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Olfactory response of the American bollworm *Helicoverpa armigera* (Hübner) moths to some volatile substances as attractants or repellents

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The American bollworm or Tomato fruitworm *Helicaverpa armigera* (Hubn.) (Lepidoptera: Noctuidae) has long been recorded as a pest of many crops in kingdom of Saudi Arabia and in Egypt, like , Tomato, Zucchini, Green pepper, Gourd, Muskmelon, Okra, Cassia, Potato, Bean, Chickpea, , Sunflower, Red beet, Tobacco, Cowpea, Turnip and Cotton. It recorded attacked about 182 plants in many parts of the world. Many problems have been encountered as a result of the extensive use of synthetic pesticides. Pesticide resistance has developed as a result of long- term and extensive use of chemical pesticides. Essential oils and their constituents from terpenes may provide a new safe alternative to congenital chemical insecticides. In this study, the behavioral olfactory response of *H.armigera* moths of (Saudi Arabian strain.) Towards the plant essential oils and some terpenes were investigated in an air flow olfactometer in order to identify their role as attractants or repellents, so as to identify safe, biodegradable alternatives to chemical insecticides. Obtained results can be summarized as follows. Some of plant volatile oils showed attraction response of moths in varying degrees of intensity of reaction for females in descending order, for volatile oils and terpenes, they were Fenugreek = Geranium = Citronellol = α- Pinene= 40% > Nerol = 30% > Cloves = Dodonea = Ethylcinnamate = 20%. , while others were attractant to males but not to females. Terpineole = α- Ionone = Nerol = Caryophyllen = 50% > Citronellol = Fenugreek = 40% > Harmal = Cardamon = Dodonea = 10%. The oils and terpenes repellent to females were arranged in ascending order of intinisty of reaction as follows, Eucalyptus = Cardamon = Mint = Caryophyllen = -20% < Lemongrass = α- Ionone = Mustard = -30% < Harmal = Farnesol = -40% < Terpineol = -50% < Gieraneol -70%, while others repellent to males but not to females, Lemongrass = Farnesopl = -10% < Cloves = -30% < Mustard = Fenugreek = -70%. Generally the attractant oils and terpenes for both sexes were Dodonea, Citronellol and Nerol, while Mustrad, Lemongrass and Farnesol were repellents. Dodonea oil was attractant for both sexes of *H. armigera* moths, while Harmal and Lemongrass were repellents. Clove and Dodonea were weakly attracttants for females, while Cardamon, Harmal and Dodonea oils were very weakly attractant for males. For terpens, Citronellol and Nerol were attractant, while Farnesol was repellent to both sexes. The research amied to shed some light on the possibility using the volatile oils or terpenes as a mean for controlling *H.armigera* as attractants or repellents between other program.

Keywords: *Helicovirpa armigera*, attractants and repellents, natural volatile oils, terpenes, olfactometer.

INTRODUCTION

Helicoverpa armigera Lepidoptera: Noctuidae, is one of the most important economic and serious pest of cotton crops worldwide (Kranthi et al., 2005). The larvae of this pest feed on a wide range of the economically important crops including corn, tomato, sunflower, legumes, tobacco and several cucurbitaceous and citrus crops (Xiulian et al., 2004). Extensive long-term use of chemical insecticides can result in pesticide resistance, damage to the environment and non-target organisms, disruption of ecological balance and threaten to human health(Bird and Akhurst., 2007; Neupan and Ahrestha 2015). Much efforts hav been directed towards developing management aims to use Biopesticides in control programs. Many plants contained various bioactive secondary metabolites, such as terpenes, flavonoids, volatile oils (Chen et al., 2015; Sharififar et al., 2016; Jarmusch et al., 2016) which act as natural pesticide source. Increase attention has been directed to botanical pesticides due to their natural origin, the wide range of potential material resources , as well as many secondary plant metabolites that are degradable, harmless and non-resistant (Valmas and Ebert, 2006). Many aromatic plants contain volatile compounds namely allelochemicals which are known to possess insecticidal activity (Wilson and Shaya, 1998).to possess insect repellents or attractive activities. All insects use olfactory cues and responses to orient first towards a potential host and second towards their host (Kim et al., 2015). The present research was directed inorder study the olfactory response of *H. armigera* moths towards some plant volatile oils and their constituents from terpenes to find out their role as attractants or repellents. The results will provide a basis for utilization of the tested materials from volatile oils and terpenes in the control program of the insect in the future. Some natural plant essential oils and terpenes were chosen to test their effects on the olfactory response of *H. armigera* moths' inorder to identify their role as attractants or repellents; using an air flow olfactometer..

MATERIALS AND METHODS

Olfactometer:

An air flow olfactometer has been designed according to specifications of (Osgood and Kempster, (1971) with some modifications to be suitable for the tested moths. The device is shown in (Figs. 1), it is a transparent Plastic box (Perspex material), olfactometer test cage 30cm.long, 15 width and 20cm.height). The box or the screen cage (6) has a central hole (1) provided with a stopper to prevent air escape. The cage is divided into two equal compartment or air ways by incomplete plastic wall partition (2) (20cm. long and 15 cm height) leaving basal space for the moths movement and choosing between the two test compartments, leaving 10cm longx15cm.width at the back side of the box for ease of movement of the tested moths (8) and choice between the two air streams that passing through the two test chambers (3), one chamber contained the odor of the tested material and the other without odor as a control. At the front side of the box and at the end of each air way there were two circular openings (7) 5cm. diameter, fastened on each opening an inverted plastic funnel with end opining of 2. cm. Diameter (4) to prevent the responded and catched moth from returning back to the box after they have entered the test chambers. Two circular test chambers (10cm. diameter.) (3) mounted above each funnel at the front side of the screen cage along with the flight way, at the terminal side of each test chamber there is a wide opening10 cm. (5) covered with a wire screen for air entrance from outside the device towards the screen cage then exhausted to outside the device throughout the exhausting holes (7) at the back side of the test box. The two openings covered with metallic wire to prevent the insects from escapecing to outside the test box.. The test samples are placed on a filter paper inside the test chamber. The device fastened on the air current direction (A) moved by an electric fan coming from outside passing through the test chamber carrying the odor molecules of the volatile materials through the flight way to the screen cage in which the test moth adults of *H. armigera* were housed in. The odor stimulate the insect which responded as attracted and captured into the chamber with odor or as repellent by escaping into the control chamber without odor, the remaining insect in the movement area or not entered any of the two chambers is considered non-responding. The air flow goes outside through the two exhausted holes.

Olfactory Response of the adult moths of *H.armigera*.

Apreliminary test was made to **determine** the end point of the adult response to the volatile material, 10 µl. of the tested volatile oils introduced on the filter paper inside the test chamber. Ten moths' one sex males or females were used separately in the screen cage.

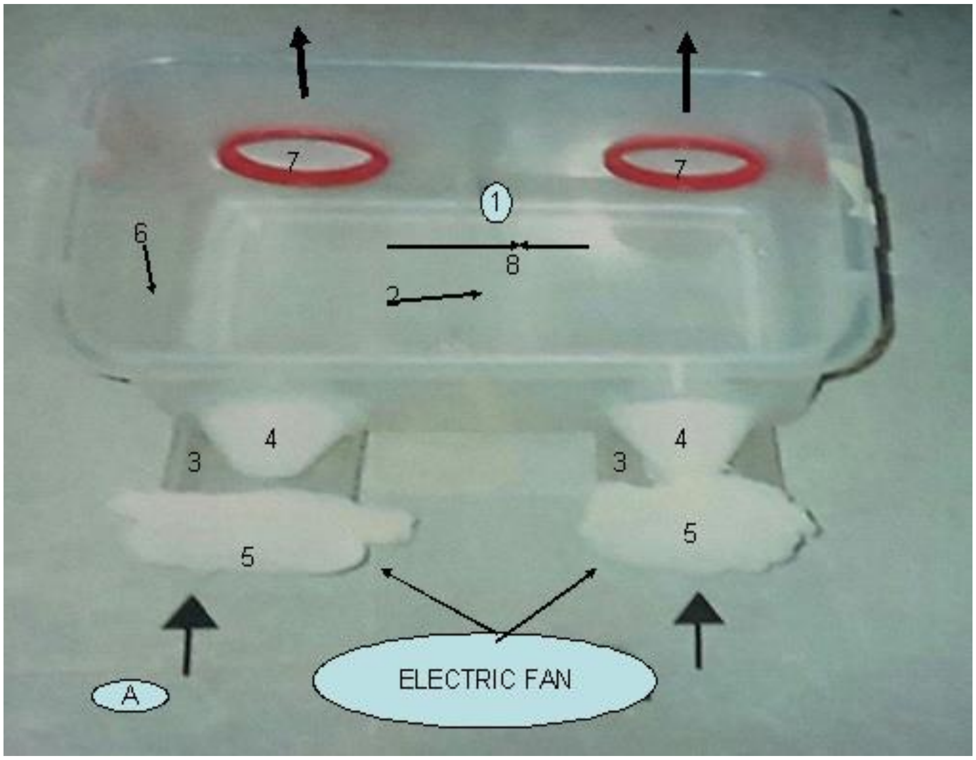

Figure 1: Olfactometer device. (1) hole in the upper side of the test box for entrance the tested adult moths, (2) incomplete plastic partition, (3) test chambers, (4) inverted funnels, (5) posterior end of the test chamber provided with filter paper and covered by a wire, at the front side of the device, (6) test box, (7) holes at the back side of the test box covered with wire, (8) space at the back of the test box for moths movement freely , (A) current air .

. After the olfactometer was turned on the tested moths showed movement with wing vibration, and flew for several centimeters, then enter each of the two air ways. After an average time of 1 hr. the insect stopped movement in the screen cage. This was the end point of the test.

A filter paper was impregnated with 10 µl. of the tested oil then introduced inside one of the test chambers of the olfactometer while the other chamber was left blank as a control. The tested adult moths were introduced into the screen cage through the central hole. Newly emerged moths (males ♂and females ♀) were starved 1. h . before each test. The insect responded to the odor and discriminated between the two air ways by attraction or repulsion. For each volatile material 10 ♂ and 10♀were tested individually. Mean numbers of the total insects that entered each of the air way of test chamber was recorded. The average intensity of reaction (IR) was calculated according to [Gunn and Cosway, 1938)] from the equation.

$$IR = 100 * \frac{S - C}{S + C}$$

While IR= Intensity of reaction, S = Number of insects attracted to the scented chamber of the olfactometer. C= Number of insects moving to the non- scented control chamber. From 1- 10% the odor was very weakly attractive, 11-20% weekly attractive, 21-40%was attractive, and greater than 40% was highly attractive. If less than 1% the odor was considered repellent. After each test the olfactometer was thoroughly washed with distilled water then left to dry to get rid of the odor. All experiments were conducted in constant temperature dark room 28±2ºC.

Natural plant oils & Commercial terpenes.

Ten extracted essential oils were obtained from EL-Seraty Factory in Makah, Saudi Arabia these were (Mint, Clove, Mustard and Cardamom) the other six oils (Fenugreek, Harmal, Lemon grass, Eucalyptus, Geraneum, and Dodonea), were obtained from Egyptian company for aromatic extracts . Eight terpenes from that found in many plant flowers and leaves were obtained from Aldrich chemical company-Ltd- England (Terpiniol, Caryophyllen, Citronellol, Gieraniol, α-Ionone, Farnesol and Nerol

Insect culture.

The *H. armigera* insect Saoudi Arabian strain were reared in laboratory on artificial diet described by (Sharaby and AL-Dhafer 2018), all tests conducted in this study were performed under controlled condition with temperature (27± $2C^0$ and 75%± 5 R.H , and a light – dark cycle 14: 10 hr.

RESULTS AND DISCUSSION

The olfactory response of *H.armigera* moths towards ten of natural plant essential oils tested using the previously described olfactometer device were recorded in Table (1) that cleared the positive intensity of reaction (attractants, kiromones) and the negative reaction (repellents, allomones) for the tested oils (Lemon grass, Cardamon, Harmal, Fenugreek, Mastard, Dodonea, Geranum, Clove, Eucalyptus and Mint). Data indicated that Fenugreek and Geranium oils attractants for female moths with a positive intensity of reaction reached (40%), while they were not attractive for males with negative reaction (-70 & -020%) respectively. Clove and Dodonea were weakly attractive with reaction intensity of (20%) for females. The repellent oils for females could be arranged in adescending order with the negative intensity of reaction as follows. Harmal (-40%) > Mustard and Lemon grass (-30%)> Cadamon, Eucalyptus and Mint (-20%). While Cardamon,Harmal and Dodonea oils were very weakly attractive for males their attraction did not exceeded 10% . The repellent order for males only could be arranged descending as a negative intensity of reaction as follows. Fenugreek and Harmal (-70%) > Clove (-30%) > Geraneum (-20%) > and Lemongrass (-10%). Generally, in conclusion Dodonea oil was attractive for both sex of *H. armigera* moths, while Harmal and Lemongrass were repellents. Table (2) showed the intensity of reaction as positive attractions or negative repellents for eight pure terpenes that is a constituent of many plant essential oils were Caryophyllen, Nerol, α- Ionone, Geraneol, Farnesol, Ethylcinnamate, Citronellol, Terpeneol and α- pinene.

Data indicated that Citronellol , α- pinene and Nerol were attractive for females with 40, 40 and 30% intensities of reaction respectively, while Ethylcinnamate was weakly attractive with (20%) . The repellent terpens for females only was arranged in descending order. Geraneol (-70%) > Terpineol (-50%) > Farnesol (-40%) > α-Ionone (-30%) > Caryophyllen (-20%). For males the highly attractive oils were Caryophyllen, Nerol, α-Ionone and Terpineol the intensities of reaction were positive with 50% for each, while Citronellol terpen was attractive for males with (40%), while Farnesol was repellent with negative intensity of reaction (-10%). Generally, Citronellol and Nerol were attractive, while Farnesol was repellent to both sexes. Our findings agreed with that mentioned by Salama et al., (1987) they recorded that some terpenes and essential oils were attractive and others were repellents for *H. armigera*, Citronellol was attractive for females while Caryophyllene was attractive for males, and disagreed with those of Farnesol, and α-Ionone that were attractive for females, this may be as a result of variation in the insect habitat or strains (Saudi strains may differfron Egyptian). Abdel-Aziz and Sharaby (1997) recorded that 2.5% emulsion of Mustard oil obtained from *Brassica alba* sprayed on cotton plants under field conditions significantly decreased the number of eggs laid by *S. littoralis* females, the percentage of female repellency for egg laying reached 89.4%. Rostelien et al., (2000) recognized the olfactory receptors on *H. virescens* antennae and their role in response to some sesquiterpenes found in some plants adopting of electrophysiological methods (GC- SCR) indicated that 80% of the receptors were sensitive to one kind of Germacrene D which recognized by chromatographic analysis. Liao et al., (2017) recorded that essential oils from aromatic plants may provide a new and safe alternative to conventional insecticides. Essential oils possess aromatic properties make insects disgusted by food, reducing or stop feeding (Arsu et al., 2013). Ribeiro et al., (2015) noticed that the constituents of terpinen-4-ol had distinctly deterred feeding activity on *H.armigera*. The structure activity relationships of the essential oil constituents indicated that the constituents with hydroxyl or aldehyde groups are strong than constituents belonging to hydrocarbons, which is consistent with the report by Seo et al., (2009). It is helpful for the future synthesis of a new type of insecticides or insect repellents. Setiawati et al., (2011) mentioned that in laboratory experiments the highest concentration. of citronella oil reduced egg laying by 53-66%. Ovicidal activity was concentration dependent, and egg hatchability decreased by 15- 95% compared to control. Field experiments showed that treatment of citronella oil at 2 Ml. significantly decreased fruit damage by *H.armigera*, therefore the study revealed that citronella oil has a potential to be incorporated into the controlling program of *H.armigera.*

Table (1): Olfactory response of *H.armigera* moths to different natural plant essential oils .

Intensity of reaction		Tested natural plant essential oils	
Female ♀	**Male ♂**	**Scientific name**	**English name , Family : Order**
-30	-10	*Cymbopogon Schoenanthus*	Lemongrass , Poaceaee : Poales
-20	+10	*Elettaria cardamomum*	Cardamom , Zingiberaceae : Zingiberales
-40	+10	*Rhazya stricta*	Harmal , Apocynaceae : Gentianales
+40	-70	*Trigonella foenumgraecum*	Fenugreek , Fabaceae : Fabales
-30	-70	*Brassica alba*	Mustard , Brassicaceae: : Brassicales
+20	+10	*Dodonea viscose*	Dodoneaeae , Sapindac : Sapendalies
+40	-20	*Glossonema varians*	Geranium , Apocynaceae : Gentianales
+20	-30	*Dianthus caryophyllous*	Cloves , Caryophyllaceae : Caryophyllales
-20	0	*Eucalyptus rostrata*	Eucalyptus , Myrtaceae : Myritiflorae
-20	0	*Mentha vrdis*	Mint , Lamiaceae : Lamiales

Values of Intensity of reaction:
Non attractive or repellent (Less than 1%) ,Very weakly attractive(1- 10%) ,Weakly attractive (11- 20%) ,Attractive (21- 40%) ,Highly attractive (41- 100%)

From the aforementioned results could concluded that some natural plant essential oils and their constituents from pure terpenes may be used in the attractive baits or sprayed as repellents on the host plants to prevent the insect from reaching their host as a means of biocontrol method against *H. armigera* , decreasing the presence of the insect, and their chance for mating.

CONCLUSION

In this study, the behavioral olfactory response of *H.armigera* moths of (Saudi Arabian strain) towards the plant essential oils and some terpenes were investigated in an air flow olfactometer inorder to identify their role as attractants or repellents , so as to identify safe, biodegradable alternatives to chemical insecticides. Aim of this research was to explore the future synthesis of a new type of insecticides or insect repellents for protection the host plant from the insect infestation, and attractants used in bait traps against the insect as a biological method in IPM program.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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All dataset on which abstracted of the study have been drawn are presented in the main manuscript.

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AUTHOR CONTRIBUTIONS

All author declare that the manuscript has not been send for consideration for publication or for publication anywhere else before , the manuscript is original research data.

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