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STUDIES ON PRESENCE OF SEMIOCHEMICALS FROM *PHENACOCCUS SOLENOPSIS* (TINSLEY)

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ABSTRACT

The mealybug, *Phenacoccus solenopsis* (Tinsley), is a serious pest in Vadodara agricultural fields in India. In an attempt to find solutions to reduce the infestation by this pest,simple behavioural bioassays were conducted under the laboratory conditions using semiochemicals obtained through n-hexane solvent- volatile extract of female *Phenacoccus solenopsis.* Observations were made at the rate of attraction of male and female of mealybug towards female volatile extracts which was isolated by both confinement and adsorbent methods. The result obtained during behavioural bioassay showed that the attractive index of males towards female volatile extract collected from adsorbent method was higher (0.86) than the females. This indicated the presence of a semiochemicals as a sex attractant in the female volatile extract which could attract comparatively more males. The further isolation of volatile extract by using GC-MS shows the presence of ester and terpenes compound. Hence, the information contained in this paper pave way to the identification of proper management practices to maximize lure methods during effective reproductive period and also useful in providing good scope for further development of ecofriendly methods for *Phenacoccus solenopsis* control.

KEY WORDS: ***Phenacoccus solenopsis,*** **Attractive index, GC-MS, Semiochemicals**

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INTRODUCTION

The earlier studies on the populations of the mealybug, *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae) reveal its economic importance as a major concern for all economically important crops in agriculture fields of Vadodara[1]. It was reported that *P. solenopsis* is the major threat to the agricultural and horticultural crops in many tropical and subtropical countries[2], attacking 159 hosts plant species belonging to 21 different families[3]. In North India (Punjab, Haryana and Rajasthan), mealybug has taken a leading position among sucking pests whereas in Central India (Gujarat and Maharashtra) and South India, it showed a moderate incidence of damage[4]. *P. solenopsis* causes yield loss by directly infesting leaves, stems, fruits, roots and also cause damage indirectly by secreting honey dew which cause development of sooty mold as well as attracts ants as transporting agents of the mealy bugs to different host plants[5]. During 2006, the mealybug caused economic damage, reducing the cotton yield by up to 40-50 % in infested fields in several parts of Gujarat[6]. To protect their crops from damages of mealybug infestation, growers often apply synthetic chemical insecticides such as organophosphates, carbonates, synthetic pyrethroids and nicotonoids. Although continuous usage of synthetic insecticides caused health hazards, development of pest genotypes resistant to pesticides, resurgence and upset by pests and environmental pollution[7] as well as hazardous to the environment and non-target insects such as pollinators and predators[8,9]. The outbreaks of *Phenacoccus solenopsis* in Indian agricultural fields necessitate the studies on the development of biorational alternative for control of *Phenacoccus solenopsis.*

MATERIALS AND METHODS

a) Mass multiplication of Phenacoccus solenopsis under laboratory condition

The rearing and breeding of *Phenacoccus solenopsis* was carried at room temperature (24.3-31.2^{o}C, 48.9-71.7% R.H.) in the laboratory. The cotton twigs, harboring *Phenacoccus solenopsis* colonies, were collected from the fields and brought to the laboratory. The mealybugs present on these cotton twigs were gently removed with the help of soft camel hair brush and were released on the cotton twigs (Twigs end was wrapped with wet cotton) kept in plastic box (20 x 15 cm^2) in laboratory. Newly hatched crawlers were collected from the ovipositing female of laboratory culture and were placed onto the cotton twigs for mass rearing. Culture obtained was used for collection of volatiles and behavior bioassays experiments.

b) Collection of volatile extract from mealybugs Phenacoccus solenopsis

Though many methods are available, the following methods were used in isolating the volatile from mealybugs:

i. Confinement and wash method

More than 1000 of adult female mealybugs were kept in the sterilized conical flask of 200 ml, having Whatmann filter paper at the base and covered by the silver foil. After 24 hrs, the females were removed and entire surface of the conical flask was washed and rinsed with little quantities of the solvent n-hexane. The crude extract was also used for the simple bioassay.

ii. Adsorbent method[10]

The airborne volatile collection apparatus was constructed by JANCOS, Vadodara. This method involved placing of adult female mealybugs into an airborne volatile collection apparatus, through which filtered air passed on to a narrow glass tube whose inside diameter of 11.5mm, length of 125 mm and packed with an adsorbent (2g Tenax-TA 60-80 mesh, Sigma-Aldrich, Bangalore) for 4-5 days. Insects were exposed to air flow for 24hrs with equal light and dark regimes at 24± 2^{o}C. The trapped volatiles were extracted from adsorbent using 10 ml of n-hexane. This solution was then used for bioassay studies.

c) Behavioral Bioassays[11]

The studies conducted in dark room with live male and female to avoid any visual contact, indicated the presence of pheromonal attraction between sexes. Simple bioassay was conducted by using petri plate having two small filter papers. One was treated as control

having n-Hexane and other was treated as test which was applied uniformly to female volatile extracted obtained by using confinement and adsorbent methods respectively. In the first trial, 60 females were released in an experiment set consisting of female volatile extracted by using confinement method. The above experiment was also conducted by using female volatile obtained through adsorbent method. In the next set of experiments, 60 males were released in the experimental petri plate containing female volatile extract obtained by using confinement methods and adsorbent method. Each experiment was repeated three times. The response of each sex to the collected volatile by different method was studied by counting the number of adults attracted towards it.

Based on the data collected as described above, the attractive index (AI) was worked out as below:

AI= (No. of insects responded to test material - No. of insects responded to control) ÷ (No. of insects released -No. of insects responded to control)

The collected data were statistical analyzed by IBM SPSS statistics - 19 software for χ^2 (chi square) goodness-of- fit.

d) Identification of semiochemicals

This was helpful to analyze the complex multi component blend of the semiochemicals present in minute quantities. GC – MS used is one of the so-called hyphenated analytical techniques. A gas chromatography- mass spectrometry (Perkin Elmer, Auto system XL GC+, Turbo mass 4.1- software, in SICART, India) was used. The temperatures of the GC-oven and ion source were 210-220^{o}C, respectively. Silica base capillary column (250 micrometer diameter x 100m length, 0.25 µm thickness of cp- silica 8) was used under a programmed oven temperature condition of 50^{o}C for 1 min to increase to 250 at 5^{o}C / min. Helium was used as carrier gas at a flow rate of 1.2 ml/min.

RESULTS

In the first trial, 60 females were released in to the experiment set consisting of female volatile extracted by using confinement method. In this, 11 females got attracted towards the volatile but, it was only for a few second. So, it was concluded that the volatile collected from female did not attract females. The above experiment was also conducted by using female volatile obtained by using adsorbent method. During this only 2 females was attracted towards the volatile, which indicate that female was also not get attracted towards the volatile obtained by adsorbent method. In the next set of experiments, 60 males were released in the experimental pertriplate consisting of female volatile extract obtained by using confinement method. In this 44 males easily got attracted towards the female volatile extract. The same experiment was also conducted by using female volatile obtained by using adsorbent method. During this, 52 males get attracted towards the female volatile (Table I). This showed that the males get attracted towards the volatile obtained from females using both confinement and adsorbent methods The responsiveness of the mealybug was converted to AI (Attractive index) and subjected to statistical analysis. The maximum value 0.86 AI and 0.72 AI were recorded for the volatile extract collected from female using adsorbent method and confinement method, which attracted the males respectively. The Attractive index value obtained by using confinement method was little less than the result obtained by using adsorbent method. But, males were showing good responsiveness percentage i.e. 71.42 and 86.2 respectively. χ^2 goodness-of-fit also confirmed that the males were attracted towards female volatile (Table I). Therefore, this provided evidence for presence of sex attractent in the female which attract male.

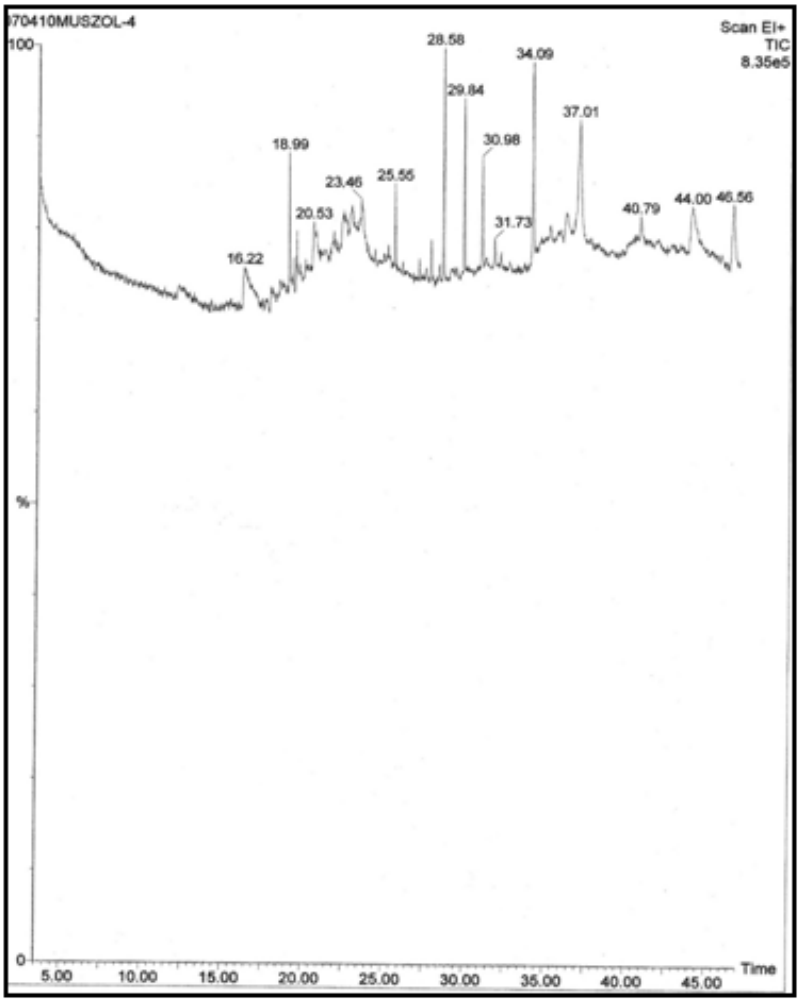
Table 1
Bioassay study using female mealybug, Phenacoccus solenopsis (Tinsley) volatiles for sex- attranctent communication

Adult released in experiment setup			Responsiveness percentage	Female volatile obtained by	Attractive index (AI)	χ^2 value & significance
Sex	**Number of released insect**	**Number of insect respond**				
♀	60	11	17.24	Confinement method	0.17	32.25 NS
C		2		n- Hexane		
♀	60	4	6.66	Adsorbent method	0.07	40.39 NS
C		0		n- Hexane		
♂	60	44	71.92	Confinement method	0.72	2.09*
C		3		n- Hexane		
♂	60	52	86.44	Adsorbent method	0.86	1.86*
C		1		n-Hexane		

= significance, NS= Non- significance, *CD (0.05) = 0.099

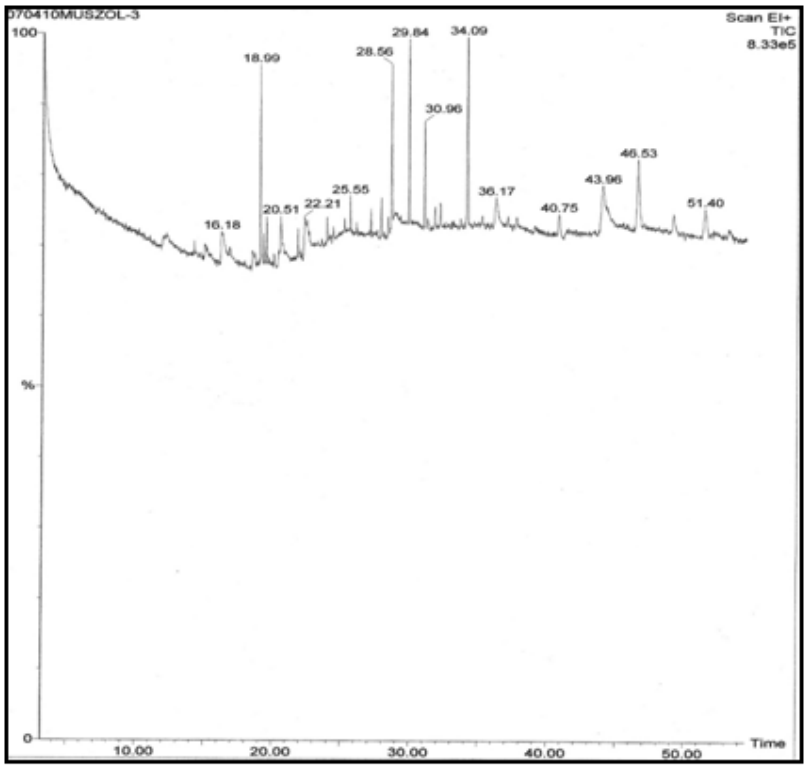
The result of behaviour studies is an encouragement for further fractionation and identification of volatile from n hexane solvent extract by using GC-MS (Graph 1 and 2). The collected air born volatiles in n-hexane were identified as ester and terpenes.

Graph 1
Representation of gas chromatography of female semiochemicals isolated from Phenacoccus solenopsisTinsley using n-Hexane as solvent by using Confinement method.



Graph 2

Representation of gas chromatography of female semiochemical isolated from Phenacoccus solenopsis Tinsley using n-Hexane as solvent by using Adsorbent method.



In which the major volatiles which are identified from both confinement and adsorbent methods are:

Isomer of cyclobutane methyl ester
3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Diterpenes)
2,6,10,15,19,23-Hexamethyltetracosa-2,6,10,14,18,22-hexaene (Triterpenes)
Carotene (Tetraterpenes)
Hexadeconoic acid
2- Cyclohexane-1-ol

DISCUSSION

Worldwide, the *Phenacoccus solenopsis* (Tinsley) is known to attack a large number of plant species including crops, vegetables, ornamental plants and weeds[12]. Even in India *Phenacoccus solenopsis* recorded from 22 plant species of 10 families comprising 7 field and vegetable crops, 3 ornamentals & 12 weeds[13]. Hence this information suggested that management interventions should be focused against reproducing adult females more to prevent the multiplication and spread of the pest. Therefore bioassay studies were done by using adult females to determine an effective management scheme by primarily with finding the evidence of sex pheromone from female. The present study gave fruitfull results of attractiveness of *P. solenopsis*male towards volatile extracted from female of same species. Similar type of behaviour bioassay conducted on *Planoccocus citri* (Risso) using ethanol, diethyl ether and petroleum ether shows high attraction and copulation responses in male mealybug[14]. Laboratory bioassay reported the presence of sex pheromone in *Matscoccus resinosae* (Bean), which attracted its male strongly towards crude extract from female using pentane as solvent[15]. The evidence of sex pheromone was obtained from *Epidiaspis leperii* (Signoret) by using behaviour assays conducted by using female volatile solvent extracts which attract 80-100 % of male[16]. The crude pheromone, extracted from virgin female of *Planococcus minor* (Maskell) when bioassayed in laboratory showed high

attraction of male[17]. Simple behaviour bioassay conducted on *Pseudococcus cryptus* (Hempel) showed the evidence of sex pheromone from female attracting male[11] which was further isolated and identified as ester compound[18]. Similarly, In Japan, ester sex attractant component from *Planococcus kraunhiae* (Kuwana) female was identified by using adsorbent method which is known to be an euryphagous pest of fruit trees, such as pear, citrus, grapes and persimmon[19]. Monoterpene as sex pheromone was identified from obscure mealybugs[20]. In U.S. 2- acetoxy, 3-methyl butanate as sex pheromone was isolated and identified from *Pseudococcus calceolariae* (Maskell) which is a major pest of citrus, grapes, sugarcane and apple[21]. In this the cyclobutane methyl ester is belonging to sex pheromone in other species of mealybug like *Macollinecoccus hirsutus*[22]. Terpenes such as 3,7,11,15-Tetramethyl-2-hexadecen-1-oland 2,6,10,15,19,23-Hexamethyltetracosa-2,6,10,14,18,22-hexaene are identified from volatile extracts are mainly act as kairomones. The role of Hexadeconoic acid and 2-Cyclohexane-1-ol are also identified which act as attractant in *Agelastica coerulea* and *Alnus glutinosa*[23]. Hence, these semiochemical (both pheromones and kariomones) can be used for attraction of male mealybugs, which can reduce the population in the fields. Therefore, the results from behaviour bioassays and isolation of semiochemical compounds encourage for further proper identify, isolate and synthesis of sex-pheromone in future by collabrating with various R & D institute from mealybug species. This will be helpful for development of ecofriendly control methods leading to an ultimate contrubution to minimizing the load of pesticides from agricultural fields.

CONCLUSION

Mealybug, *Phenacoccus solenopsis* spread more easily than many other insect species. Since, they possess a waxy coating on the dorsal side that protect them from insecticides and natural enemies, have a high reproductive rate, their ability to hid in soil, cracks and crevices of plants and propensity to spread quickly proved that mealybug have immense potential to emerge as crop pest and thus pose great threat to agriculture in the Vadodara. The present study results show that by finding the evidence of the presence of pheromone and various kariomone in *P. solenopsis* can be further characterized and synthesised for the development of pheromone trap for *P. solenopsis*, a major polyphagous pest in world.

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