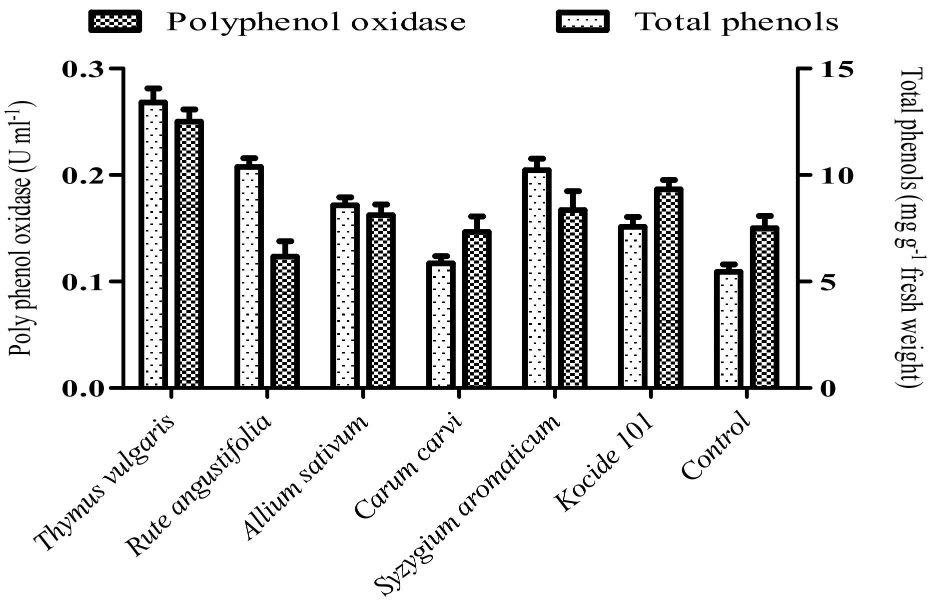

Fig. 2. Total phenols and polyphenol oxidase activity in faba bean plants as affected by essential oils spraying after 70 days from sowing.

Discussion

Plant essential oils are potentially useful source of antimicrobial compounds. Our results indicated that all tested essential oils (except black cumin oil) showed an antifungal activity against growth and sporulation of *B. fabae* with highest effect in case of thyme, rue, garlic, caraway and cloves oils. In this respect, a complete inhibition in fungal growth and sporulation was observed with 1.0 µl ml^{-1} of thyme and caraway and with 2.5 µl ml^{-1} of rue, garlic and cloves essential oils. This is in agreement with that of Daferera *et al*. (2003) and Combrinck *et al*. (2011) who reported thyme oil as the most effective inhibitor, totally inhibiting *Botrytis cinerea* and all of the pathogens tested at relatively low concentrations. Whereas, the *in vitro* efficacy of cloves, thyme, caraway and rue oils was reported by Al-Askar & Rashad (2010), Arslan and Dervis (2010) and Abdel-Kader *et al*. (2011) against different pathogens.

Because of the great number constituents, essential oils seem to have no specific cellular targets (Carson *et al*., 2002). But the general mode of action could be explained based on one or more of the following; as typical lipophiles, they pass through the cell and cytoplasmic membrane, disrupt the structure of their different layers of polysaccharides, fatty acids and phospholipids and permeabilize them. Cytotoxicity appears to include such membrane damage (Bakkali *et al*., 2008). However, recent work shows that in eukaryotic cells, essential oils can act as pro-oxidants affecting inner cell membranes and organelles such as mitochondria. Depending on type and concentration, they exhibit cytotoxic effects on living cells but are usually non-genotoxic. In some cases, changes in intracellular redox potential and mitochondrial dysfunction induced by essential oils can be associated with their capacity to exert antigenotoxic effects (Bakkali *et al*., 2005 & 2006).

The antifungal activity was strongly associated with monoterpenic phenols, especially thymol, carvacrol and eugenol, in the oils. Natural isopropyl cresols, thymol (2-isopropyl-5-methylphenol) and carvacrol (5-isopropyl-2-methylphenol) produced mainly from thyme are credited with a series of pharmacological properties, including antimicrobial and antifungal effects (Ahmad *et al*., 2011). These two compounds showed a complete growth inhibition of *Botrytis cinerea*, *Alternaria arborescens* and *Rhizopus stolonifer* (Plotto *et al*., 2003). Also, the inhibitory effect of thyme oil was found against some pathogens of both fruits (Combrinck *et al*., 2011) and root rots of faba bean (Abdel-Kader *et al*., 2011). Thymol and carvacrol as thyme oil components affect the surface electrostatics of the cell membrane and membrane integrity (Lambert *et al*., 2001 and Sánchez *et al*., 2004), as well as, damaging the cell wall, cell membrane and cellular organelles (Rasooli and Owlia, 2005).

The main constituent of caraway oil is carvone which produced by over 70 different plants (Burdock, 1995). It can be used as antimicrobial agent and as a potato sprout inhibitor (Carson and Riley 1995). In the same time, it did not show negative effects on plant quality (Hartmans *et al*., 1995). On the other hand, the efficacy of clove oil was echoed by the activity of eugenol. Pure eugenol was found to be extremely effective against all postharvest pathogens of fruits (Combrinck *et al*., 2011), which inhibitors enzyme processes and related compounds as methyl- or acetyleugenol (Pepeljnjak *et al*., 2005). Its antimicrobial activity can be attributed to the presence of an aromatic molecules and a phenolic OH-group that are known to be reactive and can form hydrogen bonds with -SH groups in the active sites of target enzymes, resulting in deactivation of enzymes in fungi (Velluti *et al*., 2003 and Alma *et al*., 2007).

However, at all concentrations of the different oils, there were obvious variations in the final pH, which means the different responses of *B. fabae* towards different oils. The positive correlation coefficient between growth and sporulation is expected and agreed with the nature growth curve of the pathogen. The negative correlation between final culture pH from one side and growth and sporulation from the other side is also expected since the growth and sporulation of a fungus in the growth medium is always accompanied with the secretion of different metabolites, which alters the reaction behaviour of the growth medium towards the acidic conditions.

The essential oils tested *in vivo* significantly reduced DI and DS of faba bean chocolate spot disease, along the tested period, compared to untreated control treatments. Garlic oil, however, was superior in this respect. The fungicide Kocide 101 application showed, generally, the highest reduction in the disease parameters in comparison with all other treatments and those results are in agreement with those of Abdel-Kader *et al*. (2011). The mode by which microorganisms are inhibited by EOs seems to involve different mechanisms attributed to phenolic toxicity (Gutierrez *et al*., 2008), their hydrophobicity and partition in the microbial plasmic membrane (Bakkali *et al*., 2008). Microscopic observations, however, revealed that they may cause considerable morphological degenerations of the fungal hyphae such as cytoplasmic coagulation, vacuolations, hyphal shrivelling and protoplast leakage as well as loss of conidiation (Soylu *et al*., 2010).

Yield loss from chocolate spot has been attributed to the reduced pod set and subsequent reduction in number of pods per plant from pathogen infection (Hanounik and Bisri, 1991). Yield increases given by EOs applications came mainly from increases in the weight of individual grains; those yield increases given from better pod retention.

The infection of faba bean plants by *B. fabae* significantly decreased the content of photosynthetic pigments (Chlorophyll a, b, carotenoids and total pigments). The application of the selected EOs or the fungicide Kocide 101 significantly increased the levels of the photosynthetic pigments compared to check treatment. The reduction in the content of photosynthetic pigments in the infected leaves may be due to the effect of the pathogen on the chloroplasts or on chlorophyll content directly or on the enzymes concerned with photosynthesis (Aldesuquy and Baka, 1992). Also, the reduction in chlorophyll content of infected plants may be due to destructive effect of phytopathogens or their metabolites on chloroplasts (Hassan *et al*., 2006) or protein synthesis (Pundir *et al.,* 1991). Such influence might subsequently lead to an inhibition in chlorophyll formation and chloroplast development (Das, 1973). Increasing photosynthetic pigments will expected to increase carbohydrate content in plant tissues. This important action greatly affects pectin that consider a barrier against plant pathogens invasion and phenolic compounds, which play a major and important role in plant defence (Hahlbrock and Scheel, 1989). In addition, the enhancement in chlorophyll content increases disease resistance as well as decreases photophosphorylation rate, which occurred after infection (Amaresh and Bhatt, 1988). In this connection, Rhodes and Wooltorton (1978) indicated that, the adaptation of plants to biotic and abiotic stress is due to the stimulation of protective biochemical systems and synthesis of secondary metabolites such as phenolics.

The present study demonstrated essential oils of rue and thyme as the best inducer for phenol and polyphenol oxidase contents. Hassan *et al*. (2006) suggested that phenolic compounds are implicated in disease resistance and although they are found in healthy as well as in diseased plants, their synthesis or accumulation seems to be accelerated after infection. Phenolics are well known as antimicrobial compounds occur naturally in plants (Sivaprakasan and Vidhyasekaran, 1993). According to Matern and Kneusel (1988), the first step of the defence mechanism in plants involves a rapid accumulation of phenols at the infection site, which restricts or slows the growth of the pathogen. This effect might be due to the impact of these substances on enzymatic activity and translocation of the metabolites to faba bean plant. This accumulation in phenolic compounds in leaves may be due to inhibition of catalase activity, which in turn induces phenylalanine lyase gene expression and synthesis of phenolic compounds (Vermerris and Nicholson, 2006). Yet, total phenols have long been considered as important defence-related compounds whose levels are naturally high in resistant varieties of many crops (Gogoi *et al*., 2001 and Khaleifa *et al*., 2006).

Considering the reduction in the mycelial growth and spore formation *in vitro* and incidence of disease symptoms on treated plants, we concluded that essential oils of thyme, garlic, caraway, cloves and rue could be used as possible biofungicides alternative to synthetic fungicides against chocolate spot fungus. However, further studies need to be conducted to evaluate the cost and efficacy of these essential oils on wide range of diseases.

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تأثير الزيوت الطيارة لبعض النباتات الطبية والعطرية على نمو ومكافحة الفطر *Botrytis fabae* مسبب مرض التبقع الشيكولاتى في الفول

خالد محمد إبراهيم غنيم* ، وسام الدين إسماعيل على صابر ، سـحر محمـود البـاز*****

* قسم بحوث أمراض البذور- معهد بحوث أمراض النباتات- مركز البحوث الزراعية - الجيزة - مصر.

** قسم الميكروبيولوجى- معهد بحوث الأراضي والمياه والبيئة- مركز البحوث الزراعية - الجيزة- مصر.

*** قسم بحوث الفطريات وحصر الأمراض - معهد بحوث أمراض النباتات - مركز البحوث الزراعية – الجيزة - مصر.

تهدف هذه الدراسة إلى إيجاد بدائل لاستخدام المبيدات الفطرية في مكافحة مرض التبقع الشيكولاتي فى الفول المتسبب عن الفطر *Botrytis fabae*. وقد تم دراسة تأثير اثنا عشر نوعاً من الزيوت المستخلصة من أجزاء مختلفة من النباتات الطبية والعطرية في مقاومة الفطر معملياً. أدى استخدام زيتي الزعتر والكراوية إلى حدوث تثبيط تام في نمو وإنتاج الجراثيم للمسبب المرضى عند تركيز (١ميكرولتر/مل)، كما أظهر استخدام زيوت السذب، الثوم والقرنفل تأثير تثبيطي قوى وذلك بتركيزات أعلى نسبياً (٢,٥ ميكرولتر/مل). وتحت ظروف التجريب بالحقل، كان لاستخدام زيوت الزعتر، السذب، الكراوية والثوم تأثير معنوي واضح في وقاية نباتات الفول البلدي من الإصابة الطبيعية بمرض التبقع الشيكولاتي. كما أظهر استخدام زيت الثوم، متبوعا بزيتي الكراوية والقرنفل انخفاض معنوي كبير في حدوث وشدة إصابة نباتات الفول البلدي بالمرض خلال مراحل النمو المختلفة. كما أظهر استخدام زيت الثوم تلاه زيت الكراوية تأثير معنوي في زيادة بعض الصفات الظاهرية (طول النبات وعدد الأفرع وعدد الأوراق لكل نبات) ومكونات المحصول (عدد القرون لكل نبات ووزن ١٠٠ بذرة) لنباتات الفول المعاملة. كما أدى استخدام معاملات الزيوت السابقة إلى حدوث زيادة معنوية في محتوى نباتات الفول من صبغات البناء الضوئي (كلوروفيل أ وكلوروفيل ب والكاروتينيدات) وكذا إنزيم البولي فينول أوكسيديز والفينولات الكلية. وتظهر هذه الدراسة أهمية النتائج المبشرة لاستخدام الزيوت الطيارة كمبيدات حيوية في وقاية نباتات الفول البلدي من الإصابة بفطر *Botrytis fabae*.