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Oviposition deterring and antifeeding activities of certain essential oils of medicinal plants against *Pieris rapae* (Lepidoptera: Pieridae)

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Abstract:

Cabbage (*Brassica oleracea* var. capitata L.) is one of the most important cruciferous in Egypt. Cabbage white butterfly *Pieris rapae* L. (Lepidoptera: Pieridae) consider a serious pest affecting cabbage production. The present study was conducted to evaluate the repellent, antifeedant and oviposition deterrent effects of eight essential oils on *P. rapae* under laboratory conditions. Data revealed that all tested oils affected on oviposition and feeding of *P. rapae* and their effects were concentration dependent. At 1000 µg/L, mint and fenugreek oils showed the highiest repellent rates with 73.89 and 77.83%, respectively, with insignificant differences between them. Also, the highest antifeedant effect was recorded in garlic oil (87.21%) in bar with mint oil (86.93%) and fenugreek oil (79.13%). At 1000 µg/L, mint oil reduced oviposition of *P. rapae* females by 91.97% followed insignificantly by thyme oil (84.26%) and garlic oil (76.71%) in choice test. In case of no choice test, the highest oviposition deterrence index was obtained in mint (69.36%), followed insignificantly by thyme (66.46%) and garlic (57.01%). So, the application of garlic, mint and thyme oils can be combined with different biological and agricultural methods in an integrated pest management program could reduce the use of synthetic insecticides, especially in the early stage of cabbage.

Introduction

Cabbage or headed cabbage (*Brassica oleracea* var. capitata L.) is one of the most important cruciferous crops which cultivated with the aim of using leaves as human food during the various seasons. Cabbage white butterfly *Pieris rapae* L. (Lepidoptera: Pieridae) is cruciferous specialist phytophagous and consider as a serious pest affecting cabbage production in Egypt, the feeding injury caused by its larvae may reduce production to zero (Awadalla *et al.*, 2013 and Embaby and Lotfy, 2015). In conventional agriculture, farmers use pesticides to control *P. rapae*, however, with the increasing popularity of organic farming in Egypt, there is a need to use environment-friendly tools to overcome the problem of insect pests. One of the most important and crucial event in the life cycle of Lepidoptera is the selection of a suitable site for oviposition and larval feeding. The oviposition deterrents and antifeedants have attracted a lot of research in the recent years as the first line defense against insect infestation (Ikeura *et al.*, 2012; Kordan and Gabryś, 2013; Ali *et al.*, 2017 and Zhang *et al.*, 2017). Essential oils are secondary metabolic products of plant which are volatile, natural and complex compounds. Essential oils can act as toxins, antifeedants and oviposition deterrents to many insect pests (Pavela, 2005; Yazdani *et al.*, 2014 and Kumari and Kaushik, 2016). The present study was conducted to evaluate the effects of 8 essential oils on oviposition and feeding of *P. rapae* under laboratory conditions.

Materials and methods

All experiments were conducted in the Laboratory of Plant Protection, Shandweel Agricultural Research Station, Sohag Governorate at room conditions during November and December 2019, where ambient temperature ranged between 10 and 25 ºC, and RH.% varied from 44% to 50%.

1. Insect culture:

The stock culture of *P. rapae* was maintained under laboratory conditions. For this purpose, larvae were collected from the cabbage crop in the Farm of Shandaweel Agriculture Research Station and reared in plastic cups 15 cm diameter with 10 cm in deep and fresh cabbage leaves were provided daily to the larvae till pupation. After pupation, the pupae were transferred to a wooden rearing cage (1x1x1 meter). When adults emerged, a small cotton-wool wick soaked in 10% honey solution and placed in the cage as a source of carbohydrate for adults. Cabbage plants at eight to ten-leaf stage were placed inside cage for oviposition. Cabbage leaves with eggs collected and kept in petri dish (9 cm diameter) the filter papers. Newly hatched larvae were transferred to plastic cups.

2. Essential oils:

The mainline value in the selection of the tested essential oils was their commercial availability and their phylogenetic distance to Brassicaceae on the angiosperm. Oils were obtained as ready-made oil from El-Captain Company for Extraction of Natural oils, Plants and Cosmetics, Cairo, Egypt. The essential oils of garlic (*Allium sativum* L.: Liliaceae), mint (*Mentha piperita* L.: Labiatae), thyme (*Thymus vulgaris* L.: Lamiaceae), camphor (*Cinnamomum camphora* L.: Myrtaceae), colocynth (*Citrullus colocynthis* L.: Cucurbitaceae), cumin (*Cuminum cyminum* L.: Apiaceae), fenugreek (*Trigonella foenum graecum* L.: Fabaceae) and orange (*Citrus sinensis* L.: Rutaceae) at three concentrations were used for each oil (250, 500 and 1000 µg/L).

3. Repellent activity:

Repellent assay was conducted on two cabbage leaf disks (3 cm diameter) on petri dish (15 cm diameter), one of them was dipped for 10 seconds in oil solution at the required concentration and the second distilled in water only as a control. The 4^{th} instar larva was placed between the two disks and the disc chosen by the larva was noted. The experiment replicated six times (replicate include 10 larvae). The repellent rate is expressed according to the formula of Ikeura *et al.* (2012) as follows: Repellent rate = 100 (T / (T+C)), where T and C are the mean number larvae choosing treated and control discs, respectively.

4. Antifeedant activity:

Antifeedant activity was evaluated using a leaf disc bioassay in no choice test. Leaf discs (15.40 cm^2) were cut from fresh cabbage leaves and soaked in solutions with three concentrations of different oils for 30 seconds (control discs received distilled water only), then dried at room temperature. The fourth instar larvae were starved for 8 h. and introduced singly into the center of each petri dish. Each treatment was repeated five times. After 24 hours, the area of feeding on the leaves was measured using LI-3000A Portable Area Meter. The antifeeding index (AFI%) was calculated using the formula: AFI% = [(C - T)/(C + T)] x 100, where C and T are the areas consumed by the control and treated leaf disks, respectively (Zhang *et al.*, 2017).

5. Oviposition deterring activity:

Two different assays were used, choice and no choice tests were adopted to evaluate the effect of eight oils at three concentrations on the oviposition of *P. rapae*. The two experiments were conducted using wooden cage (60x60x60 cm). Each cage was supplied with a piece of cotton soaked in 10% honey aqueous solution to facilitate feeding. A replicate consisted of one cage with three graved females (five days old) and six replications were performed for each bioassay. In choice test, two cabbage plants of 6–8 leaves were put in each cage, one of them was sprayed with one of the eight studied oil at the required concentration and the other was sprayed with water as control. However, in no choice test, each cage contained one plant with same treatment. The eggs were counted after 24 hours and the oviposition deterrent index (ODI%) was calculated with formula of Huang *et al.* (1995) as follows: ODI% = 100 {(C-T)/(C+T)}, where C and T were the mean number of eggs laid on control and treated plants, respectively.

6. Statistically analysis:

The data of deterrence rate, antifeeding index (AFI) and oviposition deterrent index (ODI) were statistically analysis using one-way analysis of variance (ANOVA). The differences between concentrations were subjected by L.S.D. test, however, Duncan Multiple Range Test was used to find significant differences in the activity among the studied oils **(Snedecor, 1956)**.

Results and discussion

1. Repellent activity:

Data in Table (1) showed that the tested oils differed significantly in regardless to concentration. Also, the same results were obtained for concentration in regardless to oil type. The highest and the lowest repellent activity were recorded in 1000 µg/L and 250 µg/L concentrations, respectively. It is clear that the repellent activity was concentration dependent. At low concentration (250 µg/L), no repellent effects were observed in all oils (FDI < 50%). However, in 500 µg/L and 1000 µg/L concentrations, only mint and fenugreek oils showed repellent rates more than 50%, with 63.66% and 59.13%, respectively, at 500 µg/L and 73.89 and 77.83%, respectively, at 1000 µg/L, with insignificant differences between them. No significant difference was found between 1000 µg/L and 500 µg/L for the two oils. The previous results are in agreement with Ikeura *et al.* (2012) who found that spearmint have a notable feeding repellent effect against *P. rapae* larvae with 72%. Also, the repellent effect of fenugreek oil against insect was reported by Meghwal and Goswami (2012).

Table (1): Repellent and antifeedant activities of eight essential oils against *Pieris rapae* larvae.

Oil	Repellent rate% 250 µg/L	500 µg/L	1000 µg/L	F. value	L.S.D.	Antifeedant index% 250 µg/L	500 µg/L	1000 µg/L	F. value	LSD
Garlic	19.11**b BC**	39.09**ab B**	48.15**a B**	4.88	20.27	24.66**b B**	76.64**a A**	87.21**a A**	65.8	12.72
Mint	32.08**b AB**	63.66**a A**	73.89**a A**	8.67	22.31	27.49**b AB**	71.90**a A**	86.93**a A**	33.3	16.67
Thyme	12.22**b C**	27.51**ab B**	44.18**a B**	7.07	18.13	24.96**c B**	50.34**b BC**	66.11**a C**	31.5	11.53
Camphor	16.80**b BC**	23.60**b B**	37.42**a B**	7.48	11.58	35.46**b A**	64.09**a AB**	72.51**a BC**	19.7	13.42
Colocynth	18.58**b BC**	24.43**b B**	37.50**a B**	5.65	12.28	25.94**c AB**	46.47**b C**	73.76**a BC**	57.0	9.71
Cumin	20.73**b BC**	24.71**b B**	47.02**a B**	6.53	16.72	14.22**c C**	22.84**b D**	36.44**a D**	23.5	7.10
Fenugreek	36.51**b A**	59.13**ab A**	77.83**a A**	4.01	31.13	19.33**c BC**	51.79**b BC**	79.13**a AB**	74.4	10.73
Orange	18.19**b BC**	21.46**b B**	34.92**a B**	5.78	11.12	20.41**b BC**	26.91**b D**	41.71**a D**	14.9	8.81
F. value	**2.77**	**7.34**	**4.92**	**---**	**---**	**3.92**	**17.60**	**36.4**		

Mean in the same column sharing similar capital letters are not significantly different by Duncan Test at P-0.05
Mean in the same row sharing similar small letters are not significantly different L.S.D. Test at P-0.05

2. Antifeedant activity:

Data in Table (1) revealed that the differences between oils were significant in regardless to concentration. Also, the effect of oil concentration was significant in all tested materials. The highest and the lowest antifeedant activity were recorded in 1000 µg/L and 250 µg/L concentrations, respectively. It is clear that the antifeedant activity was concentration dependent. The present study showed that all tested oils decreased the leaf area consumed by single larvae at the three concentrations (Figure, 1). At low concentration (250 µg/L), no antifeeding effects were observed from all tested oils (AFI < 50%). With concentration increase (500 µg/L), high antifeeding effects were recorded in garlic (76.64%), mint (71.90%) and camphor (64.09%), with insignificant differences between them. Also, thyme and fenugreek gave 50.34% and 51.79%, respectively. At high concentration (1000 µg/L), only cumin and orange did not have antifeedant effects. The highest AFI was recorded in garlic oil (87.21%) in bar with mint oil (86.93%) and fenugreek oil (79.13%), followed significantly by colocynth oil (73.76%), camphor oil (72.51%) and thyme oil (66.11%).

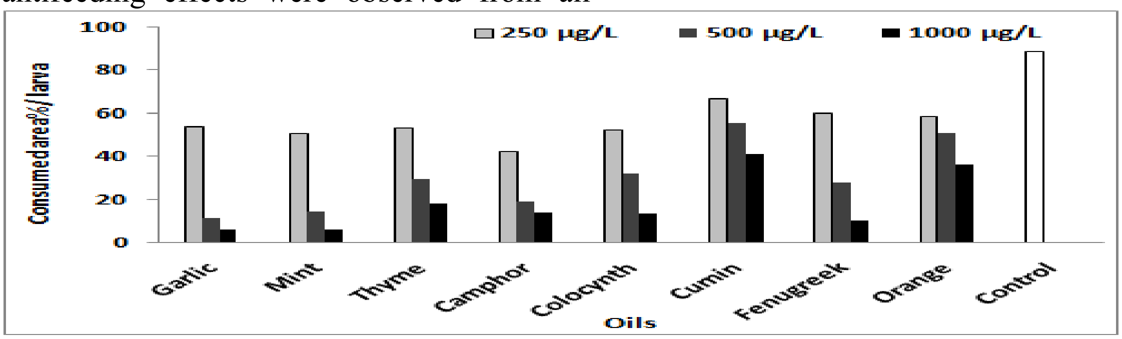

Figure (1): Leave area consumption percentages of *Pieris rapae* after the application of eight essential oils at three concentrations.

Depending on the consumed leaf area percent in Figure (1), garlic, mint and camphor strongly inhabited feeding of *P. rapae* larvae at 500 µg/L and 1000 µg/L, however, thyme, colocynth and fenugreek oils had a strong antifeeding effect at 1000 µg/L only. At 1000 µg/L, the larvae consumed approximately 13 times less food than in the control in case of garlic and mint oils. No significant difference was found between 1000 µg/L and 500 µg/L for garlic and mint oils, however, 1000 µg/L concentration differed significantly from 500 µg/L and 250 µg/L in fenugreek oil.

In previous studies, garlic and mint extracts decreased food consumption of *P. brassicae* (Khan and Siddiqui, 1994 and Ali *et al.*, 2017). Also, Sharaby and El-Nojiban (2015) reported that the garlic and mint oils exhibited antifeedant and starvation effects on *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) larvae. Many studies suggested that sulfide derivatives are the most active compounds in insect repelling in garlic oil (Dugravot *et al.*, 2004 and 2005 and Mann *et al.*, 2011). However, menthol and menthone may be the most active compounds in the case of mint (Gracindo *et al.*, 2006 and Tsai *et al.*, 2013). Also, Kordan and Gabryś (2013) showed that monoterpenes such as thymol in thyme oil had active deterrent effects against *Pieris brassicae* (Linnaeus) (Pieridae: Lepidoptera).

3. Oviposition deterrent activity:

3.1. Choice test:

Data in Table (2) showed that the differences between various tested oils were significant at the three concentrations used. It is clear that the highest oviposition deterrent index was obtained from the highest concentration, while the lowest one recorded in the lowest concentration. The recorded oviposition deterrent indexes were lower than 50% (7.16% to 31.85%) at 250 µg/L. Mint oil decreased the mean number of eggs by 59.24% followed insignificantly by thyme

(49.60%) at 500 µg/L, however, the rest oils showed low oviposition deterrent activity (ODI < 50%). At high concentration, mint oil reduced oviposition of *P. rapae* females by 91.97% followed insignificantly by thyme oil (84.26%) and garlic oil (76.71%). Also, the oviposition deterrent activities of camphor and colocynth oils increased with increase of concentration to record 68.14% and 57.56%, respectively. On the other hand, the oils of cumin, fenugreek and orange seem to be ineffective on *P. rapae* oviposition. At 1000 µg/L, the untreated plants received approximately 5, 26, 14, 5 and 3 folds more than the treated plants with garlic, mint, thyme, camphor, colocynth, respectively (Figure, 2).

Table (2): Oviposition deterrent activity of eight essential oils against *Pieris rapae* females in choice and no choice tests.

Oil	Oviposition deterrence index Choice 250 µg/L	500 µg/L	1000 µg/L	F. value	L.S.D.	No choice 250 µg/L	500 µg/L	1000 µg/L	F. value	L.S.D.
Garlic	8.94**c B**	35.11**b BC**	76.71**a ABC**	25.2	20.541	4.74**c B**	27.20**b ABC**	57.01**a AB**	69.8	9.46
Mint	31.85**c A**	59.24**b A**	91.97**a A**	18.3	21.213	25.39**c A**	46.11**b A**	69.36**a A**	65.3	8.21
Thyme	25.50**c A**	49.60**b AB**	84.26**a AB**	19.3	20.262	20.74**c A**	40.49**b AB**	66.46**a A**	14.7	18.02
Camphor	9.36**c B**	30.88**b C**	68.14**a BC**	31.1	16.071	8.11**b B**	19.75**ab BC**	42.96**a BC**	4.25	25.94
Colocynth	8.16**c B**	38.97**b BC**	57.56**a CD**	37.4	12.303	6.01**b B**	25.07**ab ABC**	42.85**a BC**	4.47	26.28
Cumin	7.16**c B**	28.17**b CD**	39.76**a DE**	18.9	11.470	5.52**a B**	25.11**a C**	26.08**a CD**	3.06	N.S.
Fenugreek	11.41**b B**	13.83**b DE**	28.70**a E**	12.8	7.8894	6.11**b B**	12.56**ab C**	19.92**a D**	5.30	9.05
Orange	7.85**b B**	10.65**b E**	29.60**a E**	11.4	10.560	7.35**a B**	10.84**a C**	20.83**a D**	2.87	N.S.
F. value	6.87	9.76	14.6	---	---	18.45	3.40	10.16	---	---

Mean in the same column sharing similar capital letters are not significantly different by Duncan Test at P-0.05

Mean in the same row sharing similar small letters are not significantly different L.S.D. Test at P-0.05

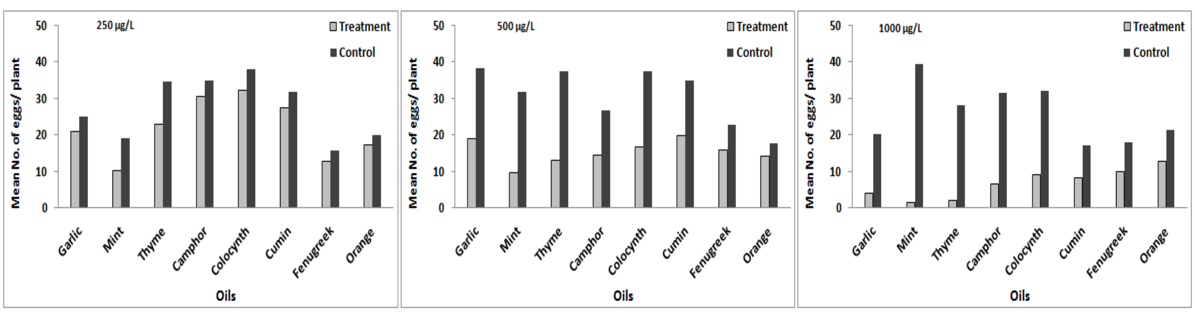

Figure (2): Mean numbers of eggs laid by females of *Pieris rapae* on cabbage plants treated with eight essential oils at three concentrations in choice test.

3.2. No choice test:

In the same line, the tested oils varied significantly at the three concentrations used. The differences between concentrations were significant in all essential oils, except in cumin and orange. The highest oviposition deterrent index was obtained from the highest concentration, while the lowest one recorded in the lowest concentration. At 250 µg/L and 500 µg/L, the eight tested oils showed ineffective oviposition deterrence by ODI less than 50%. The oils ranged between 4.74 to 25.39% at 250 µg/L. and between 10.84% to 46.11% at 50ko µg/L. Only at 1000 µg/L concentration, three oils were reduced oviposition of *P. rapae* females with ODI more than 50%. The highest effect was obtained in mint (69.36%), followed insignificantly by thyme (66.46%) and garlic (57.01%) (Figure, 3).

The present results are in agreement with Lundgren (2008) who found that extracts of thyme and onion decreased *P. rapae* oviposition on cabbage. Also, Ribeiro *et al.* (2015) reported that garlic, mint and thyme essential oils gave oviposition repellence (IDO>80%) rates against *Anticarsia gemmatalis* Hubner (Lepidoptera; Noctuidae) , especially in the free-choice experiment. Magierowicz *et al.* (2019) found that the lowest number of *Acrobasis advenella* (Zinck.) (Lepidoptera, Pyralidae) eggs was observed for thyme treatment. Non host plant chemical compounds may play a significant role in the rejection of plants as hosts by female butterflies (Hussain, 2015), such as sulfide compounds in garlic oil (Mann *et al.*, 2011), menthol and menthone in mint oil (Tsai *et al.*, 2013) and thymol in thyme oil (Kordan and Gabryś, 2013).

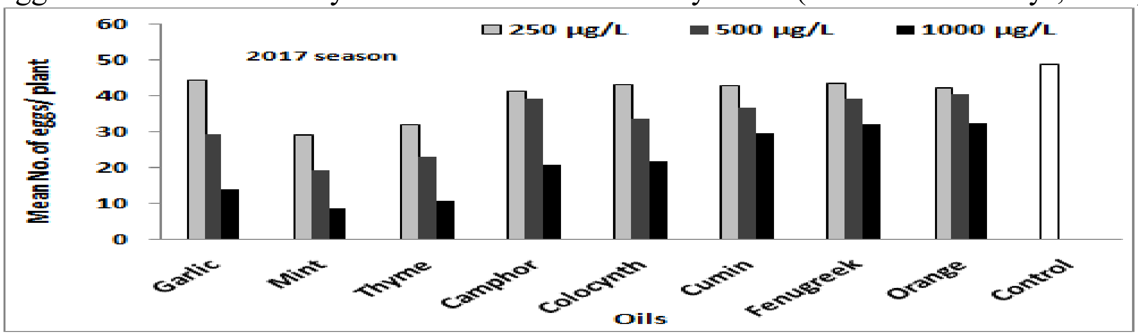

Figure (3): Mean numbers of eggs laid by females of *Pieris rapae* on cabbage plants treated with eight essential oils at three concentrations in no choice test.

From the present data, it is clear that the essential oils of garlic, mint and thyme reduced food consumption of *P. rapae* larvae and decreased the oviposition of its female. So, the application of these oils can be combined with different biological and agricultural methods in an integrated pest management programs could reduce the use of synthetic insecticides, especially in the earlier stage of cabbage. However, further research is needed to investigate the effect of plant essential oils that have the strongest effect in the field conditions.

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