

Efficacy of sequential spray of some essential oils against faba bean chocolate spot disease under laboratory and field conditions

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ABSTRACT

Studies were carried out *in vitro* and *in vivo* to determine the antifungal activity of different sequential spray systems of two essential oils; clove and rose in addition to the fungicide Mancozeb against faba bean chocolate spot disease which caused by *Botrytis fabae*. *In vitro*, results revealed that both of essential oils at five tested concentrations; 25, 50, 100, 250 and 500 ppm decreased the mycelial growth of *B. fabae*. Clove and rose oils and Mancozeb at 500 ppm completely inhibited the fungal growth up to 7 days from inoculation. Also, conidia germination % and its germ tube length (μm) decreased by increasing oil concentrations and the best reduction effect was cleared in the case of 500 ppm for both tested oils. Clove oil reduced conidia germination and germ tube length from 0.52 to 79.59 % and from 6.29 to 56.19 %, respectively and at the same time rose oil reduced it from 3.59 to 95.39 % and 29.22 to 66.33 %, respectively according to the oil concentrations used. *In vivo*, faba bean chocolate spot disease severity was decreased in faba bean cultivars; Giza 843, Misr 1, Sakha 1 and Nubaria 1 by using sequential spray system Mancozeb / Mancozeb followed by Rose / Mancozeb and Rose / Rose in both growing seasons. Pods number / plant and seeds number / plant were the highest in case of Mancozeb / Mancozeb followed by Rose / Mancozeb but this sequence was upside down with seeds weight / plant. Also, the other sequential spray systems containing rose oil had promising results.

Keywords: Faba bean, cultivars, chocolate spot, essential oils, clove oil, rose oil.

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the most important leguminous crops in Egypt (Tawadros, 1986). Chocolate spot disease caused by *Botrytis fabae*, Sard., is one of the most common foliar diseases of faba bean in

Northern sector of Egypt. This disease is a major factor contributing to the notorious variation from one season to another in the productivity of bean crop (Hawtin and Hebblethwaite, 1983). Seed yield loss in faba bean due to the disease infection was found to be 58 % (Abd El-Hak *et al.*, 1984). In humid conditions, the fungus becomes more aggressive, spread rapidly and cause necrosis (Leach, 1955) and high yield loss (75 %) (Hebblethwaite, 1983). Many attempts were made to control this disease using non systemic fungicides (Hanounik, 1981; Bainbridge *et al.*, 1985 and Khaled *et al.*, 1995) or fungicides (Elliott and Whittington, 1980; Bainbridge *et al.*, 1985 and Khaled *et al.*, 1995) and each of them gave some degrees of chocolate disease control.

Since World War II, traditional agricultural practices have been replaced by the use of synthetic chemicals for the management of plant pathogens, pests and weeds. This has, no doubt, increased crop production but with some deterioration of environmental quality and human health (Cutler and Cutler, 1999). In addition, to the target pathogen, pesticides may also kill various beneficial organisms and their toxic forms can persist in soil (Hayes and Laws, 1991) at the same time, resistance by pathogens to fungicides has rendered certain fungicides ineffective (Wilson *et al.*, 1997). Because of these problems there is a need to find alternatives to synthetic pesticides. Such products from higher plants and microbes are relatively broad-spectrum, bio-efficacious, economical and environmentally safe and can be ideal candidates for use as agrochemical (Macias *et al.*, 1997 and Cutler, 1999). Among these, essential oils from number of plants have been reported to show activity against wide array of plant pathogenic fungi (Mukherjee, 1974; Singh *et al.*, 1978; Dikshit *et al.*, 1979; Kurita *et al.*, 1981; Omar *et al.*, 1993 and Rice, 1995). The compounds generally were inhibitory to growth and spore germination of the fungi and were potent at very low concentrations (Toxopeus and Bouwmeester, 1992; Isman, 2002; Soliman and Badeaa, 2002; Tripathi and Dubey, 2004 and El-Zemity and Ahmed, 2005). Singh *et al.*, (1980) found that essential oils from *Cymbopogon martini*, *C. oliveri* and *Trachyspermum ammi* exhibited strong antifungal activity against *Helminthosporium oryzae*. In this respect, clove oil and prostanthera oil have fungicidal properties against several pathogenic fungi such as *Aspergillus parasiticus* (Salmeron and Pozo, 1991) and *Cladosporium cucumerinum* (Dellar *et al.*, 1994).

The present work was designed to determine the antifungal activity of different sequential spray systems of clove oil and rose oil in addition to the

fungicide Mancozeb against faba bean chocolate spot disease which caused by *Botrytis fabae*.

MATERIALS AND METHODS

Laboratory and field experiments were carried out in Etay El-Baroud Agric. Res. Station during two successive seasons 2004/2005 and 2005/2006.

laboratory experiments:

Isolation: *Botrytis fabae* was isolated from infected faba bean leaves on faba bean leaf extract agar (FBLEA) medium and identified as *B. fabae*.

Preparation of essential oil concentrations: Clove or rose oil was tested at five concentrations for its fungicidal activity against *B. fabae* using food poison technique (Grover and Moore, 1962). Oil was emulsified using Tween 80 (0.05 %) and used at 25, 50, 100, 250 and 500 ppm in FBLEA medium. Also, Mancozeb® was used as a standard fungicide at the same concentrations mentioned above. Addition of Tween 80 (0.05%) alone to the medium was served as check treatment. Fifteen ml of the medium which contains the tested essential oil or Mancozeb was poured into each sterile Petri dish (90 mm diameter) at 40-45° C under aseptic conditions and left to solidify. Mycelial discs (5mm diameter) of *B.fabae* were taken from 8 days old culture on FBLEA and transferred to the center of Petri dishes after solidification of the medium. Each treatment was repeated three times and incubated at 25°C with photoperiod 12 h light/12 h dark. The antifungal activity was determined by measuring the radial growth (cm) after 3, 5 and 7 days from inoculation. Percentage of mycelial growth inhibition after 7 days was calculated from the formula: Mycelial growth inhibition = $[(dc - dt) / dc] \times 100$ (Pandey *et al.*, 1982) where;

dc= Average of diameter of fungal colony in the check treatment

dt = Average of diameter of fungal colony in the oil treatments

Effect of different concentrations of essential oils on conidia germination and germ tube length: Fifteen ml / dish of FBLEA medium was poured into Petri dishes. Agar plugs were cut with a sterile cork borer (10 mm diameter) and placed into Petri dishes (3 plugs /dish). An aliquat of 30 µl of *B.fabae* suspension was then pipetted onto the surface of each agar plug. Plugs were dried four 10 min followed by application of 30 µl / plug

of the tested essential oil at one of the four concentrations (25, 50, 100, 250 and 500 ppm) which prepared using Tween 80 (0.05%) in sterile distilled water. The Petri dishes were closed immediately and sealed with plastic film and incubated at 25°C and 12 h photoperiod. Sixteen hours after incubation, percentage of conidia germination was determined from counts of 100 conidia selected from a random sweep across the diameter of each agar plug. A conidium was considered germinated if the germ tube length was equal to 1- 1.5 times of the length of the conidium (Antonov *et al.*, 1997). Average germ tube length (μm) was determined on the base of random measurement of five germ tubes on each agar plug.

Field experiments: Field experiments were carried out in a randomized complete blocks design with three replicates. Faba bean cultivars; Giza 843, Misr 1, Sakha 1 and Nubaria 1 were sown, sprayed and evaluated under natural infection conditions during seasons 2004 / 2005 and 2005 / 2006. Each experimental plot (5.4 m²) had three ridges, 60 cm in between and 3 meters long. Planting took place at 15th October at the recommended seed rate (33 plants / m²) in two sides / ridge with 2 seeds / hill, 20 cm a part. Oils were sprayed two times at 1st and 15th February at concentration 0.5 ml/L + 0.3 ml Tween 80 to emulsify the oil and Mancozeb was sprayed at 2.5 g / L. Sequential spray treatment systems were: Clove / Clove, Clove / Rose, Clove / Mancozeb, Rose / Rose, Rose / Clove, Rose / Mancozeb, Mancozeb / Mancozeb and Tween 80 (0.3 ml / L) as check.

Severity of chocolate spot disease due to natural infection was determined just after 15 days from spraying with each oil or the fungicide according to the key of Bernier *et al.*, (1984). At harvest, five guarded plants were taken at random on which the following characters were recorded: pods number / plant, seeds number / plant and seeds weight / plant (g).

Data were statistically analyzed according to Snedecor and Cochran (1981). Treatment means were compared by L.S.D at 5% level of probability.

RESULTS AND DISCUSSION

Within the large reservoir of natural fungicides that exist in plants and microorganisms, it is reasonable that examples exist that would serve as safe and effective alternatives to synthetic fungicides. Such compounds, if properly formulated and applied, could be used directly or could serve as

templates for synthetic analogs (Wilson *et al.*, 1997). Essential oils have long been recognized as having good fungitoxic effects (Singh *et al.*, 1980).

Laboratory experiments: Table (1) and Fig (1) show that clove and rose oils at five concentrations; 25, 50, 100, 250 and 500 ppm significantly decreased the mycelial growth of *B. fabae* on FBLEA medium. This result is in agreement with the findings of Rahhal (1997), Letessier *et al.*, (2001), Ramezani *et al.*, (2002), Plotto *et al.*, (2003) and Oxienham *et al.*, (2005). In this respect, Beg and Ahmad (2002) reported that clove oil possessed fungicidal activity against some phytopathogenic fungi.

They added that above 0.05 %, lysis of conidia and inhibition of mycelial growth were detected and at higher clove oil concentration (10%), up to 20 % of conidia were lysed within 24 h of incubation. Also, Mancozeb showed the same inhibitory effect. From the above table, it is clear that oils and Mancozeb at 25, 50 and 100 ppm reduced the fungal

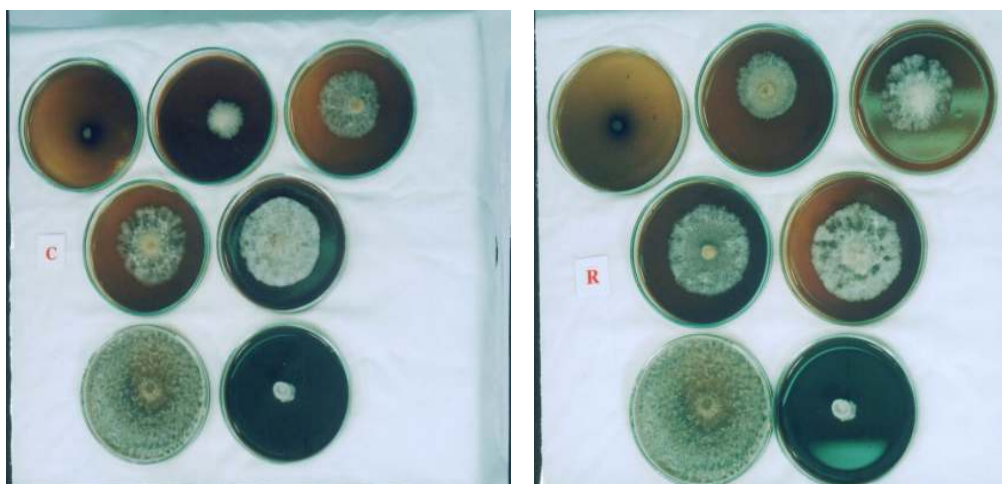


Fig (1): Effect of clove oil (C) and rose oil (R) at five tested concentrations on the growth diameter of *Botrytis fabae* on FBLEA medium after 7 days. First row from left to right 500, 250 and 100 ppm, second row from left to right 50 and 25 ppm, and the third row from left to right 0 ppm (check) and Mancozeb at 500 ppm.

Table (1): Growth diameter (cm) of *Botrytis fabae* after 3, 5 and 7 days from inoculation on FBLEA medium treated with six concentrations of clove, rose oils and Mancozeb (*in vitro*).

Treatment	Days	Concentrations (ppm)						$\bar{x}$
		0	25	50	100	250	500	
Clove	3	3.00	1.90	1.27	0.00	0.00	0.00	1.03
	5	6.57	4.20	3.13	1.40	0.00	0.00	2.55
	7	9.00	5.77	5.27	3.67	1.37	0.00	4.18
		--	(35.89)*	(41.44)	(59.22)	(84.78)	(100.00)	
Mean		6.19	3.96	3.22	1.69	0.46	0.00	
Rose	3	2.60	2.00	1.47	0.00	0.00	0.00	1.01
	5	6.30	3.97	3.23	1.87	1.53	0.00	2.82
	7	8.93	6.00	5.20	4.03	3.03	0.00	4.53
		--	(32.81)	(41.77)	(54.87)	(66.07)	(100.00)	
Mean		5.94	3.99	3.30	1.97	1.52	0.00	
Mancozeb	3	2.80	1.70	0.83	0.63	0.13	0.00	1.02
	5	6.30	3.00	1.67	0.90	0.20	0.00	2.01
	7	8.93	4.23	2.20	1.27	0.27	0.00	2.82
		--	(52.63)	(75.36)	(85.78)	(96.98)	(100.00)	
Mean		6.01	2.98	1.57	0.93	0.20	0.00	

() * Reduction % after 7 days $[(dc - dt)/dc] \times 100$

dc= diameter of fungal growth in check treatment

dt= diameter of fungal growth in the oil or fungicide treatments

	L.S.D 0.05	L.S.D 0.01
O (oil)	0.072	0.095
C (concentrations)	0.101	0.134
D (days)	0.072	0.095
O x C	0.175	0.232
C x D	0.175	0.232
O x D	0.124	0.164
O x C x D	0.304	0.401

growth rate compared to check treatment. In case of 250 ppm, there is no detectable growth with oil treatments until the 3rd day, but slow growth was noticed after the 5th day with clove oil treatment and after the 3rd day with rose oil treatment. On the other hand, Mancozeb at this concentration drastically decreased the fungal growth until the 7th day and this result is in agreement with the finding of Abou-Zeid *et al.* (1990). The average of fungal growth at this concentration with clove, rose and Mancozeb were 0.46, 1.52 and 0.20 cm, respectively. Also, it is clear that the fungal growth

increases with increasing the incubation period and the effect of the two oils used closed together, but the growth rate in case of Mancozeb treatment was slower than in the oil treatments. From these results it can be concluded that oils at the above four concentrations act as fungistatic. In contrast, clove, rose oils and Mancozeb at 500 ppm completely inhibited the fungal growth until the 7th day from inoculation. In this respect, the above data are in agreement with the results obtained by Jobling (2000). He reported that tea tree oil concentrations between 100 and 500 ppm were able to prevent the growth of *B.cinerea in vitro*. Likewise, several essential oils as clove, rose, bergamot, eucalyptus, nioul and lavender (Rahhal, 1997) and caraway, fennel and thyme (El-Zemity and Ahmed, 2005) proved to be very effective oils and gave different inhibitory effects against number of phytopathogenic fungi. Generally, Mancozeb treatment had the best inhibitory effect where it ranged after 7 days between 52.63–100 % growth inhibitions of *B. fabae* followed by clove and rose oils with averages ranged between 35.89-100 % and 32.81-100 %, respectively.

Tables 2 and 3 represent the effect of clove and rose oils at five concentrations (0, 50, 100, 250 and 500 ppm) on germination percentage of *B. fabae* conidia and its germ tube length (μ m). It is noticed that the germination % decreased by increasing the concentration of oils (Table 2). Generally, the highest inhibitory effect was cleared in case of 500 ppm for both tested oils where the reduction % was 79.59 and 95.39 % for clove and rose oils compared to check treatment, respectively. The reduction of conidial germination may be due to the lysis of conidia as mentioned by Beg and Ahmad (2002). The statistical analysis showed that the differences between oils, concentrations and the interaction between them were highly significant. At the same time, Table 3 cleared that germ tube length of *B. fabae* conidia decreased by increasing oil concentrations. Generally, clove oil reduced germ tube length from 6.29 to 56.19 % and rose oil reduction was from 29.22 to 66.33 % according to the oil concentration. The differences between oils, concentrations and the interaction between them were highly significant. These results are in agreement with the findings of Caccioni and Guizzardi (1994); Antonov *et al.*, (1997); Wilson *et al.*, (1997) and Letessier *et al.*, (2001) who tested different essential oils against spore germination and germ tube length of a wide range of plant pathogenic fungi.

Table (2): Germination % of *Botrytis fabae* conidia at five concentrations of the tested essential oils after 16 h from incubation on PDA at 20°C.

Oils	Concentrations (ppm)					$\bar{x}$
	0	50	100	250	500	
Clove	96.75	96.25	80.75	64.74	19.75	71.65
	--	(0.52)*	(16.54)	(33.08)	(79.59)	
Rose	97.50	94.00	84.50	37.00	4.50	63.50
	--	(3.59)	(13.33)	(62.05)	(95.39)	
Mean	97.13	95.13	82.63	50.88	12.13	

()* Reduction % = $[(gc - gt) / gc] \times 100$

Where; gc= germinated conidia in check

gt = germinated conidia in oil treatments

L.S.D 0.05 L.S.D 0.01

O (oil)	2.42	3.27
C (concentrations)	1.91	5.18
O x C	5.42	7.32

Table (3): Mean germ tube length (µm) of *Botrytis fabae* conidia at five concentrations of some essential oils after 16 h from incubation on PDA at 20°C.

Oils	Concentrations (ppm)					$\bar{x}$
	0	50	100	250	500	
Clove	19.40	18.18	14.18	12.53	8.50	1.46
	--	(6.29)*	(26.91)	(35.41)	(56.19)	
Rose	19.75	13.98	10.00	8.48	6.65	1.18
	--	(29.22)	(49.37)	(57.06)	(66.33)	
Mean	19.58	16.08	12.09	10.51	7.58	

()* Reduction % = $[(lc - lt) / lc] \times 100$

lc= conidia germ tube length of check treatment

lt= conidia germ tube length of oil treatments

L.S.D 0.05 L.S.D 0.01

O (oil)	0.070	0.094
C (concentrations)	0.110	0.149
O x C	0.156	0.210

Field experiments: Tables 4 and 5 represent chocolate spot disease severity as a response to sequential spray systems of clove and rose oils in addition to Mancozeb compared to check treatment in the two growing seasons. Generally, sprayed faba bean cultivars with Mancozeb two times, 15 days

intervals reduced significantly chocolate spot severity (Table 4) and it had the first grade with an average 2.08 %. The fungicidal effect of Mancozeb on this disease also found by Yeoman *et al.*, (1987).

Table (4): Efficacy of sequential spray of the tested essential oils on severity of chocolate spot disease on some faba bean tested cultivars under natural field infection during (2004/2005) growing seasons.

Cultivars	Giza 843		Misr 1		Sakha 1		Nubaria 1		— x
Record Oils	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	
Clove/Clove	1.99	3.26	2.27	3.24	2.00	3.14	2.17	3.09	2.65
Rose/Rose	1.69	2.89	2.07	2.90	2.32	3.02	1.89	2.64	2.43
Clove/Rose	2.10	2.79	2.46	3.15	2.43	3.27	2.25	2.70	2.64
Clove/Mancozeb	2.00	2.58	2.11	2.65	2.25	2.96	2.13	2.78	2.43
Rose/Clove	2.51	3.76	2.50	3.39	1.84	2.98	2.08	3.08	2.77
Rose/Mancozeb	2.23	2.70	2.25	2.76	1.96	2.80	2.03	2.61	2.42
Mancozeb/Mancozeb	1.66	2.39	2.05	2.52	1.51	1.94	1.95	2.64	2.08
Check	2.41	4.58	3.72	5.09	2.43	3.59	2.62	4.52	3.62
Mean	2.07	3.12	2.43	3.21	2.09	2.96	2.14	3.01	

	L.S.D 0.05	L.S.D 0.01
O (oil)	0.21	0.28
C (cultivars)	0.15	0.20
R(record)	0.11	0.14
O x C	0.06	0.57
C x R	N.S	N.S
O x R	0.30	0.40
O x C x R	N.S	N.S

The sequential spray treatment; Rose / Mancozeb had the second grade with an average 2.42 % followed by Clove / Mancozeb and Rose/Rose with the same average (2.43 %). Mancozeb / Mancozeb, Clove / Mancozeb and Rose / Mancozeb treatments had the best grades respectively in case of Giza 843 and Misr 1, but this arrangement differed lightly in case of Sakha 1 and Nubaria 1. The other sequential spray treatments also decreased disease severity with different values. This result is in agreement with the findings of Singh *et al.*, (1980) who reported that essential oils have long been recognized as having good fungitoxic compounds. Results in Table (5) show the efficacy of sequential spray systems in the second season and it clear that Mancozeb / Mancozeb, Rose / Mancozeb and Rose / Rose had the first three grades as it showed in the first season and the other essential oil sequential spray treatments followed it with light differences in its

arrangements than in the first season, but it decreased significantly the disease severity compared to check treatment.

Table (5): Efficacy of sequential spray of the tested essential oils on severity of chocolate spot disease on some faba bean tested cultivars under natural field infection during (2005/2006) growing seasons.

Cultivars	Giza 843		Misr 1		Sakha 1		Nubaria 1		— x
Record	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	
Oils									
Clove/Clove	1.77	2.83	1.47	2.63	1.07	2.07	0.97	1.73	1.82
Rose/Rose	1.97	2.20	1.43	2.17	1.47	2.07	1.10	1.83	1.78
Clove/Rose	1.77	2.67	1.53	1.93	1.53	2.47	1.30	2.27	1.93
Clove/Mancozeb	1.93	2.20	1.73	2.63	1.70	2.20	1.20	1.73	1.92
Rose/Clove	1.73	2.40	1.76	2.47	1.73	2.67	0.97	1.50	1.90
Rose/Mancozeb	1.53	2.03	1.57	2.70	1.43	2.17	0.83	1.37	1.70
Mancozeb/Mancozeb	1.23	1.70	1.67	2.43	1.23	1.87	1.00	1.60	1.59
Check	2.00	3.43	2.97	3.80	2.73	3.37	2.33	3.72	3.04
Mean	1.74	2.43	1.77	2.60	1.61	2.36	1.21	1.97	

	L.S.D 0.05	L.S.D 0.01
O (oil)	0.14	0.19
C (cultivars)	0.10	0.13
R(record)	0.07	0.09
O x C	0.28	0.37
C x R	N.S	N.S
O x R	0.20	N.S
O x C x R	0.39	0.52

The different arrangement between the sequential spray treatments in the two seasons may be due to the influence of the environmental conditions (Antonov *et al.*, (1997).

Results of agronomic characters of the tested faba bean cultivars due to sequential spray systems of clove, rose oils and Mancozeb in the first season is presented in Table (6). Pods number / plant, seeds number / plant and seeds weight / plant were the highest in case of Mancozeb / Mancozeb treatment followed by Rose / Mancozeb, Rose / Rose and Clove / Rose. This means that faba bean cultivars which received rose oil at the first, the second spray or at both had the best grades. Generally, The highest pods number/plant for all faba bean tested cultivars was cleared in case of Mancozeb / Mancozeb treatment followed by Rose / Mancozeb, Rose/Rose treatments with averages of 15.94 , 15.25 and 13.85 and in case of seeds

Table (6): Efficacy of different sequential spray systems of the tested essential oils on some agronomic characters of faba bean tested cultivars under field conditions during (2004/2005) growing seasons.

Parameters	Pods number /plant					Seeds number /plant					Seeds weight / plant (g)									
	Giza	Musr	Sakha	Nubaria	- x	Giza	Musr	Sakha	Nubaria	- x	Giza	Musr	Sakha	Nubaria	- x					
Cultivars	843	1	1	1	1	843	1	1	1	1	843	1	1	1	1					
Oils																				
Clove/Clove	10.90	11.80	10.73	9.73	10.79	28.60	27.73	33.50	32.80	30.66	268.27	247.93	314.00	369.99	300.05					
Rose/Rose	13.93	16.57	14.37	10.53	13.85	36.37	38.53	42.50	35.53	38.23	340.80	308.73	383.07	398.93	357.88					
Clove/Rose	12.13	14.63	13.73	10.67	12.79	31.70	34.37	44.80	35.67	36.64	297.37	342.93	315.43	398.93	338.67					
Clove/Mancorab	13.47	13.27	13.17	10.20	12.53	35.40	31.23	41.17	34.20	35.50	331.50	277.33	365.90	383.87	339.65					
Rose/Clove	13.50	16.67	15.03	9.93	13.78	35.60	39.53	46.87	33.50	38.88	333.43	352.87	395.50	377.70	364.88					
Rose/Mancorab	15.33	17.03	17.10	11.53	15.25	40.47	39.97	53.07	38.87	43.10	379.77	357.20	399.20	438.03	393.55					
Mancorab/Mancorab	16.10	16.80	16.33	12.53	15.94	42.53	39.77	56.73	42.10	45.28	398.83	353.90	421.03	474.20	411.99					
Check	8.97	10.33	10.83	8.83	9.74	23.60	24.60	33.93	29.70	27.96	221.50	217.90	279.00	334.57	263.24					
Mean	13.04	14.64	14.16	10.49		34.28	34.47	44.07	35.30		321.43	307.35	359.14	397.03						
	LSD 0.05					LSD 0.01					LSD 0.05					LSD 0.01				
0 (oils)	0.53					0.70					1.97					18.86				
C (cultivars)	0.37					0.50					1.40					13.34				
0 x C	1.06					1.40					3.94					37.72				

number/plant also, Mancozeb/Mancozeb, Rose/Mancozeb and Rose/Clove treatments were the best with averages of 45.28, 43.10 and 38.88 and the same results were cleared in case of seeds weight/plant with averages of 411.99, 393.55 and 364.88, respectively. Mancozeb/Mancozeb treatment increased agronomic characters by reducing disease incidence. In case of the sequential spray systems which contain rose oil, the increment of the above agronomic characters is in agreement with the findings of Currah and Ockendon, (1984) and Doweker *et al.*, (1985) Also, Al-Sahaf, (2002) mentioned that spraying onion plants with rose water (0.01% rose oil) three times increased the number of visiting insects to the flowers and they spent long periods foraging or grooming, so the flower set percent was elevated and this reflect on the number of seeds, plant seed yield (g/plant) and total seed yield (kg / ha). Table (7) shows the results of the second growing season and it close to that of the first one but with light differences in the arrangement of some treatments. The first and second grades were found between Mancozeb/Mancozeb and Rose/Mancozeb treatments. Mancozeb/Mancozeb had the first grade in case of pod number / plant and seed number/plant with averages for all cultivars 17.18 and 49.21, respectively and Rose/Mancozeb had the second grade with averages 15.47 and 47.59, respectively. Seed weight/plant was the highest in case of Rose/Mancozeb followed by Mancozeb/Mancozeb with averages 457.83 and 439.00, respectively and the sequential spray systems contains rose oil come next.

Based on the present study, it could be concluded that the tested essential oils; clove and rose, according to its concentrations, possess fungistatic or fungitoxic activities worth exploiting for the bio-management of plant diseases. These can serve as natural fungicides or at least templates for the synthesis of novel fungicides.

Table (7): Efficacy of different sequential spray systems of the tested essential oils on some agronomic characters of faba bean tested cultivars under field conditions during (2005/2006) growing seasons.

Parameters	Yield number /plant					Seeds number /plant					Seeds weight /plant (g)				
	Giza	Misr	Salda	Nubaria	-	Giza	Misr	Salda	Nubaria	-	Giza	Misr	Salda	Nubaria	-
Cultivars	843	1	1	1	x	843	1	1	1	x	1	1	1	1	x
Oils															
Clove/Clove	12.83	13.30	12.60	9.47	12.05	32.23	33.30	45.37	32.50	35.85	326.33	282.00	453.67	328.67	347.67
Rose/Rose	15.40	19.67	15.60	10.47	15.29	40.57	45.40	51.03	34.60	42.90	361.67	364.33	487.67	418.00	407.92
Clove/Rose	13.07	15.93	16.63	10.30	13.98	35.03	35.60	48.23	35.57	38.61	336.33	337.67	453.67	421.67	387.34
Clove/Mancorsh	14.37	14.07	14.83	11.90	13.79	41.23	33.33	42.80	37.07	38.61	343.00	344.00	387.67	466.33	385.25
Rose/Clove	14.90	19.20	16.37	11.57	15.51	38.07	46.73	49.83	36.33	42.74	342.33	352.33	498.33	430.00	405.75
Rose/Mancorsh	13.70	17.20	17.27	13.63	15.47	45.50	48.13	58.57	44.63	47.59	405.33	351.00	523.67	476.00	457.83
Mancorsh/Mancorsh	17.10	18.33	19.67	13.70	17.18	39.13	44.97	53.57	52.67	49.21	360.33	409.33	493.67	567.00	439.00
Check	11.37	12.53	11.37	8.57	10.96	27.67	23.30	37.53	28.33	29.21	303.33	262.33	337.67	300.67	301.00
Mean	14.09	16.26	15.59	11.20		37.43	38.85	48.37	37.71		347.33	337.87	454.50	426.04	
	LSD 0.05					LSD 0.01					LSD 0.05				
0 (oils)	0.86					1.14					15.60				
C (cultivars)	0.61					0.81					11.03				
0 x C	1.71					2.28					31.21				

REFERENCES

- Abd El-Hak, T. K.; F. El-Raies Mansour and Ikhlas, Shafik (1984). An appraisal of losses caused by chocolate spot (*Botrytis fabae*) of faba bean. Proc. 6th Congress. In: Phytopathology Mediterranean. Cairo, Egypt.
- Abou-Zeid, N. M.; M. S. H. Moustafa; A. M. Hassanien and I. Ez-Eldin (1990). Control of chocolate spot disease of faba bean and the effect of fungicides on the behavior of the causal fungus. Agric. Res. Rev. 68 (3): 411- 421.
- Al-Sahaf, F. H. (2002). Effect of planting method and rose water spray on seed production in onion (*Allium cepa* L.). Emir. J. Agric. Sci. 14: 14-23.
- Antonov, A.; A. Stewart and M. Walter (1997). Inhibition of conidium germination and mycelial growth of *Botrytis cineria* by natural production. Proc. 50th N. Z. Plant Protection Conf. 159-164.
- Bainbridge, A.; B. D. L. Fitt; N. F. Creighton and G. R. Cayleyl (1985). Use of fungicides to control chocolate spot (*Botrytis fabae*) on winter field bean (*Vicia faba*). Plant Pathology, 34: 5-10.
- Beg, A. Z. and I. Ahmed (2002). *In vitro* fungitoxicity of the essential oil of *Syzygium aromaticum*. World Journal of Microbiology and Biotechnology 18 (4): 317-319.
- Bernier, C. C.; S. B. Hanounik; M. M. Hussein and H. A. M. Hamed (1984). Field manual of common faba bean diseases in the Nile Valley. ICARDA Information Bull. No.3.
- Caccioni, D. R. L. and M. Guizzardi (1994). Inhibition of germination and growth of fruit and vegetable post harvest pathogenic fungi by essential oil components, J. Essential Oil Res. 6 (2): 173-179.
- Currah, L. and D. J. Ockendon (1984). Pollination activity by blowflies and honeybees on onions in breeders cages. Ann. Appl. Biol., 105 (1): 167-176.

- Cutler, H. G. (1999). Allylopathic in the biological control of plant diseases. In: Macias, F. A.; J. C. B. Galindo; J. M. G. Molinillo and H. G. Cutler ed. Recent advance in allelopathy. Vol. 1. A Science for the future. Servicio de Publications, Universidad de Cadiz, Spain. Pp. 397-414.
- Cutler, H. G. and S. J. Cutler (1999). Biological active natural products: Agrochemicals. CRC Press, Boca Raton, USA. 299 p.
- Dellar, J. E.; M. D. Cole; A. I. Gray; S. Gibbons and P. G. Waterman (1994). Antimicrobial sesquiterpenes from *Prostanthera melissifolia* and *P.rotundifolia*. Phytochem. 36 (4): 957-960.
- Dikshit, A.; A. K. Singh; R. D. Tripathi and S. N. Dixit (1979). Fungitoxic and phytotoxic studies of some essential oils. Bio. Bull. India, 1: 45-51.
- Dowker, B. D.; L. Crrah; J. F. Horobin, J. C. Jacson and G. J. Faulkner (1985). Seed production of an F1 hybrid onion in polyethylene.J.Tunnels, Hort.Sci.,60(2): 251-256.
- El-Zemity, S. R. and Soad, M. Ahmed (2005). Antifungal activity of some essential oils and their major chemical constituents against some phytopathogenic fungi. J. Pest Cont. & Environ. Sci. 13 (1): 61-72.
- Elliott, J. E. M. and W. J. Whittington (1980). The control of chocolate spot (*Botrytis fabae*) infection of field beans (*Vicia faba* L.) by the fungicides Benlate, Bavistin, Carceobin and BAS352F. Journal of Agricultural Science 94,461-464.
- Grover, R. K. and J. D. Moore (1962). Toxicometric studies of fungicides against the brown rot organisms *Sclerotinia fructivola* and *S.laxa*. Phytopathology, 52: 876-880
- Hanounik, S. (1981). Influence of Ronilan on the severity of chocolate spot and yield of faba bean. Faba Bean Information Service, News-Letter, 3: 50-51.

- Hawtin, G. C. and P. D. Hebblethwaite (1983). Background and history of faba bean production. In: The Faba Bean (*Vicia faba* L.) . Ed. P. D. Hebblethwaite, pp 3-33. Butter Worth London.
- Hayes, W. J. and E. R. Laws (1991). Handbook of Pesticide Toxicology. Vol. 1. Academic Press, New York. Pp. 55-56.
- Hebblethwaite, P. D. (1983). The faba bean (*Vicia faba* L.). Butter worth. London, pp 573.
- Isman, M. (2000). Plant essential oils for pest and disease management. Crop Protection, 19, 8-10: 603-608.
- Jobling, Jenny (2000). Essential oils: A new idea for post harvest disease control. Good and Vegetable magazine 11 (3): 50, August (Melbourne, Australia).
- Khaled, A. A.; Samiha, M. H. Abd El-Moity and S. A. M. Omar (1995). Chemical control of some faba bean diseases with fungicides. Egypt. J. Agric. Res., 73 (1): 45-56.
- Kurita, N.; Makoto; R. Kurane and Y. Takahara (1981). Antifungal activity of components of essential oils. Agric. Biol. Chem. 45: 945-952.
- Leach, R. (1955). Recent observation of the *Botrytis* infection of beans. Trans. Brit. Mycol. Soc. 38: 171.
- Letessier, M. P.; K. P. Svoboda and D. R. Walters (2001). Antifungal activity of the essential oil of Hyssop (*Hyssopus officinalis*). Journal of Phytopathology. 149: 11-12, 673.
- Macias, F. A; D. Castellano; R. M. Oliva; P. Cross and A. Torres (1997). Potential use of allelopathic agents as natural agrochemicals. Brighton Crop Prot. Conf. Weeds:33-38.
- Mukherjee, N. (1974). Antifungal activities of some oils and detergents. 1- Action on spore germination, growth and sporulation of some plant pathogenic fungi. Z.Pflkrankh. PflSchutz., 81 (8): 468-471.

- Omar, S. A.; M. M. Rahhal; A. A. Hilal and A. Zayed (1993). Influence of intercropping of broad bean with some medicinal plants on disease severity of chocolate spot and rust and seed yield. 4th Conf. Agric. Dev. Res., Ain-Shams Univ., Cairo, Feb. 13-18.
- Oxienham, S. K.; K. P. Svoboda and D. R. Walters (2005). Antifungal activity of the essential oil of Basil (*Ocimum basilicum*). Journal of Phytopathology, 153: 3, 174-180.
- Pandey, D. K.; N. N. Tripathi; R. D. Tripathi and S. N. Dixit (1982). Fungitoxic and phytotoxic properties of the essential oil of the *Hyptis suaveolens*. Z. Pflkrankh. PflSchutz., 89 (6): 344-349.
- Plotto, A.; D. Roberts and R. G. Roberts (2003). Evaluation of plant essential oils as natural post harvest disease control of tomato (*Lycopersicon esculentum*). Acta Horticulturae, 628: 737-745.
- Rahhal, M. M. H. (1997). Antifungal activities of some plant oils. Alex, Sci. Exch., 18 (2): 225-230.
- Ramezani, H.; H. P. Singh; D. R. Batish; R. K. Kohli and J. S. Dargan (2002). Fungicidal effect of volatile oils from *Eucalyptus citriodora* and its major constituent citronellal. New Zealand Plant Protection. 55: 327-330.
- Rice, E. L. (1995). Biological control of weeds and plant diseases. University of Oklahoma Press, Norman. 439 p.
- Salmeron, J. and R. Pozo (1991). Effect of cinnamon (*Cinnamomum zeylanicum*) and clove (*Eugenia caryophyllus*) on growth and toxigenesis of *Aspergillus* gr *flavus*. Microbiologie Aliments Nutrition, 9(1): 83-87.
- Singh, A. K.; S. C. Tripathi and S. N. Dixit (1978). Fungitoxicity of volatile fractions of some angiospermic plants. Proc. Symp. Environmental Science & Human Welfare, Ujain, p. 13.
- Singh, A. K.; A. Dickshit; M. L. Sharma and S. N. Dixit (1980). Fungitoxic activity of some essential oils. Econ. Bot. 34: 186-190.

- Snedecor, G. W. and W. G. Cochran (1981). Statistical Methods. Seventh Edition, Iowa State Univ. Press, Ames, Iowa, USA.
- Soliman, K. M. and R. I. Badeaa (2002). Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food and Chemical Toxicology, 11: 1669-1675.
- Tawadros, H. W. (1986). Effect of irrigation at different stages of growth on the yield and water use by faba beans. FABIS Newsletter No. 14, April 22-25.
- Toxopeus, H. and J. Bouwmeester (1992). Improvement of caraway essential oil and carvone production in the Netherlands. Industrial Crops Product, 2-4: 295-301.
- Tripathi, P. and N. Dubey (2004). Exploitation of natural products as alternative strategy to control post harvest fungal rotting of fruit and vegetables. Post Harvest Biology and Technology. 32, 3: 235-245.
- Wilson, C. L.; J. M. Solar; A. El-Ghauth and M. E. Wisniewski (1997). Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. Plant Disease, 2: 204-210.
- Yeoman, D. P.; D. H. Lapwood and J. M. Ewen (1987). Effect of a range of fungicides used to control rust (*Uromyces vicia faba*) on spring sown field field beans (*Vicia faba*) in the UK. Crop Protection, 6: 90-94.

فاعلية الرش المتعاقب لبعض الزيوت الطيارة لمكافحة مرض التبقع الشيكولاتي في الفول البلدي في المعمل والحقل

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أجريت الدراسة لتقدير التأثير التضادي لزيت القرنفل وزيت الورد بالإضافة للمبيد الفطري مانكوزيب باستخدام تركيزات مختلفة علي نمو والنسبة المئوية لانبات وطول أنبوبة إنبات كونيديا فطر بوترايتس فابي معمليا. كذلك درس حقليا تأثير بعض نظم الرش المتعاقب لزيت القرنفل وزيت الورد بالإضافة للمبيد الفطري مانكوزيب لمكافحة مرض التبقع الشيكولاتي علي أربعة أصناف من الفول البلدي هي: جيزه 843 ، مصر 1، سخا 1 ، نوباريه 1 حقليا خلال الموسمين 2004 / 2005 ، 2005 / 2006 .

أثبتت النتائج المعملية أن التركيزات المستخدمة من الزيوت المختبره ومبيد مانكوزيب وهي 25، 50، 100، 250 جزء في المليون قللت النمو الميسليومي للفطر. كما أن زيت القرنفل وزيت الورد والمانكوزيب بتركيز 500 ج ف م أدى إلي تثبيط تام للنمو الفطري حتي 7 أيام من التحضين. كذلك أنقص زيت القرنفل النسبة المئوية لانبات الكونيديات وطول أنبوبة الإنبات لها بنسبه تتراوح بين 52، 59 – 79% و لزيت الورد بين 29، 6 – 19، 56% علي الترتيب . وجد تحت الظروف الحقلية أن نظام الرش المتعاقب مانكوزيب/مانكوزيب قد أنقص شدة الإصابة بالتبقع في الأصناف المستخدمة من الفول البلدي ثم تلاه نظامي الرش زيت الورد/ مانكوزيب وزيت الورد/ زيت الورد خلال كلا موسمي الزراعه. أيضا زاد عدد القرون/ نبات و عدد البذور/ نبات في النظامين مانكوزيب/الأعلي يليه مانكوزيب ، زيت الورد/ مانكوزيب . أما في حالة وزن البذور/ نبات فكان نظام الرش زيت الورد/ مانكوزيب مانكوزيب/مانكوزيب. وبصورة عامه فقد لوحظ أن النظم المتعاقبه الأخرى والمحتويه علي زيت الورد أعطت نتائج مبشره.