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FIELD APPLICATION OF TICK-DECOY COATED WITH PHEROMONE TECHNOLOGY AS A MEAN OF *BOOPHILUS ANNULATUS* TICK CONTROL

(With 5 Tables and 2 Photos)

By

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التطبيق الحقلى لتقنية مصائد القراد المغطاة بالفيروفون كوسيلة لمقاومة قراد البووفيلس انيولاتس

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فى هذا البحث تم استخدام تقنية "مصائد القراد" فى مقاومة قراد الأبقار نوع "بووفيلس انيولاتس" حيث تم التعرف وتحليل الفيرمونات (الجاذبات الجنسية) المستخلصة من اناث القراد باستخدام طرق التحليل الكروماتوجرافى (الالواح الرقيقة وجهاز الكروماتوجرافى ذو الضغط العالى) ووجد استجابة عالية فى اجتذاب ذكور هذا القراد نحو الفيرمون المحضر للاناث وصلت الى ٦٦و٥٦% ووصلت نسبة موت ذكور القراد عند استخدام هذه المصائد البلاستيكية المغطاة بالفرمونات الى ٣٣و٨٣% وقد أجريت تجربة حقلية لمعرفة كفاءة هذه المصائد المغطاة بفيرمونات هذا النوع من القراد (محتوية على مبيد الاكتومين ١٠%) على ثمانية بقرات مصابة بعدوى طبيعية بالقراد (٢٥ من الذكور، ٢٥ من الاناث على كل حيوان) وقد تم تقسيم هذه الأبقار الى مجموعتين، مجموعة مكونة من خمس بقرات (وضع على كل منها ٥٠ كبسولة بلاستيكية) بالاضافة الى مجموعة ضابطة مكونة من ثلاث بقرات.وبعد وضع الكبسولات البلاستيكية لوحظ انجذاب معظم ذكور هذا القراد نحوها مما أدى الى موتها(٧٦%) نتيجة لاختلاطها بالمبيد. اما بالنسبة لاناث هذا القراد فتم حفظها فى حضانة بيولوجية حيث وضعت بيضا غير مخصب.

SUMMARY

Eradication of the cattle tick "*Boophilus annulatus*" was achieved by using the tick decoy technology. The identification and analysis of the female tick pheromone composition were carried out using thin layer chromatograph (TLC) and high pressure liquid chromatography (HPLC). Bioassays were done to determine the *B.annulatus* male responses to the prepared *B.annulatus* female pheromone. The results showed high male responses (56.66%). Application of manufactured pheromone –

pyrethroid decoys of the male *B.annulatus* ticks gave great mortality of ticks (83.33%). A field trial to evaluate the efficiency of tick decoys coated with *Boophilus annulatus* pheromones was done on eight cows. Decoys containing 10% ectomin were used. Each cow was naturally infested with 25 female and 25 male *Boophilus annulatus* ticks. Fifty pheromone ectomin impregnated decoys were glued in the predilection seats to each cow of the experimental group. Following decoys attachment, male ticks began to detach, attracted to the decoys and died with mortality rate reached 76%. Female ticks after incubation laid unfertile eggs.

Key words: *Boophilus annulatus, pheromone, Decoys, Thin layer chromatography (TLC) and High pressure liquid chromatography (HPLC).*

INTRODUCTION

Control of ticks in Egypt, usually depends upon traditional methods e.g. Dipping, spraying and pour on using different acaricides e.g. chlorinated hydrocarbons, organophosphates and naturally as well as synthetic pyrethrins (Ristic, 1988).

Biological control is now tried as a safe, economic and effective method for tick eradication. Methods of biotechnology and recent advances in instrumentation have enabled scientists to begin to appreciate the incredible diversity of compounds incriminated in chemical communication used by animals to transmit information between individuals.

Pheromones are chemicals emitted by living organisms to send messages to individuals of the same species (Karlson and Luscher, 1959). The use of pheromones offers an exciting, innovative approach to future development of tick control techniques. Pheromones are likely to be most effective when used in combination with toxicants. Pheromones may be used as lures (Gladney *et al.,* 1974), sexual confusants (Ziv *et al.,* 1981), assembly agents or even as repellents (Sonenshine, 1985).

Mating behavior in ticks is regulated by sex pheromones produced by female ticks which are used to attract conspecific males. Tick control can be achieved with pheromone traps with a technique called "tick decoy", in which synthetic pheromone impregnated decoys lead to mating disruption and the number of mating and off springs is reduced. Fifty percent of *Amblyomma variegaturn* female population were able to find upwind-positioned targets containing the synthetic

aggregation – attachment pheromone of this species or the pheromone component O- nitrophenol alone (Hess and De-Castro, 1986)

Three low molecular weight compounds were found in hexane: diethyl ether extracts of fed males of the African ticks, *Amblyomma variegatum* (tropical bont tick) and *A. hebraeum* (bont tick), namely, o-nitrophenol, methyl salicylate and 2,6-dichiorophenol (Lusby *et al.*, 1991).

Unfed adults and nymphs of the bont tick *Amblyomma hebraeurn* were attracted to hosts on which fed males emitting an aggregation-attachment pheromone (AAP), were present. Pheromone /acaricide mixtures (decoys) have the potential to selectively attract and kill these ticks. The effects of three acaricides, amitraz (an amidine), flumethrin (a synthetic pyrethroid) and chlorfenvinphos (an organophosphate), combined with AAP, on the attraction and attachment of the unfed adults were investigated. Flumethrin caused rapid and high mortality in attached and unattached ticks and was superior to the other two acaricides (Norval *et al.*, 1991 a,b).

Unfed adults of the African ticks, *Amblyomma hebraeuni* and *A. variegaturn,* were exposed to volatile compounds in an olfactormeter in efforts to identify both tick-produced or synthetic chemicals capable of eliciting an attraction response. They concluded that ticks respond positively to a variety of volatile chemicals (synthetic pheromone), which may conceivably be used to attract them to traps, animals or acaricides in efforts to control ticks or the diseases they transmit (Yunker *et al.*, 1992).

The presence of a mounting sex pheromone on the surface of fed female dog tick *(Rhipicephalus appeniculatus)* was demonstrated. This pheromone, which is present on the female cuticle, allows the male to recognize the female. The pheromone was removed by cleaning the female in hexane, resulting in the loss of male mating behavior in vitro experiments. Male mating behavior was resumed when extract made from fed female cuticle was replaced on cleaned females. When the extract was transferred to inanimate objects typical male mating behavior was released. Preliminary chemical analyses indicated that the active component of the extract was contained in the sterol ester fraction of the extract (Hamilton *et al.*, 1994).

Studying the pheromone composition using TLC and HPLC explained that cholesteryl esters were found to constitute a major component of the lipids coating the body cuticle of females of the camel tick, *Hyalomma dromedarii* and the brown dog tick, *Rhipicephalus*

sanguineus. One or more cholesteryl esters, alone or in combination, have been shown to serve as the mounting sex pheromone of several species of ixodid ticks (Sobbhy *et al.*, 1994).

The use of decoys coated with *H. dromedarii* female pheromones and impregnated with cyfluthrin experimentally on infested rabbits showed the attraction of fed males toward decoys and 72.77% mortality among ticks were recorded. Also the eggs from the survived females reached only 20 – 23 hatching (Fahmy and Aggour, 1995).

A test to evaluate the efficacy of tick decoys impregnated with *Hyalormma dromedarii* pheromones on nine camels was performed as a first trial in Egypt. They used tick decoys containing 10 % cyfluthrin of decoy weight. Each camel was naturally infested with about 25 female ticks and 25 male ticks. Six of animals were served as the experimental group while other three were served as the control group. About 50 pheromone / cyfluthrin impregnated decoys were glued to each camel in the experimental groups (total 300 decoys). Following decoys attachment, male ticks began to detach and were attracted to the decoys, many died and dropped off. Female ticks after incubation either died without oviposition or laid infertile eggs, presumably because they were never inseminated (Aggour and Abd El-Gawad, 1995).

MATERIALS and METHODS

Materials

1. Ticks (for pheromone preparation): About 2000 ticks were collected from naturally infested cows from different investigated farms, and identified according to Hoogstraal (1956).

2. Tick extract solvent: Hexane: diethyl ether (90: 10) GC-grade.

3. Standards GC-grade (from sigma): Cholesteryl esters, Cholesterol, 2,6- Dichiorophenol (DCP), Triglycerides and Fatty acids. Each standard was put in a labeled vial, sealed and kept in freezer.

4. SOLVENTS for THIN LAYER CHROMATOGRAPHY (TLC): Petroleum ether, diethyl ether and acetic acid.

5. PLATES: High-performance precoated silica gel, glass backing plates were used. HPTLC type (LHP-K).

6. Drummond micropipette: for application of samples on the plates.

7. High Pressure Liquid Chromatography (HPLC): Waters HPLC system comprising a model 721 system controller for the UV detector), Paired model 510 pumps, Model U6K injector, Model 996 photodiode array detector (PA), Whatman ODS-3 reversed phase analytical column

25 cm x 6.35 m.m and control of all PA and pump operations was done with the Waters Millennium system.

Methods:

1. Comparison between standards (cholesteryl esters, cholesterol, triglycerides and fatty acids) and tick extract using thin layer chromatography (high performance thin layer chromatography plates) according to Fried and Sharma (1982)

Purpose:

to determine the lipids present in tick extract, especially cholesteryl esters, cholesterol, free fatty acids and triglycerides.

Procedures:

1.1. Preparation of tick extract: 1000 engorged female *Boophilus* ticks were placed in a mixture of pure GC- grade Hexane: diethyl ether (90: 10) in a glass container with a ground glass stopper and put on shaker for 2-3 minutes. This mixture was concentrated to about 1-2 ml under a gentle stream of nitrogen gas, then transferred to a glass ampoule with a conical bottom and sealed carefully (using Teflon and paraflim paper), after that stored in freezer until ready for use (at -20 °C).

1.2. Preparation of standards: 1 mg / ml (1 µg / 1 µL) hexane using a chromatographic microbalance (sensitivity 0.1 µg). gc/ms grade. The standards (Cholestryl esters, cholesterol, 2,6, DCP, Triglycerides and Fatty acids) obtained from Commercial Supplier (Sigma) were weighed out and adjusted to a concentration of 1 mg / ml. 2 ml total for each standard was put in a labeled vial, sealed and kept in freezer.

1.3. Preparation of solvents: The solvent system was prepared as follows: total 300 ml for each. according to System I (Mangold, 1969): Petroleum ether :diethyl ether :acetic acid (80: 20 : 1) or System II (Skipsi *et at.*, 1965):

a) Petroleum ether: acetic acid (96: 4)

b) Petroleum ether: diethyl ether: acetic acid (90: 10: 1)

The solvent was placed in a rectangular glass tank with lid. The tank was lined on three sides with thick filter paper that is thoroughly soaked with the solvent. The tank allowed to stand for 30 min. to one hour to allow the inside atmosphere to be saturated with solvent vapor.

1.4-Plates: High-performance (HP) precoated silica gel, glass backing plates were used. Clean HPTLC plates by placing them in the tank containing any solvent prepared and then dry (oven or air dry), then divided into 5-6 lanes.

1.5- Application of samples: 5 µl of each sample was applied by using Drummond micropipet. Dry samples thoroughly before development

(hair drier is used). Plates were placed in the tank containing the solvent. After development, the plate was removed and oven dried at 100°C for 3 mm. Next the plate is sprayed with 50 % H_2 $S0_4$ and 50 % Methanol and dried in an oven (90 - 100 °C) and cooled to room temperature. The spots visualized in this manner are examined and the spots in the sample lane are compared with the spots in the different lanes for the standards. Spots that have the same R*f* value (± 5 %) are considered to be the same class of compound. Tick extract spots are compared with those of the standard, to determine their identification to class.

2. Analysis using high pressure liquid chromatography. (HPLC)

Purpose:

- Measuring the retention time and maximum wave length of peak of tick extract.
- Comparison between curves of standards and curves of tick extract, using the Waters photodiode Array Detector and Millennium Software System.

Procedures:

2.1. Preparation of solvents: The solvents (Isopropyl alcohol & Acetonitrile) were filtered and degassed for at least 20 minutes. The flow rate was 120:80 respectively.

2.2. Analysis of data was done using millennium.

3. Manufacturing and testing of tick decoys: According to (Aggour and Abdel-Gawad, 1995)

Procedures for manufacture of tick decoys: 220 grams of PVC resin (Goodrich Geon 138) were mixed with 220 cc dioctylphthalate. Then 20 ml Drapex 6.8 stabilizer was added together with 6 ml of Mark Red anti-oxidant. All ingredients were mixed thoroughly with the electric mixer until the mixture was unifonnly mixed and could be poured. The mixture was allowed to stand overnight. Sufficient 2,6-dichiorophenol was added to equal 0.1 % of the final product (approximately 220 mg). Sufficient acaricide (Ectomin) was added to equal 10 % of the 220 grn. The mixture was poured into molds and placed in the oven at 145 °C for 20 minutes. a mixture of standards proved to be found in the tick extract in hexane was prepared to be deposited on the plastic decoys. The mixture contained also butylated hydroxytoluene 1 % by weight of the final mixture.

4. Bioassay: Two types of bioassay were carried out according to (Hamilton and Sonenshine, 1988) on conspecific and on, inanimate objects (decoy). All male ticks were prescreened to ensure that only that were responding to females were used in bioassays. Females were also

prescreened to ensure that they we-re capable of exciting a mating response by the male before being cleaned. The females were cleaned and delipidized using Hexane to ensure that they would no longer attract males before the extract was applied. Bioassays were performed in the laboratory at approximately 25°C on filter paper in a 9-em-diam. Petri dish. The response of the male when presented to the imobilized female was scored by a system based on the mating behavior sequence described by Sonenshine (1985). The female was immobilized by attaching its anterior end to adhesive tape. The mating behavior sequence used in these experiments was as follows: Orientation of the male towards the female or "dummy" female (decoy), awareness of the female (touching with appendages, mounting of the female, turning on the dorsal surface of the female and movement of the male to the ventral surface of females. Each stage was given one point when the male had achieved that stage. The number of points gained by each male was recorded. The first two stages were not included in the final analysis as they are a measure of the attractiveness of the 2,6-DCP. Thus a completely successful male would gain three points. The number of points gained by the males in their response to female extracts was totaled and compared to the control for comparing percentages.

5- Field application of tick decoy technology:

Animals: Eight cows naturally infested with *Boophilus annulatus* ticks; five of them were used as experimental group (ticks and decoys) while the other three ones served as control group (ticks only).

Ticks: Each cow was naturally infested with 25 semi-engorged female and 25 active male *Boophilus annulatus* ticks

Sex Pheromones: *Boophilus annulatus* sex pheromones were synthetically prepared from authentic standards (Sigma).

Decoys: The plastic decoys used were prepared at Biotechnology Dept., Animal Health Research Institute Giza, Egypt; according to (Aggour and Abd EL-Gawad, 1995). Mixture of the pheromone constitutes in hexane was deposited on the plastic decoys shortly before application.

Experiment: The experiment was done according (Aggour and Abd EL-Gawad, 1995) as follow: Fifty pheromone/ectomin-impregnated decoys were attached to each cow in the experiment group by using Pattex contact cement along the back, neck, back of head, near anus, near udder and elsewhere (Photo 1).

The eight cows were isolated in a clean stall to avoid additional tick infestation; the floor was provided with double sided barrier to capture the fallen ticks for ease and accurate observation.

Observations on the behavior of the ticks were recorded within shortly after applications on the decoys and then daily for 10 days as follows:

- Number of males attempt to mount decoys.
- Number of males dropped dead on the floor.
- Number of males dropped live on the floor.
- Number of males attached dead on animals.
- Number of males attached live on animals.
- Number of mated engorged females attached to each animal.
- Number of decoys still attached on animal.
- Number of decoys dropped on ground.

The living ticks (male and females) were incubated in a biological incubator at 28° C and 75% R.H. and the engorged females were observed for oviposition and egg hatching.

RESULTS

1. Qualitative comparison between standards and pheromonal tick extract using (TLC)

Analysis of pheromonal tick extract was done in comparison with standards using High-Performance (HP) precoated silica gel, glass backing plates. The solvents used were petroleum ether, diethyle ether and acetic acid.

The results showed that the tick extract contains the following cholestryl esters: cholesterol, ch. linoleate, ch laurate, ch. linolenate, ch. oleate, ch. myristate, ch. butyrate, ch. stearate, ch. palmitate. In regard to Triglycerides, the tick extract matched with Tnuinolein, Trilinolenin, Trilaurin, Tri-ecosenoin, Tripalmitin, Tripalmitolin and Tricaprylin. Concerning the fatty acids, only three fatty acids developed and matched with the tick pheromone. Those were Linoleic acid, Linolenic acid and Arachidonic acid.

2. Quantitative comparison between standards and *B.annulatus* extract pheromone using HPLC: Quantitative analysis of the *B. annulatus* pheromone and its comparison with the different standards (Cholesteryle esters, Triglycerides and Fatty acids) were done using Waters Millennium system, using isopropyl alcohol and acetonitrile solvent at flow rate 120:80 respectively. The matching was based upon measuring the retention time and maximum wave length of peak of tick extract and those of the standards. The results obtained were displayed in Table (1).

Table 1: The component of the *B. annulatus* pheromone from the authetic standards using HPLC analysis

Cholesteryl Esters	Conce. µg/ tick	Triglyceride	Conce. µg/ tick	Fatty acids	Conce. µg/ tick
Ch Oleate	4.6	T. Laurin	121. 4	Stearic	8. 4
Cb Hexanoate	10. 1	T. Caprin	20. 3	Oleic	5 .8
Ch Lrnolenate	8. 1	T. Elaidin	17. 4	Palmitic	4 .3
Ch Butyrate	9. 2	T.Palmitin	20. 6	Arachidonic	1 .7
Ch Palmitate	5. 6	T. Stearin	22. 1	Arachidic	4. 9
Ch Hnoeate	3. 8	T. Caproin	15. 9	Linoleic	22. 5
Ch.Myristate	4.9	T.Linolein	67. 8	Linolenic	12.8
Ch.Stearate	4.7	T.Caprylin	60. 2	Lauric	14.1
Ch Laurate	1. 2	T. Palmitolein	143. 6		
Cholesterol	12. 2	T. Ecosenoin	117. 4		
2,6-DCP	1.0				

3. Bioassay

The results of tests of *B. annulatus* male responses to the synthetic mixture representing the *B. annulatus* pheromone were summarized in Table (2). Untreated and delipidized (body surface washed with hexane to remove lipids) females as well as manufactured decoys were used in the test. Bioassays were done with sexually active *B. annulatus* males and scored. 2,6- dichlorophenol was applied (10-20. µg) to all test subjects to excite male mate seeking behavior. The first and second stages are not included in calculation. Responses in excess of 20% were considered positive.

Table 2: Results of bioassay of *B. annulatus*

Compound tested	No. of positive male responses	% positive
Living untreated control females	19 /30	63.33%
Synthetic mixture on delipidized females	17/30	56.66%
Synthetic mixture on manufactured decoys	16/30	53.33%
Delipidized female with hexane only	2/30	6.66%

Pheromones- acaricide mixture was added to dummy ticks (decoys) as a means of tick eradication using biotechnology. Pyrethroid (Ectomin) treated pheromonal coated (10% w/v) and non- pheromonal decoys were applied on male *B. annulatus* tiks and the results are shown in Table (3).

Table 3: Effect of Pheromone-Acaricide Decoys on Male *B. annulatus* Ticks.

Compound tested	No.of positive male responses	% of responses	No. of dead males	% of death
Pheromonal coated decoys	51 /90	56.66 %	25/30	83.33%
Non coated decoys	0/30	0 %	0	0%

Table 4: Results of testing of tick decoys for *B. annulatus* naturally infesting cattle.

*Observations	ANIMALS															
	Experimental group										Control group					
	1		2		3		4		5		6		7		8	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
No. of males mounting decoys	17	68	13	52	11	44	14	56	15	60	-		-		-	
Dropped dead♂♂	3	12	5	20	3	12	4	16	5	20	-					
**Dropped live♂♂	8	32	7	28	6	24	8	32	11	44	-					
Attached dead♂♂	2	8	1	4	3	12	2	8	3	12						
Attached live♂♂	9	36	11	44	12	48	10	40	10	40	25	100	25	100	25	100
Total dead males	15	60	13	52	12	48	15	60	19	76	-					
No of mated engorged female	8	32	12	48	7	28	9	36	6	24	14	56	17	68	18	72
No of decoys still on animal	42	84	38	76	33	66	39	78	40	80	-		-		-	
No of decoys dropped on ground	8	16	12	24	17	34	11	22	10	20	-		-		-	

* The observations period was ten days from the beginning of the experiment

** All dropped males were died after 1-5 days post-incubation

Table 5: Biological periods of *Boophilus annulatus* females in experimental and control groups.

Biological periods (in days)	Preoviposition Mean ± SE	Oviposition Mean ± SE	Pre-hatching Mean ± SE	Hatching Mean ± SE	Hatching % Mean ± SE
Experimental group	4-8 5.88(±1.41)	5-6 5.45(± 0.5)	21-24 22.73(±0.91)	12-15 13.38(±0.98)	0-25 (13 ± 1.07)
Control group	3-4 3.65(± 0.48)	8-9 8.53(±0.50)	17-21 19.67(± 1.16)	7-10 8 .4 (±0.99)	70-100 82.18(± 1.68)

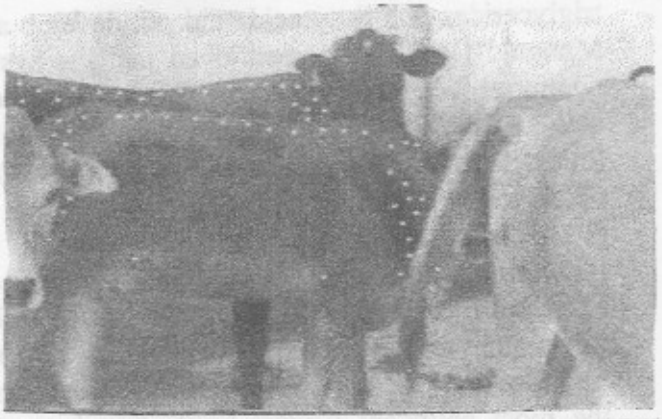

Photo 1: Distribution of tick decoys on the body of experimental cows.

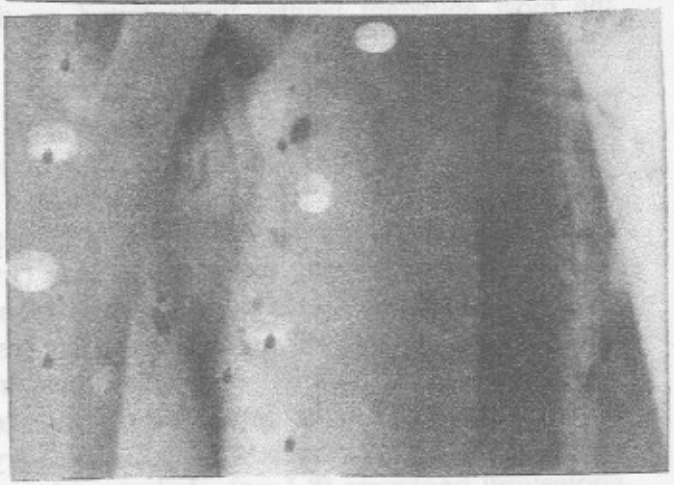

Photo 2: Attraction and mounting of male tick to pheromone impregnated tick decoys and natural females

DISCUSSION

Pheromones are compounds released by individuals of tick species which regulate the behavior of others of that same species (Karlson and Luscher, 1959). Methods of biotechnology and recent advances in instrumentation have enabled us to begin to investigate the pheromones of *B. annulatus* and enabled us to prepare it synthetically to be used in combination with effective acaricides in the form of what is called decoy trying to control the tick.

Thin layer chromatography using (HP) precoated silica gel and petroleum ether, diethyl ether and acetic acid solvents according to the systems of Mangold (1969) and Skipsi *et al.* (1965) was used for the qualitative analysis of the *B. annulatus* pheromone and the results revealed that the pheromone contains cholesterol and 8 cholesteryl esters in addition to 7 triglycerides together with 3 fatty acids. Some standard

triglycerides and fatty acids did not develop at all, and this agrees with Mangold (1969) who mentioned that the system does not separate all commonly occurring neutral lipids in biological materials. Several authors used the same technique for analysis of biological compound for lipid material among them: Scott (1969) recognized glycerides, phospholipids, cholesterol and cholesteryle esters, Fried and Pucci (1976) who perfonned various analytical TLC studies on arthropods and flatworms and had detected mainly triglycerides, free sterols, and phospholipids. Also Sobbhy *et al.* (1994) applied thin layer chromatography (TLC) on extract of *H. drornedaril* and *Rhipicephalus sanguineus* ticks and found that cholesterol and cholesteryl esters were the most abundant neutral lipids found on the body surfaces of fed females of these two species.

High pressure liquid chromatography (HPLC) is used for the analysis of the *B. annu/atus* pheromone and its comparison with the different standards (cholesteryl esters, triglycerides and fatty acids) using Waters Millennium HPLC system with isopropyl alcohol and acetonitrile solvent at a flow rate of 120 :80 respectively. The matching was based on measuring the retention time (from the beginning of the run till the mid base of the peak) and maximum wave length of peak of tick extract and those of the standards. The results, which done for the first time on *Boophilus annulatus* ticks in Egypt, revealed that *B. annulatus* pheromone composed of cholesterol (12.2 µg), 2,6-DCP (1 µg), cholesteryl oleate (4.6 µg), cholesteryl hexanoate (10.1 µg), cholesteryl linolenate (8.1 µg), cholesteryl butyrate (9.2 µg), cholesteryl palmitate (5.6 µg), cholesteryl linoleate (3.8 µg), cholesteryl myristate (4.9 µg), cholesteryl stearate (4.7 µg), cholesteryl laurate (1.2 µg) and trilaurin (121.4 µg), tn caprin (20.3), trielaidin (17.4 µg), tripalmitin (20.6 µg), tristearin (22.1 µg), tricaproin (15.9 µg), trilinolein (67.8 µg), tricaprylin (60.2 µg), tripalmitolein (143.6), tri-11-ecosenoin (117.4) and stearic acid (8.4 µg), oleic acid (5.8 µg), palmitic acid (4.3 µg), arachidonic acid (1.7 µg), arachidic acid (4.9 µg), linoleic acid (22.5 µg), linolenic acid 912.8 µg) and lauric acid (14.1 µg).

Studies on tick pheromones especially those involving sex attractants of more or less similar chemical structure is promising. These studies provide an essential theoretical base of knowledge that may lead to practical application in controlling ticks of medical and veterinary importance.

To the best of our knowledge, there are no data regarding sex pheromone released by *B. annulatus* ticks. Female produced pheromones

have been reported in at least four genera of Ixodidae, the first conclusive proof of a pheromone in ticks was presented by (Berger, 1972) who detected 2,6-dichiorophenol from feeding *Amblyomma* americanum females. A sex attractant pheromone 2,6- DCP which stimulates localized attraction of males to females has been identified in a wide variety of ticks (Sonenshine, 1985). However, sex pheromones released by feeding females of ixodidae ticks stimulate feeding males to detach. These pheromones provide directional information for orienting to recognizing and copulating with the emitting female (Berger, 1972; Kellum and Berger, 1977). Leahy *et al.* (1981) demonstrated the presence of two female pheromones in the metastriate tick *Hyalomma dromedarii.* These two pheromones were attractant sex pheromone and mounting sex pheromone (MSP). Later on, (Sobbhy *et al.* 1994) were able to extract and identify the mounting sex pheromone of *H. drornedarii* to be a mixture of six different cholesteryl esters.

The results of our bioassays clearly indicate the presence of a cuticular sex pheromone, the mounting sex pheromone (MSP), produced by *B. annulatus* (Table 3). Following their attraction by 2,6-DCP, males encountering this pheromone mount the females and apply their legs and mouth parts against the female body surface so that the male body is in direct contact with the female. Then, while still in close contact, they move to the female's venter and probe. Thus, this sex pheromone mediates the mounting phase of *B. annulatus* courtship behaviour, as described by Sonenshine (1985) on ixodid ticks. Our findings showed that this cuticular sex pheromone is essential for recognition of the females as a potential mating partner. Males are unable to recognize females lacking this cuticular pheromone as potential mates, and these males terminate their courtship activities.

However, the male responses were restored by depositing the extract onto the bodies of surface-delipidized females or even transferred onto inanimate objects (dummy ticks), evidence which supports the hypothesis that this represents pheromone-mediated behavior.

Attractant decoy technology has many advantages over conventional methods for tick control as spraying, dipping or pour-ons. Firstly, we use only very tiny amount of the acaricides when compared with other methods. So, we can avoid or minimize the pollution of the environment with these toxic chemicals as well as this was beneficial from economic view. Also the toxicity which resulted from using these acaricides due to cumulative effect of their residues in the blood and tissues of animals and human can be reduced. Moreover, the decoys

were easy to manufacture and require no specific training for application , making it farmer-friendly technology.

The attractant decoy technology was applied in Egypt for the first time on experimentally infested rabbits by Fahmy and Aggour (1995) or on naturally infested camels with tick by Aggour and Abd El-Gawad (1995) where the pheromone / cyfluthrin impregnated, decoys gave a good results, A high percentage of *Hyalomma dromedarii* males ranged from 48 - 76 % were attracted by these decoys. Also, most of these males died and the mortality rate reached 72 %. Also engorged females either gave infertile eggs or laid very small egg mass with very low hatching percentage. This probably is due to killing of most males so that females could not be inseminated or even mounted.

Our results showed that using of acaricide impregnated decoys stimulates the male responses towards the decoy by 56.66 % and the eradication of ticks was achieved successfully with 83.33 % tick mortality (Table 3).

The attractant decoy technology was applied on naturally infested cattle with *B. annulatus*. These decoys gave good results as attracted high percentage of males ranged from 44-60% with mortality percentage among these males reached 76%. The engorged females collected from treated cattle with decoys either gave unfertile eggs or laid very small egg mass with very law hatching percentage 0-25 (13 ± 1.07). This referred to killing of most males, so that females could not be inseminated.

From the aforementioned results, it was clear that application plastic attractant decoys for controlling of *B. annulatus* in Egypt will be effective.

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