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## Field Study for Control Insect Pests by Innovation Formulated of Essential Oil

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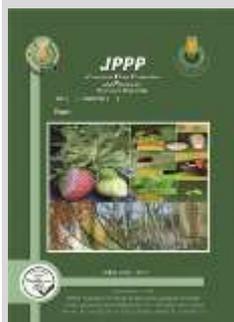
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### ABSTRACT

The current field study focused on new green technology, a new technique that attracts and kills *Lepidoptera* moths, which induced more damage to crops, by making a "formulations" mixture of essential oils extracted from medical and aromatic plants that used to attract and kill lepidopteran moths, especially females. Microliter of basil oil (*Ocimum basilicum* Linn.), coriander oil (*Coriandrum sativum* L.), cumin oil (*Cuminum cyminum* L.), and chamomile oil (*Matricaria chamomilla* L.). The mixture of four essential oils was mixed at a rate of 50µl of each oil, then placed in the transparent capsule, then placed into a pheromone trap of cotton leafworm (*Spodoptera littoralis*). The field study was carried out during the 2017 season in the Giza governorate. The observed results in Sep 2017 were a high number of moths on the treated trap camper compared to the control. Also, the studied combination attracted a kind of vital enemies from spiders to butterflies. Subsequently, increased the efficiency of sexual pheromone traps to catch more moths, also this invention was safer and protectant our environmental form populations. The patent number 30513 was granted in the year 2021.

**Keywords:** Innovation, *Spodoptera littoralis*, Sweet Basil, Coriander, Cumin and Chamomile.



### INTRODUCTION

The cotton leafworm (*S. littoralis*) (Boisd.) (Lepidoptera: Noctuidae), a destructive pest of crops, is the most economically important species in Africa and the Middle East. (Ellis 2004), known as a major polyphagous key pest in Egypt and active all year round without hibernation period, attacking plants include 73 species recorded in Egypt. (Ahmad, 1988, Amin and Salam, 2003). Sex pheromone traps were found to be more effective than the high-pressure mercury lamp. (Rizk *et al.*, 1990). In Egypt, it is necessary to design an alternative program for controlling this pest, for which the use of insecticide is considered the most effective method to control such a pest, so it is becoming very important to find out and develop a program for human health safety and better agroecosystems (El-Mezayyen *et al.*, 2019). Chemical treatments, such as the use of synthetic insecticides, are used to control flies, Long-term usage of insecticides has resulted in susceptible strains of these species developing increased resistance, as detected with permethrin and deltamethrin. (Sukontason *et al.*, 2005).

Several studies have focused on bioactive natural substances, and the use of biological insecticides is a possible method in an integrated pest management system. A small number of essential oils have biological and olfactory activity with a high added value. (Singh and Pandey, 2018). Laboratory bioassay of the target pest "female and male moths" of *Pectinophora gossypiella*, obtained that the highest total response was 83.09, 64.0 and 57.0 % for cotton leaves, flowers, and bolls oil. *E. insulana* was the highest attractive moth to cotton bolls (64.91 %), while *S. littoralis* estimated a positive response to cotton leaves (76.6 %). Ibrahim *et al.*, 2018. A lot of problems are induced by using chemical

pesticides. like the appearance of resistance, in increased residues that are more dangerous to human health and the environment. (Kljajić, and Perić, 2006). Previous research on essential oils (EOs) revealed that they have a wide range of insect pest larvicidal and adulticidal toxicity, as well as a repellent, ovicidal, and insecticidal effect on various types of insect pests. Garrido-Miranda *et al.* 2022). In aromatic plant species, essential oils are synthetic characteristics of chemical insecticides, and they play a key role in signaling processes. Over 17,000 species of essential oils also exhibit attractiveness toward pollinators. (Campolo *et al.*, 2018). Because of their high volatility at different temperatures and their sensitivity to UV light, essential oils are less persistent and stable in the environment than traditional chemical insecticides. (Koul *et al.*, 2008). Essential oils (EOs) can contain mainly hundreds of different sesquiterpenes, monoterpenes, and their derivatives. They are extracted by a steam distillation method from various plant parts, including leaves, flowers, buds, barks, peels, and resins. Also, EOs have various biological activities against different insect pests, including repellent feeding activity, inhibition of development, oviposition, and acute and chronic toxicity. (Yang *et al.*, 2020). Lipophilic plant volatiles that is released from the epidermal tissues of flowers and trichomes specialized hair-like structures may have a role in plant indirect defenses as a consequence of herbivore attracting natural enemies of pests by releasing synomones and helping them to find the attacked plants (Arab *et al.*, 2007; Abd El-Aziz *et al.*, 2007; Baldwin, 2010). Attract and kill methods, mass trapping by pheromones, aggregation, mating disruption, as well as other natural product-based approaches using semi-chemicals, can be used to successfully monitor and control the most damaging pests, such as *S. littoralis*, *S. litura*, *S. exigua*, and *S. frugiperda*. (Del Socor *et al.*, 2010 ; Guerrero *et al.*, 2013).

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Integrated pest management on cotton plants has depended on the fundamental importance of host-plant selection by phytophagous insects for several decades. Kairomones, one of the most important components of green control of insect pests, attract adult moths of both sexes and also, due to kairomones emitted from plants, the potential of natural enemies that use plants in those processes to ensure their own survival. (Nadel et al., 2012 ; Murali-Baskaran et al., 2017).

### MATERIALS AND METHODS

The present invention depends on making a formula with essential oils to attract and kill Lepidoptera moths, which cause more economic damage to crops. It consists of a mixture of more than one essential oil, such as cumin (*Cuminum cyminum* L.), basil (*Ocimum basilicum* L.), coriander (*Coriandrum sativum* L.), and chamomile (*Matricaria chamomilla*).

#### 1. Essential Oil Extraction:

Four essential oil was extracted at the laboratory of the Medicinal and Aromatic Plants Research Department, National Research Center, Dokki, Cairo.

According to Said-Al Ahl et al. (2014) and Hassanein et al. (2020), the Essential oil percentage (%) of each sample was determined with hydro-distillation for 3 hours at Clevenger-type apparatus using 25 g of both 100 g of Cumin and coriander crushed fruits, chamomile dried flowers heads and basil dried aerial parts, the volume and percentage (%) of the extracted essential oil were calculated and recorded. The extracted essential oil of each plant was collected separately, then placed on an Eppendorf tube, and dehydrated with anhydrous sodium sulphate before being stored in a deep freezer until GC-MS analyses.

#### 2. Gas Chromatography/Mass Spectrometry (GC/MS)

GC-Ms were used to analyze all essential oil samples, according to Horsfield et al., 2015 & Omer et al. (2022).

#### 3. Field trapping experiments.

Field experiments were conducted at Agricultural Research Center, during the 2017 and 2018 seasons, the mixture of four essential oils was mixed 200 microliters (50 µl of each oil) of sweet basil oil (*O. basilicum* Linn.), coriander oil (*C. sativum* L.), cumin oil (*C. cyminum* L.) and chamomile oil (*M. chamomilla* L.), then it placed in the transparent capsule, into a pheromone trap of cotton leafworm (*S. littoralis*) and another traps pheromone only " control", the data was recorded during 2017 seasons in Agriculture Research Center station(Fig 1).

The minimum and maximum temperature, Relative humidity RH%, and wind "Km/hour" according to web site" <https://www.timeanddate.com/information/> Anonymous (2017 & 2022) are shown in Table (2).



Fig.1. Uni-Trap includes sex pheromone and Akmoths mixture of oil in the cotton field.

### RESULTS AND DISCUSSION

#### Results

#### 1. Effect of essential oil mix on total moths of cotton leafworm *S. littoralis* male and female (moths/trap):

Seasonal fluctuations of *S. littoralis* moths on cotton plants as affected by treatment trapping (pheromone and essential oil mixture) and control trapping (pheromone only) are shown in Tables (1, 2, 3, and 4) and Figures (2, 3, 4, 5, 6, 7, 8, and 9).

Table 1. Seasonal fluctuation of *S. littoralis* male and female moths during the September 2017 season.

Date of Inspection 2017	No. of male and female moths/trap (Treatment)	No. of male moths/trap (control)	Temp.°C		RH %	Wind (Km/h)	Illumination %
			Min	Min			
10-Sep	0	0	26	34	36	19	80.80
12-Sep	12	0	25	46	65	19	59.50
14-Sep	13	0	24	33	74	18	35.90
16-Sep	3	0	25	33	48	14	15.90
19-Sep	1	0	25	34	40	10	0.29
23-Sep	5	7	26	31	58	17	13.40
26-Sep	2	7	24	32	66	14	38.90
28-Sep	5	4	29	31	39	21	58.40
30-Sep	2	6	26	31	44	20	77.00
Total moths/trap/3 days	43	24					

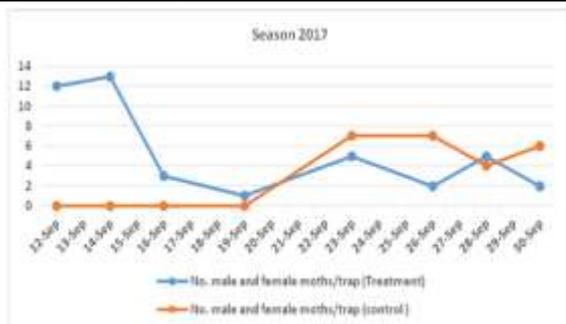


Fig.2. *S. littoralis* moths on treatment and control during Sept. 2017

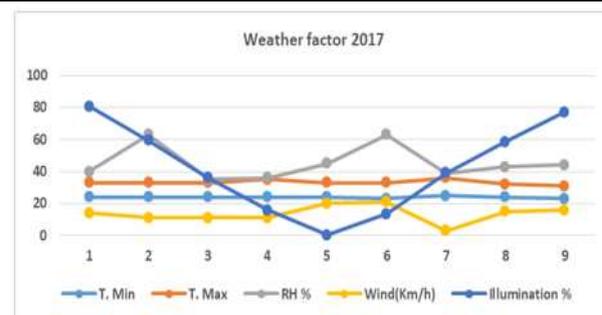
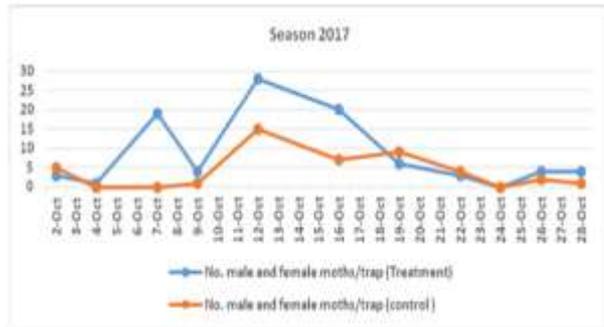


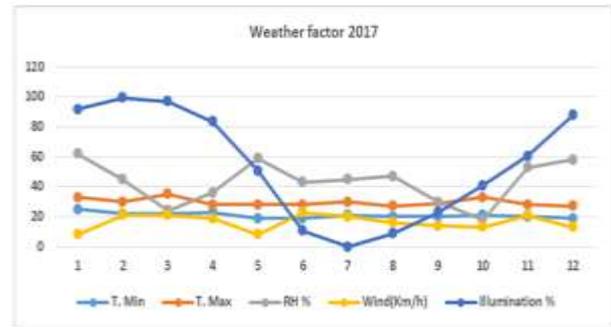
Fig.3. Weather factor of Giza governorate during Sep. 2017 season.

**Table2. Seasonal fluctuation of *S. littoralis* male and female moths during the October 2017 season.**

Date of Inspection 2017	No. of male and female moths/trap (Treatment)	No. of male moths/trap (control )	Temp.°C		RH %	Wind (Km/h)	Illumination %
			Min	Min			
2-Oct	3	5	25	33	62	8	91.80
4-Oct	1	0	22	30	45	21	99.50
7-Oct	19	0	22	35	24	21	97.00
9-Oct	4	1	23	28	36	19	83.50
12-Oct	28	15	19	28	59	8	50.60
16-Oct	20	7	19	28	43	23	10.50
19-Oct	6	9	21	30	45	20	00.0
22-Oct	3	4	20	27	47	16	8.76
24-Oct	0	0	20	29	30	14	22.70
26-Oct	4	2	21	33	18	13	40.80
28-Oct	4	1	20	28	53	21	60.60
31-Oct	13	5	19	27	58	13	87.70
Total moths/ trap/3 days	105	49					



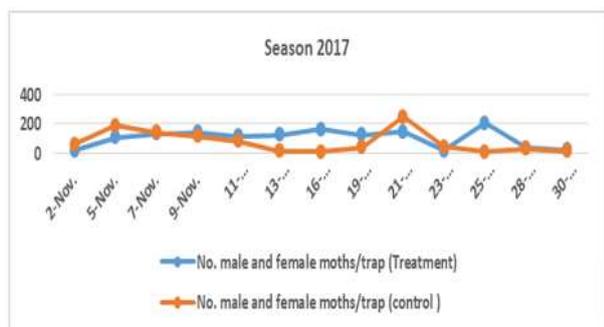
**Fig.4. *S. littoralis* moths on treatment and control during Oct. 2017**



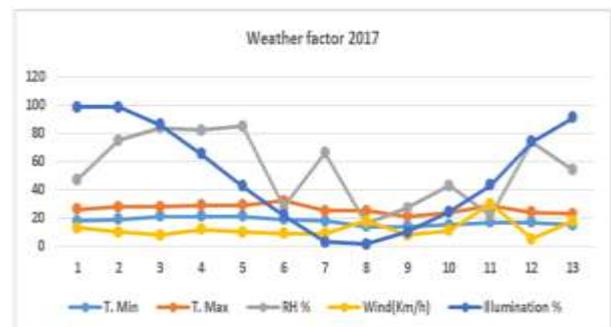
**Fig.5. Weather factor of Giza governorate during the Oct. 2017 season.**

**Table 3. Seasonal fluctuation of *S. littoralis* male and female moths during the November 2017 season.**

Date of Inspection 2017	No. of male and female moths/trap (Treatment)	No. of male moths/trap (control )	Temp.°C		RH %	Wind (Km/h)	Illumination %
			Min	Min			
2-Nov.	17	60	18	25	67	12	98.40
5-Nov.	106	187	18	26	42	17	98.40
7-Nov.	130	141	16	24	63	20	86.10
9-Nov.	137	112	16	25	45	21	65.50
11-Nov.	114	80	16	25	46	17	42.50
13-Nov.	123	10	18	27	29	26	22.00
16-Nov.	159	7	17	27	83	24	2.9
19-Nov.	123	38	17	24	62	7	1.61
21-Nov.	145	247	18	20	60	14	10.10
23-Nov.	15	41	15	22	47	11	24.50
25-Nov.	204	6	15	22	70	12	43.20
28-Nov.	33	30	15	24	33	7	74.00
30-Nov.	18	11	15	23	68	17	91.10
Total moths/ trap/3 days	1324	970					



**Fig .6. *S. littoralis* moths on treatment and control during Nov. 2017**



**Fig. 7. Weather factor of Giza governorate during the Nov. 2017 season**

**Table 4. Seasonal fluctuation of *S. littoralis* male and female moths during the December 2017 season.**

Date of Inspection 2017	No. of male and female moths/trap (Treatment)	No. of male moths/trap (control )	Temp.°C		RH %	Wind (Km/h)	Illumination %
			Min	Max			
3-Dec.	95	146	16	24	64	3	99.40
5-Dec.	70	43	17	22	62	14	95.80
7-Dec.	28	34	13	19	42	16	80.40
10-Dec.	27	74	15	21	50	19	47.90
12-Dec.	9	38	12	24	50	7	27.30
14-Dec.	27	78	13	21	78	18	11.50
Total moths/ trap/3 days	256	413					

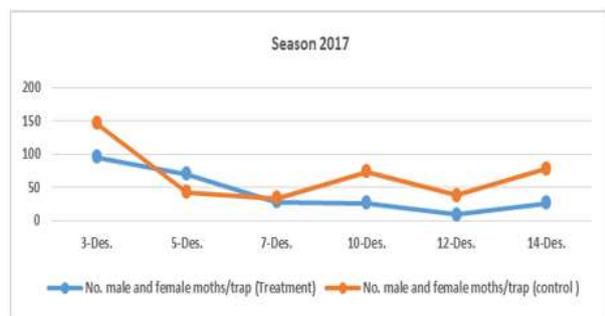


Fig. 8. *S. littoralis* moths on treatment and control during Dec. 2017

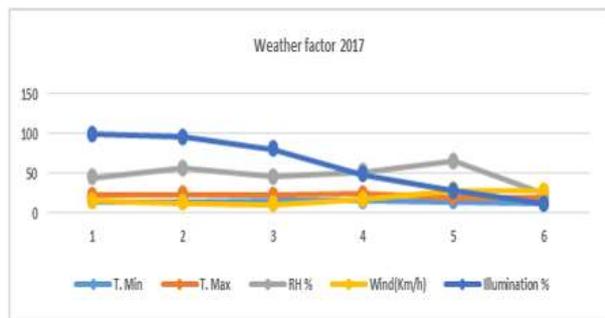


Fig.9. Weather factor of Giza governorate during the Dec. 2017 season.

The total catch of moths was 43 and 24 from the treated oil trap and control during September 2017, respectively. The corresponding moth populations during October 2017 were 105 and 49 moths per trap over three days of the cotton season for the treated oil trap and control, respectively. Moths *S. littoralis* increased to 1324 on treated oil traps in November, compared to 970 on control traps during the same period. While in December 2017, the catch of moths decreased to 256 adult moths in three days in the treated oil trap compared to 413 in the control trap (pheromone only).

**2. Essential oil percentage (%):**

Essential oil percentages were measured as percentages of EO in flowers, herbs, and dry fruits. were 0.38, 0.8, 1.8, and 0.47 % (v/w) for coriander and cumin-dried fruits, basil-dried herb, and chamomile-dried flowers, respectively.

**3. The main constituents of the essential oil:**

Data, as shown in Table 5, investigated that, the essential oil mix as identified with GC-MS, 30 compounds were identified and accounted for 100 %. The major compound was identified as Linalool (23.53 %), followed by  $\zeta$ -Terpinene and  $\beta$ -Pinene (12.71 and 12.07 %, respectively) then Bisabolol oxide A (9.44%), p-Cymene (8.86%) and Cumenic aldehyde (6.63%).

These properties can frequently be attributed to predominant essential oil constituents such as methyl chavicol (estragole), methyl eugenol, linalool, camphor, and methyl cinnamate (Baritau et al. 1992) Fruit Flies of *Ceratitis capitata*, *Bactrocera dorsalis*, and *Bactrocera cucurbita*

Results showed that this mix of essential oil is rich in total monoterpenes compounds which represented 83.94%, while total sesquiterpenes compounds recorded 16.08%. On the other hand, the total oxygenated compounds were 58.17% and the value of the non-oxygenated compound was 41.85%. These results are in harmony almost with most of the analyzed

essential oil extracted from different plants (Dhifi et al., 2016) and agreed with those obtained by Abd-ElGawad et al. (2020) on *Argemone ochroleuca* and Ibrahim et al. (2021) on *Myrtus communis*.

**Table 5. The main constituents of the mix of four oils (basil, cumin, coriander, and chamomile)**

No.	R.T.	KI	Compounds	Area (%)
1	4.48	907	$\alpha$ -Thujene	0.22
2	4.69	916	$\alpha$ -Pinene	1.27
3	5.69	955	Sabinene	0.42
4	5.87	961	$\beta$ -Pinene	12.07
5	6.16	971	$\beta$ -Myrcene	0.81
6	6.70	988	$\alpha$ -Phellandrene	0.61
7	7.35	1008	p-Cymene	8.86
8	7.41	1010	D-Limonene	1.38
9	7.58	1015	1,8-Cineole	2.64
10	8.01	1028	cis-Ocimene	0.23
11	8.44	1040	$\zeta$ -Terpinene	12.71
12	9.69	1073	Fenchone	0.16
13	10.05	1081	Linalool	23.53
14	12.06	1130	Camphor	0.51
15	13.08	1154	Borneol	0.23
16	13.35	1161	4-Terpineol	0.32
17	14.06	1176	$\alpha$ -Terpineol	0.48
18	14.32	1181	Methyl chavicol	1.67
19	16.14	1222	Cuminic aldehyde	6.63
20	17.56	1254	Bornyl acetate	0.67
21	17.99	1264	2-Caren-10-al	4.53
22	18.16	1267	3-Caren-10-al	3.99
23	23.51	1385	$\alpha$ -Bergamotene	1.43
24	24.47	1405	TRANS- $\alpha$ -BISABOLENE	1.06
25	25.51	1431	Germacrene D	0.25
26	26.85	1462	$\zeta$ -Cadinene	0.53
27	31.97	1583	$\delta$ -Cadinol	1.64
28	32.35	1592	$\beta$ -Bisabolol oxide B	0.88
29	33.42	1619	Bisabolone oxide	0.85
1682	1682	1682	Bisabolol oxide A	9.44
			Monoterpenes	83.94
			Sesquiterpenes	16.08
			Oxygenated compounds	58.17
			Non-oxygenated compounds	41.85
			Total	100

**Discussion**

From the previous results, we can notice that there was an increase in the number of moths attracted to the treatment trap (pheromones and a mixture of essential oils) compared to the control trap (pheromone only) in all months except December, which was the exact opposite.

We can also notice that the attraction of insects to the treatment increased with the progression in months from September to November, and this can be explained by the strong insect-repellent property of the volatile oil, which was attributed to the high concentration of the essential oil mix at the beginning of the period (Sept and Oct.), so the number of the attracted moths was low in these two months.

Based on the fact that Ngamo et al. (2007) stated that the activity of essential oils decreased with time due to their high volatility, it may have reached the perfect concentration in November to attract moths, but as the month progressed into December, the essential oil's continuous volatility and decrement still occurred and may have occurred in December. Because EOs is completely volatile, the moths were drawn to the control trap (pheromone only) rather than the treatment trap (pheromone and essential oil mixture).

The positive effect of the volatile oils mix may be attributed to the major components of the four essential oils mix are monoterpenes and oxygenated Compounds. The monoterpenes act as defense mechanisms to protect plants

from pathogens and herbivores by repelling, inhibiting growth, deterring oviposition, blocking predation, and mimicking juvenile hormones (Koul, 2005).

Plant terpenoids include a lot of biological activities such as deterrence, repellency, reduced palatability, enzyme inhibition, growth inhibition, altered protein availability, and direct toxicity (Harborne, 1993; Tak and Isman, 2015).

Recently, the mode of action of monoterpenoids has not been well studied. Some experiments and studies mention that monoterpenoids cause high insect mortality by inhibiting acetylcholinesterase enzyme activity (Mukherjee *et al.*, 2007; Kashima *et al.*, 2011 and Yeom *et al.*, 2015). Subsequently, essential oils with high levels of hydrogenated compounds lose their activity faster than those containing mainly oxygenated compounds; however, the oxygenated monoterpenes spread more slowly and are less volatile than the hydrogenated compounds (Huang and Ho, 1998; Regnault-Roger *et al.*, 2002).

An evaluation of the toxicity of linalool was reported against the *Rhyzopertha dominica* and rice weevil (*Sitophilus oryzae*), and the results indicated that more toxic effects of some essential oils could be attributed to major constituents such as linalool (23.53%) (Rozman *et al.*, 2007). Furthermore,  $\alpha$ -terpineol is highly toxic to *T. confusum* as a fumigant (Sener *et al.*, 2009 and Govindarajan *et al.*, 2016). While the fumigant toxicity of  $\delta$ -Cadinene  $\alpha$ -pinene and  $\beta$ -pinene against the insect's *Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles stephensi* is high, (Lee *et al.*, 2001 and 2002; Dhifi *et al.*, 2016; Sadeghi *et al.*, 2016).

Due to the fact that Male and female moths respond differently to plant volatiles due to dimorphic sexual behavior in moths, but it remains unclear whether they respond to different stimuli.

Plant volatiles' phonological, physiological, and state information conveys information about host specificity, which influences how females select host plants for egg-laying (Sun *et al.*, 2003; Reddy and Guerrero, 2004; Mowrey and Portman, 2012).

Therefore, the males of moths can use female-produced pheromones to the mat and locate receptive females, and they may also use volatiles from host plants to find areas where there is a higher probability of finding receptive females. (Reddy and Guerrero, 2004; Trona *et al.*, 2010 and 2013; Varela *et al.*, 2011; Party *et al.*, 2013).

For many years, entomologists recorded the influence of moonlight on insect flight activity; a bright light induces attraction among insects at night, so the catches of moths on traps are much higher near the period of the new moon than near the full moon (Williams and Singh, 1951).

Mizutani (1984) It was discovered that the difference in temperature between day and night, as well as the lightness of the sky at night, as well as fog density, had the greatest positive influence on insect activity, while wind velocity had the greatest negative influence.

These results are in agreement with Rice and Coats (1994) on the Southern corn rootworm (*Diabrotica undecimpunctata howardi*) and the red flour beetle (*Tribolium castaneum*).

Turlings and Wackers (2004) mentioned that plant volatiles are emitted in reaction to herbivores, attracting parasitoids and predators as a result. The majority of these systems contain crop species. In Egyptian cotton fields, three

or four species were noted, the true spider *Cheiracanthium isiacum*, Fam: Miturgidae, commonly called yellow sac spiders (Ahmed 2012 and Daniel *et al.* 2019). The new formula also attracted a species of spiders as "butterflies predator", and subsequently increased the efficiency of sexual pheromone traps to catch more moths and kill spiders. This method was more efficient in attracting the Egyptian cotton leafworm *S. littoralis*.

Furthermore, semiochemicals present in important crops created a very useful tool that can be used to control pests as well as increase their natural enemies. Different species of insects emitted small lipophilic molecules, and plants induced secondary metabolism that plays a fundamental role in chemical communication Magalhães and Laumann (2016).

## CONCLUSION

These medicinal and aromatic plants represented a new technology that was present in the Egyptian environment, which produced very high-quality essential oils at a low cost that were easy to manufacture for green production. The greater the benefits of the formula, such as increased safety and environmental protection from pollution caused by chemical pesticides used to control pests in agricultural fields, the lower the risk of disease for humans and animals.

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## دراسة حقلية لمكافحة الآفات الحشرية بتركيبة مبتكرة من الزيوت الطيارة

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### الملخص

ركزت الدراسة الحقلية على التكنولوجيا الخضراء ، هي طريقة جديدة لجذب وقتل فراشات حرشفية الاجنحة ، التي تسبب الضرر كبير للمحاصيل الزراعية من خلال عمل تركيبة من الزيوت الطيارة المستخلصة من النباتات الطبية والعطرية التي تستخدم لجذب وقتل فراشات حرشفية الاجنحة خاصة الاناث. وقد تم خلط مزيج من 4 زيوت بمعدل 50 ميكروليتر من كل زيت ثم وضعهم بكبسولة شفافة ثم توضع بالمصيدة الفرمونية بحقل القطن ، وأجريت هذه الدراسة خلال موسم 2017 بمحافظة الجيزة ، واطهرت النتائج تسجيل عدد كبير من الفراشات بالمصيدة التي بها الزيوت عن معاملة الكنترول ، بالإضافة لجذب احد الاعداء الحيوية مثل العنكبك التي تتغذى على الفراشات وزيادة كفاءة المصيدة ويعد هذا الابتكار امن ويحمي البيئة من الملوثات. وقد تم منح براءة اختراع برقم 2021/30513 بعنوان تركيبة زيوت نباتية لجذب وقتل الفراشات.