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ATTRACTION OF *Spodoptera littoralis* (Boisd.) (LEP., NOCTUIDAE) TO I-VOLATILES EXTRACTED FROM COTTON FLOWERS AND LEAVES

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ABSTRACT

Mated and virgin females and virgin males of the cotton leafworm, *Spodoptera littoralis* (Boisd.), moths responded via directed upwind flight in the glass tube olfactometer to volatiles from floral extracts of cotton,*Gossypium barbadense* L. (Malvaceae). Significantly more mated females landed on the glass plates treated with each of the extracts, than on the control.Virgin males and females landed in significant numbers on the glass plates treated with the methylene chloride extract of debracted flowers than on control glass plated treated only with the methylene chloride solvent.

As a matter of fact, the present study revealed that volatiles extracted from certain parts of cotton plants, attracted both sexes of the cotton leaf-worm moths instead of attracting only one sex as the pheromones do. It is note worthy that, this is the first time to report such results in Egypt.

INTRODUCTION

Phytophagous insects often use volatile chemicals emitted from plants to locate suitable oviposition sites and food sources for themselves or their progeny. In studies on the behavioral effects of host and nonhost

allelochemicals on *Heliothis* sp. (Lepidoptera, Noctuidae), attraction and oviposition stimulation of *H.virescens* (F.) and *H. subflexa* (Guenee) moths by host-plant extracts were recorded, (Mitchell and Heath, 1987; Mitchell *et al.*, 1990; Tingle *et al.*, 1989 & 1990).Volatiles from extracts of cotton leaves and squares (flower buds) and leaves from tobacco (*Nicotiana tabacum* L., Solanaceae) and *Desmodium tortuosum* (Swartz) de Candolle (a wild host) induced mated *H. virescens* females to exhibit a full array of behaviours (upwind flight, landing, surface examination with antennae, and oviposition on treated substrates) expected of gravid individuals seeking sites to oviposit. Virgin female and male moths were significantly less responsive to the attractant more than the mated females Mitchell *et al.* (1991) presented data suggesting that cotton squares have a greater concentration of flight - and orientation - stimulating compounds than do the leaves to *attract Heliothis virescens* (F.) females.

Wiesenborn and Baker (1990) found in the wind tunnel bioassays the *pink bollworm* moths, *Pectinophora gossypiella* (Saunders), were attracted to a hexane extract of the cotton flowers. They proposed that the flower volatiles act as a food lure, attracting moths to extra floral nectaries. Similarly, Haynes *et al.* (1991) demonstrated in the wind-tunnel assays that olfactory stimuli played an important role in the cabbage loopers *Trichoplusia ni* (Hubner), ability to locate nectar on honey suckle flowers, *Abelia grandifora* (Andre).

Chemical attractants have long played a major role in the detection and control of the Mediterranean fruit fly (Med fly), Ceratitis capitata (Wiedemann) and related tephritid flies (Chambers, 1977). Despite this, relatively few effective olfactometer designs for assessing the effects of olfactory stimuli on medfly have been reported and even less biological and behavioural information have been presented to demonstrate a clear picture of the usefulness of a particular design. The lack of sensitive and discriminating bioassay has undoubtedly hindered past efforts to identify the male sex pheromone of the medfly (Ohinata *et al.*, 1977).

The present study describes the behavioural responses exhibited by *S. littoralis* virgin and mated female ,moths and virgin male moths in glass tube oflactometer where they were exposed to extracts from the different flowering parts of cotton, *Gossypium barbadense* L.

MATERIALS AND METHODS

A glass tube olfactometer (60 cm x 4 cm i.d.) was designed to record flight and other behavioural responses by *S. littoralis* moths to crude exracts of cotton, squares (flower buds), whole flowers, debarcted flowers and leaves.

I. Preparation of cotton parts methylene chloride extracts :

The extracts were prepared from field-grown cotton (Gixa 70) by washing 400 g of fresh plant foliage material for 30 sec. in 1 liter of solvent (methylene chloride), which was filtered and stored in glass containers kept at 50°C until used (Mitchell and Heath, 1987) . Before testing, the extracts were concentrated in a rotary evaporator to 2-g equivalents (2 g E) per 200 ul methylene chloride

II- Culture of Spodoptera littoralis (Boisd) :

A laboratory strain of the Egyptian cotton leafworm *S. littoralis* was reared on castor been leaves according to El-Defrawi *et al.* (1964) and were maintained under standard lab. Conditions. Pupae were held in plastic cages with screened tops until emergence. Virgin Females and males were held separately or combined for mating (20 females and 10 males / cage). The moths were fed on 10% honey water solution in a holding room maintained at 26±10°C and 65± 5% relative humidity for two days prior to experimentation.

On the day of testing, mated and virgin Females and virgin males were confined separately in three olfactometer tubes (60 cm x 4 cm i.d.). Airflow was produced by air generated by a small fan. Crude extract of 2-gE from either cotton squares, whole flowers, debracted flowers and leaves were deposited on a small glass plate. On a second small glass plate, 200 ul plate of methylene chloride, were applied as control. After 5 min. for the solvent to evaporate, the two small glass plates were placed into the port of olfactometer upwind of the glass tubes. Five replicates, each of 10 mated females, virgin females, or virgin males, were tested individually for responses to each of the five extracts. The insect's response to the air stream alone was measured for 2 min. before every test, besides; the solvent control was also carried out. Test stimuli were placed in the port

immediately upwind of the glass tube and the insects were exposed to these stimuli for 2 min. The data were statistically analyzed using t-test (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Testing Extracts of Cotton parts :

I. Upwind movement :

Volatiles from extracts of whole cotton flowers , flowers without bracts, and the extract of leaves attracted significantly more mated and virgin females and males of *S. littoralis* than did the corresponding controls (Figure 1), these results may be due to the presence of formic acid, pyruvic acid, succinic acid, malic acid, quereetin, isoqueretin, pheophytin a and pheophytin where they proved to be as attractant constituents in cotton plant (Wallace & Mansell, 1975). When the response to each extract, as demonstrated by upwind flight toward the treated substrate, was compared with the response to each of the other extracts (unpaired t-test), the highest significant response was recorded for mated and virgin

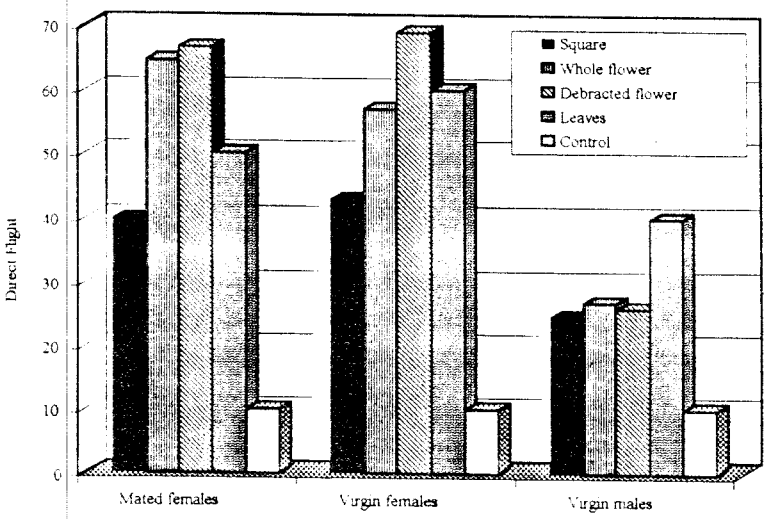

Fig.(1): Response of *Spodoptera littoralis* moths exposed in glass tube olfactometer to cotton extracts (upwind flight).

females to volatiles emitted from the extract of debracted flowers (Table 1). There were no significant differences among the different parts of cotton extracts attracting virgin males toward the treated substrates except for leaves extracts. The flight sequence was similar to that previously described by Tingle *et al.* (1989, 1990), Mitchell *et al.* (1991) and Tingle and Mitchell (1991).

Table (1): Comparison between cotton extracts for attracting *Spodoptera littoralis* moths in a glass tube olfactometer. (Upwind flight)

Treatment	Flight response (% ± SE)	Treatment			
		Square	Whole Flower	Debractd F	Leaves
Mated females					
Square	40.0 ± 4.5				
Whole flower	64.6 ± 4.5	3.9 **			
Debracted flower	66.6 ± 5.1	3.3 **	0.2		
Leaves	50.0 ± 3.7	1.7	2.3*	2.6*	
Control	10				
Virgin Females					
Square	43 ± 4.9				
Whole flower	57 ± 5.1	2.0*			
Debracted flower	69 ± 4.7	3.8**	1.7		
Leaves	60 ± 6.3	2.0*	0.38	1.3	
Control	10				
Virgin Males					
Square	25 ± 0.8				
Whole flower	27 ± 0.9	0.50			
Debracted flower	26 ± 0.5	0.34	0.3		
Leaves	40 ± 3.9	3.77**	3.20**	4.1**	
Control	10				

Response to each extract compared with response to each of the other extracts : t values determined by unpaired t-test (Steel and Torrie, 1960) :

* Significant, $P< 0.05$

** Significant, $P<0.01$

II. Moth Landing performance :

Table (2) and Figure (2) showed significantly more mated females landing on glass plates treated with each of the methylene chloride extracts of square whole flower, debracted flower and leaves than control (45, 55, 70,45 and 10% moths landing respectively) . Methylene chloride extract of debracted flower, showed significantly more landing of virgin males and females than control (60, 70 and 10% moths landing, respectively).

Table (2). Comparison between cotton extracts for attracting *Spodoptera littoralis* moths in a glass tube olfactometer. (landing)

Treatment	Landing %	T value
Mated females		
Square	45 ± 3.6	6.6**
Whole flower	55 ± 3.9	8.2**
Debracted flower	70 ± 5.7	8.7**
Leaves	45 ± 5.0	5.6**
Control	10 ± 3.9	
Virgin Females		
Square	30 ± 6.7	2.5*
Whole flower	35 ± 9.1	2.5*
Debracted flower	70 ± 7.9	6.45**
Leaves	35 ± 8.4	2.55*
Control	10 ± 5.0	
Virgin Males		
Square	25 ± 5.7	1.9
Whole flower	35 ± 4.9	2.2*
Debracted flower	60 ± 6.5	7.5**
Leaves	30 ± 6.5	2.5*
Control	10 ± 5.3	

* Significant, $P < 0.05$

** Significant, $P. < 0.01$

t-test (Steel and Torrie 1960

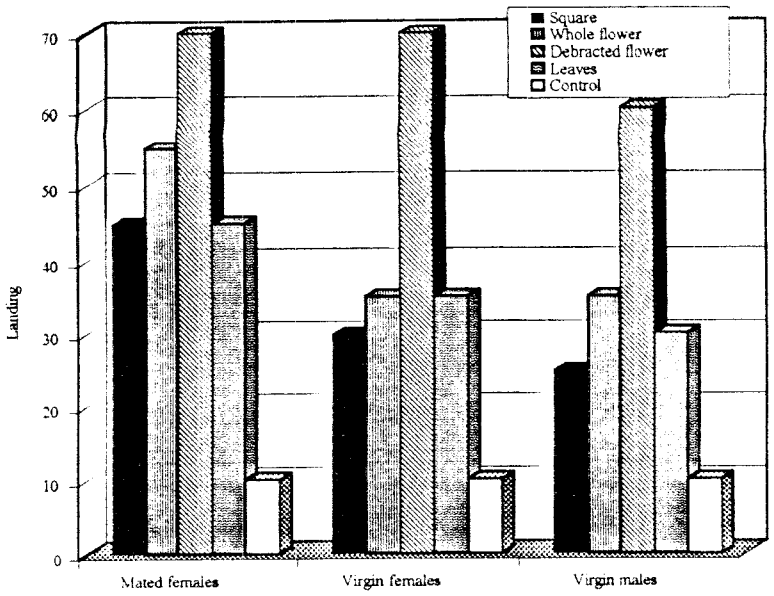

Fig.(2): Response of *Spodoptera littoralis* moths exposed in glass tube olfactometer to cotton extracts (landing).

There were no observed differences in the effect of the various extracts on oviposition (Table 3). Because it is unclear whether the chemicals that attracted the moths to respond by anemotaxis in the olfactometer are the same compounds that stimulated oviposition.

Table (3): Comparison between cotton extracts on the number of deposited eggs and the percentage of hatching .

Deposited	(a) No. of eggs	(b) % hatching ± S.E.	t- value
Control	3400	88 ± 3.2	
Whole cotton flower	4511	78 ± 10.2	1.0
Debracted cotton flower	3821	84 ± 3.1	0.5
Cotton Leaves	3735	86 ± 2.9	1.0
Cotton Square	3300	84 ± 2.8	1.4

(a) Number of deposited eggs (female 2 : 1 male moth) per treatment using five replicates .

(b) Response to each extract compared with response to each of the other extracts; t values determined (steel and Torrie 1960).

Blaney and Simmonds (1988) indicated that feeding behaviour by *H. virescens*, *H. armigera* (Hubner) and *Spodoptera littoralis* (Boisdval) adults was correlated with the electro- physiological response of the sensillae on the proboscis. They reported that responses of the sensillae to sugar and aminoacids were significantly correlated with the feeding behaviour in each of these species . Besides, Stadler and Seabrook (1975) concluded that the sensillae on the proboscis of the eastern spruce budworm, *Choristoneura fumiferana* Chem., were sensitive to sugars, which were involved in the feeding behaviour. However, they can not be considered host-specific chemicals that promote oviposition. Identification of the chemicals and blends thereof that attract cotton leafworm moths and that stimulate them to feed or oviposit may permit development of systems that can be applicable to regulate populations of this important insect pest.

The use of sex attractant pheromones as a predicitive tools to forecast pest population and their potential damage levels in specific crops, has significantly been limited because sex attractants are usually directed at only one sex. Thus, identification of plant constituents that attract *S. littoralis* adults, especially females, and that directly affect their feeding or reproductive behaviour, would greatly expand opportunities for manipulation of the biological and environmental events that control the establishment, development , and dispersal of this important crop pest.

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الملخص العربى

أستجابة فراشات دودة ورق القطن الى المستخلصات المختلفة لنبات القطن

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كان الغرض من هذا البحث محاولة الوصول الى أجزاء نبات القطن المسئولة عن جذب ذكور وإناث فراشات دودة ورق القطن. وقد تم أستخدام فى هذا البحث جهاز بسيط للشم وذلك لجذب الذكور والإناث للفراشات الحديثة الظهور والمخصبة وذلك بأستخدام مستخلص الميثلين كلوريد لأجزاء نبات القطن المختلفة. والجهاز المستخدم عبارة عن أنبوبة زجاجية يمر بها الهواء لتقييم الجذب كأستجابة لحاسة الشم فى فراشات دودة ورق القطن ومشاهدة مظاهر السلوك المختلفة الى قد تظهر نتيجة لتعرضها لهذه المستخلصات وكانت النتائج كالتالى :

- أظهرت جميع المستخلصات (براعم – زهور بالقنابات – زهور بدون قنابات – أوراق) معنوية عالية فى جذب الفراشات بالمقارنة بالكنترول.
- كان لمستخلص الميثلين كلوريد لزهور القطن تأثير كالتالى:

(I) الجذب المباشر والسريع :

١-على الإناث المخصبة : مستخلص الزهرة بالقنابات او بدون قنابات أعلى معنوية فى الجذب المباشر واسريع يليه مستخلص الأوراق ثم البراعم.

٢-على الإناث حديثة الظهور : مستخلص الزهرة بدون قنابات أعلى معنوية فى الجذب يليه مستخلص الزهور بالقنابات ثم الأوراق ثم البراعم.

٣- على الذكور حديثة الظهور : مستخلص الأوراق أعلى معنوية فى الجذب يليه باقى المستخلصات.

(II) فترة البقاء على السطح المعامل :

١- الإناث المخصبة : لوحظ ان معظم المستخلصات المستعملة ٠٠ زهور وأوراق وبراعم كان لها تأثير معنوى على بقاء الحشرة على لوح الزجاج المدهون بها فترة أطول عن الكنترول.

٢- الذكور والإناث حديثة الظهور : لوحظ أن مستخلص الزهور بدون قنابات كان له تأثير معنوى فى تفضيل الذكور والإناث للبقاء على اللوح الزجاجى المدهون بها فترة أطول من الكنترول.

(III) عدد البيض:

أظهرت النتائج أنه لم يكن هناك إختلاف معنوى فى عدد البيض الذى تضعه الفراشات نتيجة لإختلاف أنواع المستخلصات المستعملة.