

RESEARCH ARTICLE

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OVIPOSITION AND ELECTROPHYSIOLOGICAL RESPONSES OF *SPODOPTERA LITTORALIS* TO CASTOR BEAN OIL (*RICINUS COMMUNIS*) SEEDS AND LEAF EXTRACTS**ABSTRACT:**

The relationship between contact chemoreceptors and landing of female moths in the field to find out a suitable place for egg laying is very important to discover a suitable places for the immature and adults to complete their development. Chemoreceptors play an important role in mediating a diverse range of behaviours, including avoidance. The present research has been conducted to evaluate the activity of extracts from seeds and leaves of *Ricinus communis* against oviposition of *Spodoptera littoralis*. Four solvents with different polarity (petroleum ether, chloroform, ethanol, and water) were used to extract the plant material. Results showed that the fixed oil of petroleum ether and chloroform extracts from seeds were more effective in inhibiting oviposition activity than water extract. Water and alcohol extract was most effective than leaf volatile oil as inhibiting oviposition (eggs/♀). On the other hand, macerated *Nerium oleander* and the females produced the highest number of egg masses as compared to stimulated oviposition. Electrophysiological studies revealed that the chemosensitive sensilla on antenna and tarsus were sensitive to all tested extracts. The results indicated that both the frequency and the amplitude of afferents from sensilla differed according to the type and concentrations of the extract. In general, the biological activity of *R. communis* extracts was solvent type-and concentration-dependent.

KEY WORDS:

Spodoptera littoralis, castor oil, contact chemoreceptors, oviposition deterrence, oviposition behaviour.

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INTRODUCTION:

Odour-guided behaviour is dependent on the environment and on the physiological state of insects. Behavioural responses to odours can vary depending on the different processing of these odours in the central nervous system. Olfaction is one of the most important insect senses, guiding them towards their host plants, feeding sites, and mating partners (Anton and Hansson, 1994&1995; Gaaboub and Tousson, 2005). Behaviourally active plant compounds have been found for many insect species in different orders (Masante-Roca *et al.*, 2002). The female may prefer to oviposit on a host plant with poorer nutritional quality, so it offers higher protection against natural enemies' odor (Behan and Schoonhoven, 1978; Masante-Roca *et al.*, 2002; Saxena and Basit, 2008; Sadek *et al.*, 2010). Females use sensory cues emitted by plants to assess the suitability of hosts. These cues convey important information regarding nutritional quality and presence of secondary compounds that could be toxic or negatively affecting larval development (Sadek *et al.*, 2010).

Most insects have contact chemoreceptors on various surfaces of their body. This taste sense can be involved in a number of behaviours, including avoidance (Newland *et al.*, 2000; Gaaboub *et al.*, 2005; Newland and Yates, 2007; Ômura, *et al.*, 2008), detection and selection of food sites (White and Chapman, 1990; Gaaboub and Hustert, 1998; Tousson and El Atrsh, 2010) and selection of egg-laying sites (Kalogianni, 1995&1996; Tousson and Hustert, 2000; Tousson, 2004; Newland and Yates, 2007; Tousson and Hustert, 2009). Several electrophysiological studies on tarsal and antennal chemoreceptors have been carried out on moths; however, no surveys of responsiveness to a range of chemicals have been attempted. Talaat and Gaaboub (1993) reported that tarsal sensory receptors of Lepidoptera have an important role in host plant selection by responding to various chemical and mechanical stimuli. Only few studies concerning the central processing of plant volatiles have been performed on noctuid and sphingid moths. Anton and

Hansson (1994&1995) and Masante-Roca *et al.* (2002) investigated the central processing of plant odours in *S. littoralis*.

The Egyptian cotton leaf worm, *S. littoralis* is abundant in the agro-ecosystems in Egypt. It infests a wide array of host plants, belonging to 40 plant families, including several major crops in Egypt and several other Mediterranean and Middle East countries. The selection response of feeding bioassay showed that the adults of *Holotrichia obliqua* (Liu *et al.*, 2006) and *S. littoralis* (Wae Ckers *et al.*, 2001) were significantly attracted to *R. communis* in laboratory and under field conditions. The castor seeds essential oil extracts showed good repellence index when applied at high concentrations. However, greater protection could be obtained at higher concentrations but with a shorter repellence time (Soares *et al.*, 2010). The presence of oleic acid in *R. communis* repels bees and ants by simulating the "Smell of death" produced by their decomposing corpses (Matt, 2009). Therefore, the objectives of the present study were:

(1) To evaluate the effect of different extracts on oviposition of *S. littoralis*.

(2) To investigate the effect of plant extracts on the development of *S. littoralis*.

(3) To evaluate the relation between crude castors bean extracts and contact chemoreceptors on tarsus and antennae.

MATERIAL AND METHODS:

Insects:

S. littoralis was maintained in the laboratory at 27°C and 14: 10 hr L/D photo-periods at the department of Entomology, Faculty of Agriculture, Moshtohor, Banha University. Larvae were reared on castor bean leaves. Pupae were collected and kept in wooden box till adult emergence. Three to four days old moths were used in all experiments. Adults were placed in screened cages (50X50X50 cm) for mating and oviposition.

Preparation of plant extracts:

Table 1 shows the percentage of each crude extract by different solvent based on 100/g castor seeds and leaves. Fresh leaves or drains seeds of *R. communis* were collected. Hundred grams of dried and ground plant materials were treated with different solvents of increasing polarity (petroleum ether, chloroform, ethanol and water, respectively) in a flask for 72 hrs. The material was then shaken for 30 min in a shaker and the suspension was filtered through filter paper. Excess solvents were evaporated under vacuum pressure using a rotary evaporator. Residues were stored at -4°C in a refrigerator. Concentrations of 5 and 10% (w/v) were prepared from crude extracts.

Table 1. Percentage of each extract of different solvent based on 100/g castor seeds and leaves.

Solvents used	Crude extraction/ 100g seeds	Crude extraction/ 100g leaves
Petroleum ether	6.44	3.8
Chloroform	4.139	2.6
Ethanol	2.827	1.8
Water	1.41	1.6

Oviposition deterrence bioassay:

Bioassays were conducted in screened cages (50×50×50 cm) situated in a chamber at constant temperature (27 ± 1°C) and 14: 10 hr light: dark photo-period. The bioassay began with the onset of the light period and lasted for 24 hr. Three females and five males were introduced into each cage. For oviposition, moths were offered two treated and two untreated *N. oleander* branches. Each branch ended with two leaves fixed in 100-ml vial filled with water to prevent wilting of the leaves and situated in the corner of the cage. Except the bioassay tests of the solubility of the deterrent, leaves were treated with a water suspension of castor seeds or leaf extracts in which the concentration of extract ranged from 5 -10%. This suspension was prepared in a Potter homogenizer with few droplets of twin and applied to the undersurface of *N. oleander* with a brush because eggs are usually laid only on the undersurface of the leaves.

Electrophysiological study:

Responses of individual sensilla (sensilla chaeticum on the ventral side of the tarsus and sensilla trichoidea on the antennae) to chemical stimuli were recorded using the tip recording technique described by Hodgson *et al.* (1955). The potentials were amplified and filtered using AC amplifiers. A blunt glass microelectrode filled with different solutions was placed over the shaft of the sensillum. Electrodes containing salt (0.1 M of NaCl mixed with the extracts at the concentration of 5 or 10% petroleum ether, chloroform, ethanol and water extracts), were used to stimulate the chemosensory afferents. Controlled movements of this electrode were used to deflect the sensillum so as to elicit spikes in the mechanosensory afferents. The same electrode was therefore used simultaneously to evoke and record the spikes of the afferents. The displacement of a sensillum did not deform its short and stout shaft. To identify the sensory receptors on the surface of antennae and tarsus of female *S. littoralis*, scanning electron micrographs of the cuticle surface were taken. The antennae and tarsus were usually rinsed in chloroform then critical point dried following dehydration in ethanol. After drying they were coated with gold-palladium, examined and photographed on a scanning electron microscope (SEM).

Statistical Analysis:

Data were analyzed by one-way analysis of variance (GLM) using computer program (SAS, 2010).

RESULTS AND DISCUSSION:

Data presented in tables 2 & 3 indicate that, the number of egg masses deposited by *S. littoralis* on the *Nerium oleander* leaves treated with petroleum ether extract of *R. communis* (leaves or seeds) was significantly lower than the number of egg masses on control leaves. The same solvent alone did not exert the same effect. Other tested extracts induced significant provable oviposition deterrence (Figs 1 & 2).

Table 2. Mean squares of egg masses, egg numbers and egg hatching of *S. littoralis* affected by material, concentration and their interactions.

S.O.V.	df.	Nr. of Mass		Nr. of Eggs		Nr. of Hatching	
		Seeds	Leaves	Seeds	Leaves	Seeds	Leaves
Rep.	4	0.065	0.12	1.07	3.019	1.45	1.87
Material (M)	3	0.314*	0.21*	1.91**	1.66*	1.98*	2.13**
Conc. (C)	2	0.451*	0.351*	4.87**	3.66**	0.501**	0.72**
M X C	6	0.018*	0.21*	1.98**	1.81*	1.99*	2.42**
Error	44	0.116	0.21	0.825	0.962	0.33	0.38

* & ** denote significance at 0.05 and 0.01 probability levels, respectively

Table 3. Mean number of egg masses, egg numbers and egg viability of *S. littoralis* affected by material and concentration

			Mean No. of Egg Masses		Mean No. of Egg		Mean No. of Egg Hatching	
			Seeds	Leaves	Seeds	Leaves	Seeds	Leaves
Petroleum ether	5%		0.58	0.86	142.1	168.6	97.6	122.6
	10%		0.398	0.51	120	140	92.2	108
Chloroform	5%		0.76	0.89	160	174.2	146.3	138.6
	10%		0.56	0.66	140	156.1	116.2	113.8
Ethanol	5%		0.87	0.68	228.2	187.1	221	197
	10%		0.71	0.351	190.3	148.6	160.6	119.2
Water	5%		1.96	1.3	280	180	267	164
	10%		1.56	0.76	240	130	220.2	122
Control			2.21	2.01	360.5	366	341.8	330.1

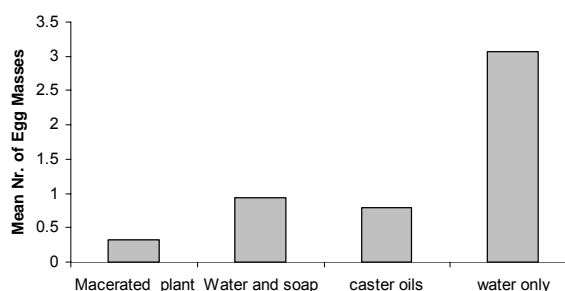


Fig. 1. Effect of castor seeds oil, macerated Castor leaves, and macerated *Nerium oleander* on the mean number of egg masses of *Spodoptera littoralis*.

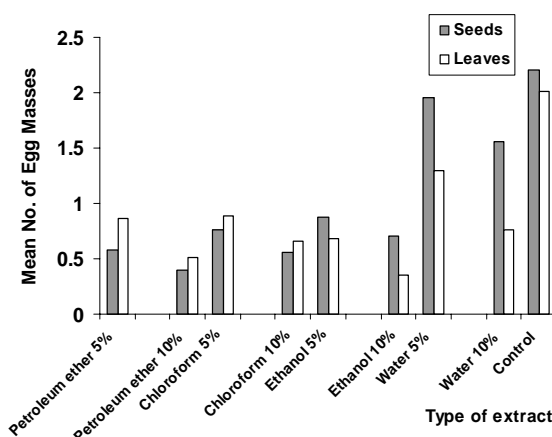


Fig. 2. Effect of castor bean oil seeds and leaf extracts on the mean number of egg masses of *Spodoptera littoralis*.

The ability of petroleum ether to solubilize the oviposition deterrent substances in *R. communis* indicated a moderate polar character of the deterring substances. The results of this study provide information on the oviposition deterrent in *S. littoralis* and the first hints of its chemical nature.

It was clearer that suspension of macerated *N. oleander* in water (100 mg/ml) did not deter oviposition in *S. littoralis*. However, in a suspension of macerated *N. oleander*, oviposition-attracting substances might compete with oviposition-deterrent compounds that were possibly set free by damaged leaves. Crude suspension extract spray at a concentration of 5 % did not significantly reduce oviposition. On the other hand, the strongest oviposition deterrence effect was caused by 10% extract suspension. The minimum percentage of crude water extract suspension exerting significant oviposition deterrence was in the range between 5 and 10%. This laboratory results need more field experiments to confirm oviposition deterrence. The fractionation of petroleum ether extract led to the separation and identification of fatty acids; oleic acid and linoleic acid, however, all of these compounds considered as mosquito larvicidal compounds.

Water leaf extract of *R. communis*, a cultivated plant in tropical countries, showed excellent insecticidal activity against *Callosobruchus chinensis*. Plant volatiles can have inhibitory or repellent effects that interrupt insect responses to pheromones and attract predators and parasitoids to the attacking species after herbivory injury. Gadi *et al.* (2004) review different interactions between plant semiochemicals and insect pheromones, paying attention to those that can result in the development of more efficient and reliable programs for pest control. Our results agree with the results of Trongtokit *et al.* (2005) and Saxena and Basit (2008), where they found that *R. communis* extract acted as inhibitory oviposition.

As previously hypothesized, crude extracts activity was dependent on the type of solvent. Possibly gravid females only avoided egg deposition in response to such changed crude extracts and volatile oil of castor crude extracts. Castor crude extract compounds would indicate unsuitable oviposition sites. Examination of this hypothesis revealed that the oviposition-deterrent activity of crude extracts and material content. These chemicals from eucalyptus, castor, tomato and coriander served as volatile inhibitory oviposition and did not reduce the arrival/stay of the insects on the host plants. Carvacrol had a slight toxic effect on the nymphs, but none of the volatiles was toxic to the adults (Saxena and Basit, 2008). Aqueous leaf extract of *Ricinus communis*, showed excellent insecticidal activity against *Callosobruchus chinensis* and the isolated flavonoids showed potential insecticidal, ovidical and oviposition deterrent activities against (Upasani *et al.*, 2003).

Perception of the oviposition deterrent by the antennae (Fig. 3A&B) did not provide evidence for olfactory perception. Gravid females often could be observed touching the leaves with their antennae. Therefore, perception by chemotactile sensilla should be considered. Electrophysiological experiments are necessary in order to determine the sensilla responding to the oviposition deterrent in *S. littoralis*. The oviposition-deterrent pheromone deposited by females of *Rhagoletis pomonella* is principally perceived by sensilla located on the tarsi (Crnjar *et al.*, 1978). In addition to tarsal and probably abdominal contact chemoreceptors, in females of *Pieris brassicae* also olfactory sensilla located on the antennae show electrophysiological responses to the inherent oviposition-deterrent pheromone of the eggs (Behan and Schoonhoven, 1978). Gaaboub (1990) investigated the antennae of *S. littoralis* males and females by means of scanning electron microscopy and found seven different types of sensilla. Electrophysiological recordings were carried out to study the afferent responses to different concentrations of *R. communis* (5 and 10%) extracted with petroleum ether, chloroform, ethanol and water extracts on the electrical activity of antennal trichoid sensilla (Fig. 3B) and tarsus chaetica sensilla (Fig. 4A&B). The investigation showed that the sensilla were sensitive to all mentioned extracts (Figs 3C & 4C). The results indicated that both the frequency and the amplitude of afferents from sensilla differed according to the type of chemical and its concentration (Figs 3 & 4).

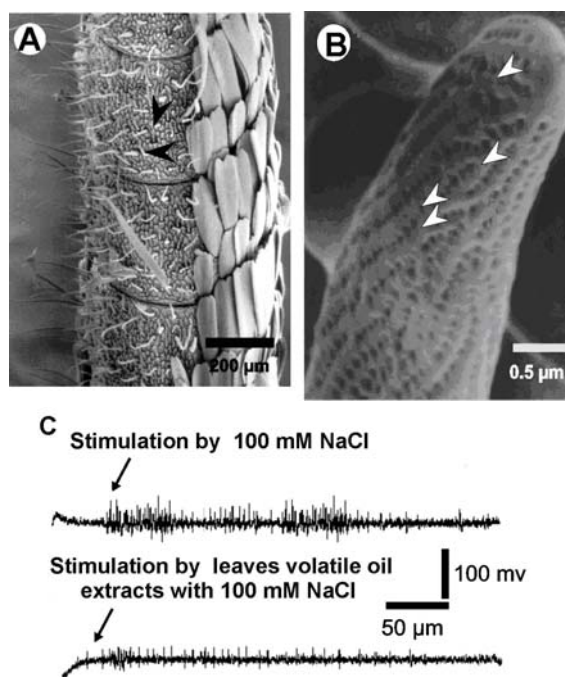


Fig. 3. A. Scanning electron micrographs of antenna of female *S. littoralis*. B. High magnification of sensillum trichodea (white arrows head shown the pores). C. Recording from antennal sensilla (sensilla trichodea) to 0.1M NaCl and also to NaCl 0.1 M with leaves volatile oil extract was used to stimulate the chemosensory afferents.

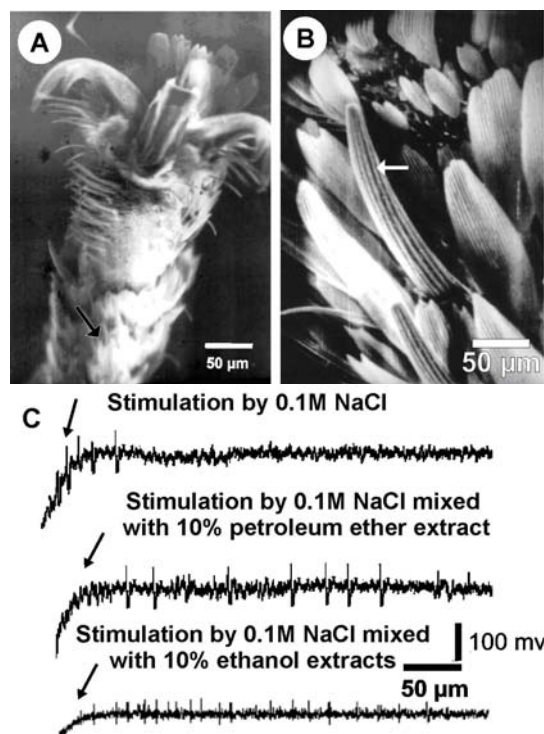


Fig. 4. A. Scanning electron micrographs of tarsus of female *S. littoralis*. B. High magnification of sensillum chaeticum (white arrow). C. Recording from a tarsus sensilla (sensilla chaeticum) to 0.1M NaCl and also to NaCl 0.1 M mixed with 10 % of petroleum ether extract and (C) NaCl 0.1 M mixed with 10% of ethanol extract were used to stimulate the chemosensory afferents.

High concentrations of the stimulation were more effective than low concentrations. Plasticity of odour-guided behaviour and matching plasticity of central nervous processing of pheromones have been shown to occur in the moth, *A. ipsilon* (Gadenne and Anton, 2000). Sensitivity of interneurons to sex pheromones was found to be age- and hormone-dependent, whereas central processing of plant volatiles occurring in food sources was age-independent (Masante-Roca *et al.*, 2002). Two different response types

occurred, in most cases the chemical sensitive neuron began to fire immediately upon stimulation, followed by a period of decreasing frequency as adaptation occurred. Some neurons, however, showed an initial latency of around 100ms, followed by a period of increasing frequency. Both types were due to the activity of a single neuron in each sensillum, and in both cases. After a suitable recovery time (10 min), it was possible to record other responses (White and Chapman, 1990).

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الاستجابات الكهربية و وضع البيض لدودة ورق القطن المصرية *Spodoptera littoralis* المعاملة بأوراق و بذور زيت الخروع

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أوضحت النتائج أن مستخلص البتروليم اثير هو الأعلى فاعلية عند مقارنته بمستخلصات الماء، الايثانول والكلوروفورم. كذلك أوضحت نتائج الدراسة الالكتروفسيولوجية بأن المستقبلات الكيماوية الموجودة على كل من الرسع وقرون الاستشعار تتأثر بجميع المستخلصات المنبّهة و بأنها تلعب دورا هاما في قدرة إناث فراشات دودة ورق القطن على تحديد المكان المناسب لوضع البيض.

المحكمون:

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تعتبر العلاقة بين المستقبلات الكيماوية بالملامسة و وضع البيض لأنثى الفراشات في الاماكن الملائمة في الحقل ذات اهمية لكل من الطور اليافع و الاطوار الغير الكاملة. يهدف هذا البحث إلي دراسة تأثير معاملة أوراق التفلة بمستخلص من أوراق و بذور نبات الخروع على دودة ورق القطن. حيث تم دراسة معدل وضع البيض و التأثير الالكتروفسيولوجي على شعيرات الرسع للطور اليافع وذلك عند المعاملة بالتركيزات المستخدمة (5% , 10%). أثبتت النتائج أن المعاملة بتركيز 10% لمستخلص ناتج من ورق و بذور نبات الخروع تحدث تأثيرا معنويا على معدل وضع البيض بينما المعاملة بتركيز 5% للمستخلص فكان غير معنويا . على الجانب الآخر فإن أقل تركيز لمستخلص ناتج من ورق و بذور نبات الخروع المعامل بأوراق التفلة تحدث تأثيرا فعالا في منع وضع البيض تراوح بين 5-10%. كما