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STUDIES ON THE CHEMICAL ANALYSIS OF APHID SEMIOCHEMICALS FROM *APHIS GOSSYPII* (GLOVER) AND *APHIS NERII* (BOYER DE FONSCOLOMBE)

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ABSTRACT

The aphids, *Aphis gossypii* (Glover) and *Aphis nerii* (Boyer de Fonscolombe) are serious pests in the agricultural fields of Vadodara, India. In an attempt to find the solutions to reduce the infestations by these pests, behavioral bioassays (using Y-tube olfactometer) were conducted under laboratory conditions. The volatile extracts were extracted through n- hexane solvent from the females of *Aphis gossypii* and *Aphis nerii*. Usually aphids secrete both alarm and sex pheromones and it is quite difficult to scan the males in the life cycle because males were found on the secondary host and once in a year. So, my observations will reveal the rate of repulsion of female aphids towards the female volatile extracts which was isolated by both Confinement and Adsorbent methods. The result showed the presence of semiochemicals as an alarm pheromone in the female volatile extract which could repel its own species and other species while it attracts the predators and other associated natural enemies. Upon further study the isolation and identification of volatile extract by using GC-MS showed the presence of a mixture of sesquiterpene compounds such as E-β-farnesene. Hence the information contained in this paper should pave the way to the identification of proper management practices to maximize lure methods. It would also be useful in providing good scope for the further development of ecofriendly methods to control the aphid infestation.

KEYWORDS: ***Aphis gossypii*, *Aphis nerii*, Semiochemicals, GC- MS, Y- tube olfactometer.**

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INTRODUCTION

The earlier studies on the populations of aphids, *Aphis gossypii* (Glover) and *Aphis nerii* (Boyer de Fonscolombe) (Hemiptera: Aphididae) reveal its economic impact as a major concern for all economically important crops/plants such as Cotton and Oleander plants in the agricultural fields of Vadodara[1]. In India, it has been reported from almost all states infesting over 569 plant species. In North India and East India, aphids have taken a leading position among sucking pests where as in West India, Central India and South India, it showed a moderate incidence of damage[2]. In 2011, it has been reported that 1326 insect species cause damage to Cotton (*Gossypium spp.*) crop in approx. 100 countries. Of those 1326 insect species, 16 species are the cause for major concern accounting for an annual loss of 50-60% of the total production in Northern Gujarat[3]. *Aphis gossypii* and *Aphis nerii* falls in those 16 insect species. It was reported that *Aphis gossypii* is the major threat to the agricultural and horticultural crops in many tropical, sub-tropical and temperate countries[4] attacking 220 host plants species belonging to 46 different families throughout the world[5].They cause yield loss by directly infesting leaves, stems, fruits, roots and also cause damage indirectly by secreting honey dew which develops into a sooty mold attracting ants which act as a transporting agent of the aphids to the different host plants[6]. Similarly, *Aphis nerii*, the Oleander aphid is cosmopolitan by nature. It is a common pest of several important ornamental plants. The host range of the oleander aphid includes several genera of Asclepiadaceae (*Gompho carpus*, *Asclepias*, and *Calotropis*) and Apocynaceae (*Nerium* and *Vinca*) and 16 other plant families. It can occasionally be found infesting plants in the Compositae, Convolvulaceae, Euphorbiaceous and Rutaceae families. This aphid is able to transmit several viruses including *sugarcane mosaic potyvirus* and *papaya ringspot potyvirus*. They ingest sap from the phloem of its host plant. It was reported that *Aphis nerii* is one of the key pests of oleander plant[7] affecting 90% of plants according to the field study. The damage caused by these aphid colonies were mainly aesthetic due to the large amounts of sticky honeydew produced by the colony members and the resulting black sooty mold that grows on the honeydew[8]. Both the aphids are polyphagous by nature which is a matter of concern. Razaq et al. (2011)[9] reported 10-90% yield loss to the economically important crops in India depends upon the severity of damage in various crop stages by aphids. During 1987, it has been reported that as high as 100% yield reduction of different bean crops was due to aphid infestation[10].To protect their crops from the damage of Aphids, farmers mainly depend on the conventional synthetic chemical insecticides such as Organophosphates, Carbamates, Synthetic pyrethroids and Nicotinoids. Although, conventional synthetic insecticides usually provide quick and adequate control for the time being, they are usually expensive[11]. Continuous usage of synthetic insecticides caused health hazards, development of pest genotypes resistance to pesticides, resurgence and upset of pests and environmental pollution[12]. The use of these insecticides is hazardous to the environment and non-target insects like pollinators and predators[13]. Several efforts were made all over the world to devise region specific integrated pest management (IPM) systems. The outbreak of *Aphis gossypii* and *Aphis nerii* in Indian agricultural fields necessitates the study of development of bio-rational alternatives to control the aphids. Our research focused on the role for using alarm pheromone as an alternative to conventional chemical control of insect pests and it will be interesting to know the role of E-β-Farnesene (EBF) in aphids and its associated insects under field conditions. It emphasizes the importance of investigating the ecological roles. The study aims at indicating a potential control and creates a new bio lure for sucking pest in the market of Vadodara.

MATERIALS AND METHODS

a) Collection, Preservation and Identification of Aphids

Aphids are polyphagous by nature traversing from Cash crops (Pigeon pea) to oilseeds (Cotton crop) to vegetables (Brinjal) to ornamental plants (Hibiscus). Samples of both the aphids species (*Aphis gossypii* and *Aphis nerii*) were collected from cotton crops and oleander plants in agricultural sites of Vadodara. Afterwards both the species were transferred in the vials containing 70-90% ethyl alcohol were brought to the laboratory, mounted on slides and then observed and identified. Aphids were identified by using taxonomic key provided by Blackman and Eastop (2000)[14]. A stereomicroscope; Leica MPS 60 Ø28/8x/MPS was used for identification and photographic record. The identified species were confirmed from Entomology Division, Anand Agricultural University, Anand, Gujarat.

b) Mass multiplication of Aphids (Aphis gossypii, Aphis craccivora and Aphis nerii) under laboratory condition

The rearing and breeding of all the three species of aphids was carried at room temperature (20-25^{o}C, 70-75% R.H.) under a photoperiod of 16L: 8D in the laboratory. The cotton twigs, harboring *Aphis gossypii* colonies, were collected from the fields and brought to the laboratory. The aphids present on these cotton twigs were gently removed with the help of soft camel hair brush and were released on the cotton twigs placed in plastic boxes (20 x 15 cm^2). *Aphis craccivora* were collected from the bean crops and were released on the bean twigs. Similarly *Aphis nerii* colonies were collected from the oleander twigs and were released on the calotropis twigs. Newly hatched crawlers were collected from the ovipositing female of laboratory culture and were placed on to their respective host plants for mass rearing. Culture obtained was used for the collection of volatiles and behavior bioassays experiments.

c)Collection of Volatile extract from Aphis gossypii and Aphis nerii

Though many methods are available for trapping the volatiles, the following methods were used in isolating the volatile from aphids:

i) Confinement / Wash Method

More than a 1000 adult virgin female aphids were kept in a 200ml sterilized conical flask which had a Whatmann filter paper at its base and was covered with a silver foil. After 24hrs, the female aphids were removed and the entire surface of the conical flask was washed and rinsed with little quantities of the solvent n-hexane. The crude extract was used for the behavioural bioassay.

ii) Adsorbent Method[15]

The airborne volatile collection apparatus was constructed by DURGA CHEMICALS PVT. LTD., Vadodara. This method consists of putting adult female aphids into an insect chamber, through which filtered air is passed on to a narrow glass tube with an inside diameter of 3mm. Another tube packed with granulated activated charcoal (AR capacity) which acts as an adsorbent. Insects were exposed to air flow for 24hrs with equal light and dark regimes. The trapped volatiles were eluted using 10ml of n-hexane. This volatile extract was preserved at -20°C in 5ml glass vials with Teflon-liners amber bottles. This solution was then used for bioassay studies.

d) Behavioral Bioassay using Y- Tube olfactometer[16]

This instrument was constructed by DURGA CHEMICALS PVT.LTD., Vadodara. The 'Y' tube consisting of two arms to which are fitted broad tubes serving as a test chamber (Size 20 cm). Air was blown from the other side of the 'Y' tube using an aerator [both A and B arms (12.5 cm)]. The air flow can be regulated by valve situated in the release chamber. The behavior of the insects was video graphed using Canon Powershot ISI- 120. Approximately 100 aphids were released into the test chamber. In case of natural enemies such as ladybird beetles grubs and adult ants, 10 test insects were released. Aerator was connected to both the arms A (Control) and B (Contains the cotton soaked volatile extract of aphids). The filtered air was passed continuously at medium speed. Reading was taken at every 0min, 15min, 30min, 45min, 1hr, 2hrs, 3hrs, 4hrs, 5hrs and 6hrs. Photographs were taken by Canon Powershot ISI- 120 Digicam. Each experiment was repeated three times and the results mentioned below are an average of three experiments. The raw data collected from the readings were transferred to an electronic format and converted into a spreadsheet layout (Microsoft excel, 2007). Graphs were generated from the spreadsheets. The repellent activity of volatile extract extracted from both the species was recorded in terms of percent repellency (**Percent Repellency = (N_c- N_T/N_c) x 100** where N_c and N_T are number of individuals in control and treatment arms of olfactometer) after different intervals of time. Repellency index (Pascual- Villabas and Robledo, 1998) calculated as **RI = (N_c - N_T)/ (N_c+N_T) x 100** where N_c = No. of insects in control and the N_T =No. of insects in treated. RI varying from – 100 (total attractancy) + 100 (total repellency) with 0 meaning no effect. Based on the data collected as described below, the attractive index (AI) for associated insects was calculated. The formula is given as: **AI (Attractive Index) = (No. of insects responded to test materials – No. of insect responded to control) ÷ (No. of insect released – No. of insect responded to control)**. The collected data analyzed by SPSS-19 Statistical Software for χ^2 (Chi square) goodness–of-fit for significance of response.

e) Identification of semiochemicals

Gas chromatography-Mass spectrometry (GC-MS) was helpful to analyze the complex multi component blend of the semiochemicals present in minute quantities. GC-MS is one of the hyphenated analytical techniques. The following GC-MS (Perkin Elmer, Auto system XL GC+, Turbo mass 4.1- software) was used at SICART, India. The GC used a fused-silicon based capillary column (30 m x 0.25 mm ID) coated with 0.25 µm thickness of CP-Sil 8 CB. Helium was used as carrier gas at a constant flow of 1.2 ml/min through the column. The heating up time of Oven was 50˚C to 250˚C in 2 minutes and cool down time was 250˚C to 50˚C in 48 minutes. Split less injector was used. Mass spectral analyses of the GC effluents were done. The detectors such as Flame Ionization Detector (FID) and Thermal Conductivity Detector (TCD) were used. The peaks in GC monitor were matched with mass spectral library to identify the compound name and structure.

RESULTS

(a) Record the Behavioral Bioassay (Using Y – Tube olfactometer)

Number of aphids released (N) into the Test chamber (Tc) of Y – Tube olfactometer=100
Arm A of Y- Tube olfactometer = Control (C_1)
Arm B of Y- Tube olfactometer = Volatile Extract extracted from the female aphid (T_1)

Experiments with *Aphis gossypii* (Glover)

A series of experiments were conducted to study the behavior of the test insect. In the first trial, 100 females were released into the experiment set consisting of female volatile extracted by using confinement method. In this, 30 females showed repelling effect towards the volatile, but it was only for the few minutes. So, it was concluded that the volatile collected from female did not show repulsion. The above experiment was also conducted by using female volatile obtained by using adsorbent method. (A) The effect of volatile extracts of female *A.gossypii* was observed against its own species. In this trial, 100 female aphids were released in the test chamber. Observations were made at 4hrs and 6hrs duration from experiment start time where in maximum number of aphids moved towards the Arm-A (Control) i.e. 51 aphids. Most of the aphids remained in the Test Chamber, only 2-3 number of aphids moved towards the Arm-B (Extract). This shows that repulsive effect was due to the presence of alarm pheromones (Graph 1). (B)The effect of volatile extract from female aphid species on one species against the other species was also tested by using adsorbent method.

Experiments conducted on the effect of volatile extract of female A.gossypii against female A. craccivora

In this experiment, 100 aphids were released in the test chamber. Out of them, 55 aphids within 6hrs moved towards the Arm-A. Most of the aphids remained in the Test Chamber, only 0-1 number of aphids moved towards the extract (Arm-B) (Graph 2).

Experiment conducted on the effect of volatile extract of female A.gossypii against A.nerii

In this experiment, 100 aphids were released into the Test Chamber. Out of them, 42 aphids within 6hrs moved towards Arm-A. Most of the aphids remained in the Test Chamber and only 2-3 numbers of aphids moved towards the extract (Arm-B) and returned back within 5 minutes (Graph 3).Above experiments confirm that the repulsive effect observed was due to the volatile extract. (C) Later the effect of the volatile extract from the female aphid species using adsorbent method was also observed on its bio-control agents (Ladybird beetle Grubs) and natural enemies (Ants).

Experiments conducted on the effect of volatile extract of female A.gossypii against Bio- control agents

In this experiment, 10 larvae of ladybird beetle (*Coccinella septempunctata* Linn.) were released in the Test Chamber. Maximum number of larvae/grubs moved towards Arm-B approximately 4-5. Only 1-2 numbers of larvae moved towards Arm-A. In the case of ladybird beetle, attraction was observed towards the volatile extract (Graph 4).

***Experiments conducted on the effect of volatile extract of female A.gossypii against* Ants**

In this experiment, 10 numbers of ants (*Camponotus compressus* Fabricus) were released in the Test Chamber. They all showed unusual behavior. All the ants moved at a fast rate inside the Y-Tube including Test Chamber, Arm-A and Arm-B. After 15 minutes, 6 of them aggregated in Arm-B. Maximum number of ants moved towards Arm-B (Extract). After 30 minutes, all ants had aggregated in the Test Chamber. So it can be concluded that ants were highly attracted towards the volatile extract (Graph 5).Hence, the outcome of behavioural studies showing the effect of volatile extracts of female *A.gossypii* on aphids, coccinellids and ants has been documented (Table 1). This showed that *A.gossypii* showed repulsion effect towards its own species and one against the other species. In case of bio-control agents and natural enemies, the volatile extract of female showed the kairomonal effect. Therefore, this provided the evidence for the presence of alarm pheromone which alerts its own species as well as one against the other species.

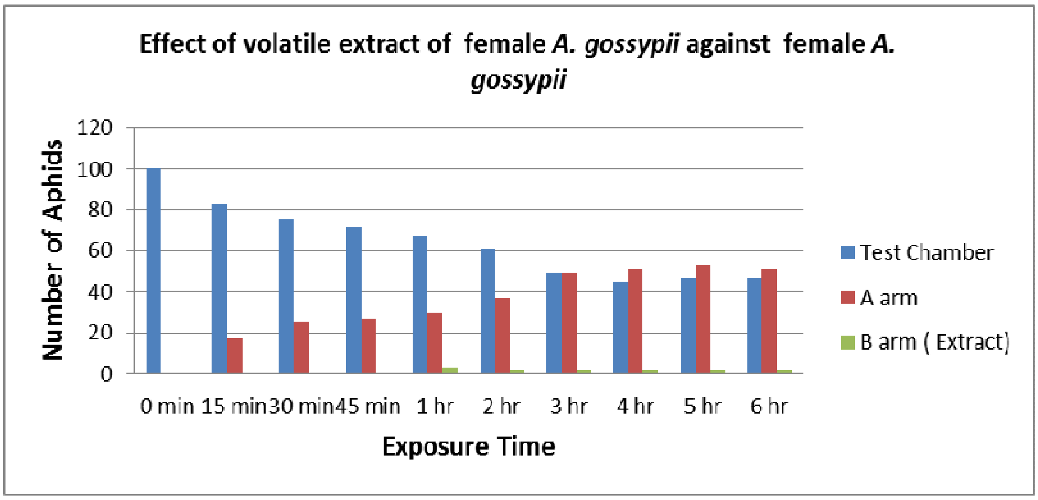
Table 1
Outcome of Behavioral bioassay showed the effects of volatile extracts of female A.gossypii (Glover) on Aphids, Coccinellid (Ladybird beetle Grubs) and Ants

Sr.No.	Volatile extracts of female *A.gossypii*	Repulsion effects	Attraction effects	Remark
	Effects of volatile extracts from female aphid species against its own species			Repulsion Effect (Semiochemicals)
1.	Volatile extracts from female *A.gossypii* against female *A.gossypii*	√	×	
	Effects of volatile extracts from female aphid species on one species against the other species			
2.	Volatiles extracts from female *A.gossypii* against female *A.craccivora*	√	×	
3.	Volatile extracts from female *A.gossypii* against female *A.nerii*	√	×	
	Volatile extracts from female aphid species on its bio-control agents and natural enemies			Kairomonal Effect
4.	Volatile extract from female *A.gossypii* against *Coccinella septempunctata*	×	√	
5.	Volatile extract from female *A.gossypii* against *Camponotus compressus*	×	√	

****√ = Yes,* X = No***

A) To observe the effect volatile extract of A.gossypii against its own species

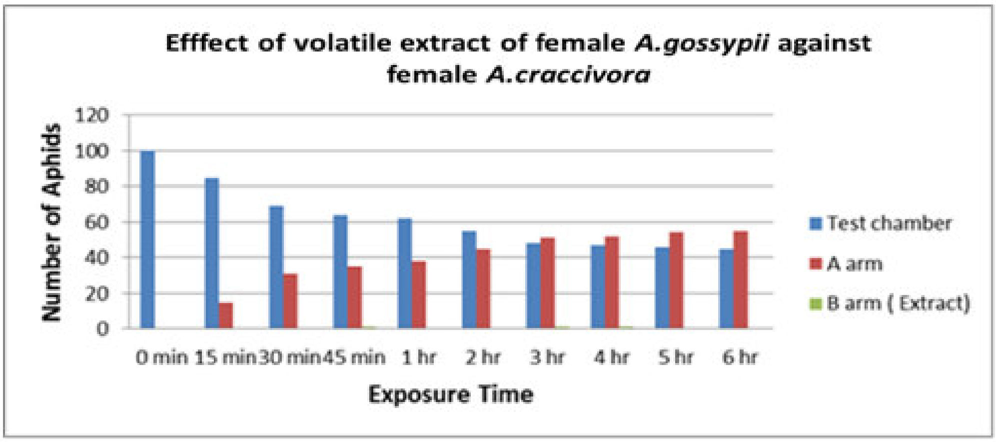
Graph 1
Effect of volatile extracts from female A.gossypii against female A.gossypii



*B) **To observe the effect of volatile extracts from female Aphid species on one species against the other species.***

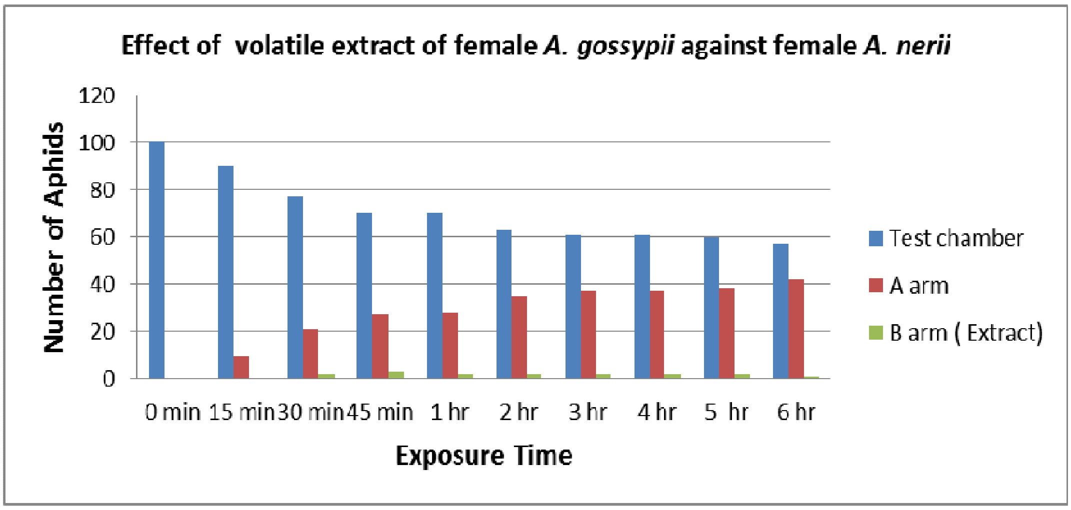
Graph 2

Effect of volatile extracts from female A.gossypii against female A.craccivora



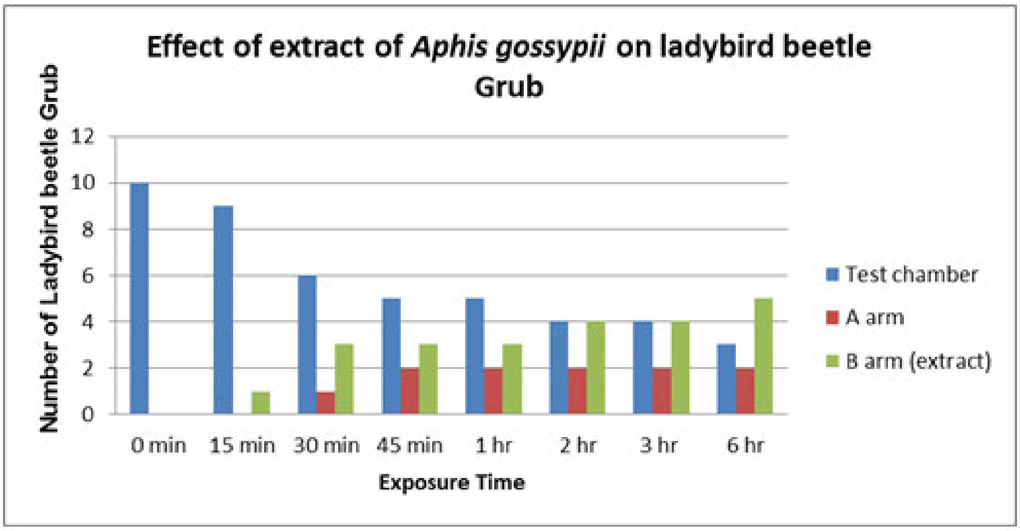
Graph 3

Effect of volatile extracts from female A.gossypii against female A.nerii



*C) **To observe the effect of volatile extract of female Aphis gossypii on bio- control agents and its natural enemies.***

Graph 4
Effect of volatile extract of female A. gossypii against ladybird beetle Grub



Graph 5
Effect of volatile extract of female Aphis gossypii against Ants

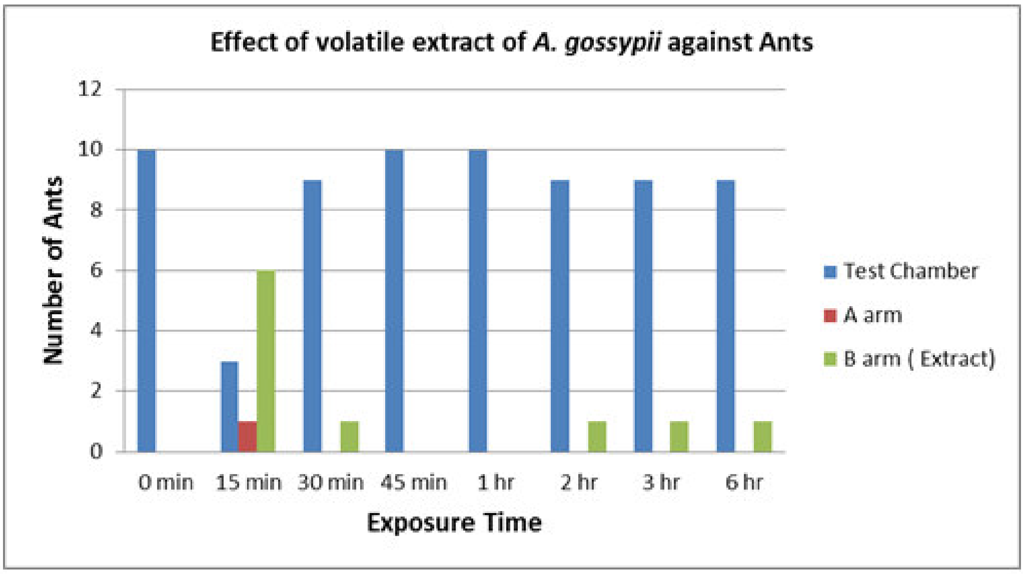


Table 2
Bioassay study using female volatile extract of aphid, Aphis gossypii (Glover) against its own, other species and natural enemies for chemical communication

Adult released in Experiment Setup				**Percentage Repellency (%)**	**Repulsive Index (RI)**	**X^2 value & Significance to response**
Species	**Number of insect released per trial**	**Number of insect responded**				
A.gossypii (Extract) against its own species	100	E	1.4	95.88%	92.09	0.046^a (*S=0.977)
		C	34			
A.gossypii (Extract) against *A. craccivora*	100	E	0.3	99.20%	98.41	0.630^a (*S = 0.730)
		C	37.6			
A.gossypii (Extract) against *A.nerii*	100	E	1.6	93.81%	88.35	2.160^a (*S= 0.340)
		C	27.5			
Bio- control agent (Coccinellid beetle) and Natural enemies (Ants)						
Species	**Number of insect released per trial**	**Number of insect responded**		**Percentage attraction (%)**	**Attractive index (AI)**	**X^2 value & Significance to response**
A.gossypii (Extract) against *Coccinella septempunctata*	10	E	2.875	52.17%	0.173	0.500^a (*S=0.779)
		C	1.375			
A.gossypii (Extract) against *Camponotus compressors*	10	E	1.25	90%	0.113	0.500^a (*S=0.779)
		C	0.125			

S= Significance, df = 2

Experiment with Aphis nerii (Boyer de Fonsolombe)

Various experiments were conducted to study the behavior of the test insect. In the first trial, 100 females were released into the experiment set consisting of female volatile extracted by using confinement method. In this, 17 females showed repelling effect towards the volatile, but it was only for the few minutes. So, it was concluded that the volatile collected from female did not show repulsion. The above experiment was also conducted by using female volatile obtained by using adsorbent method. (A)The effect of volatile extract of female *A.nerii* was observed against its own species. In this trial, 100 female aphids were released in the test chamber. Observations were made at 6hrs duration from experiment start time where in maximum number of aphids moved towards the Arm- A (Control) i.e. 49 aphids within 45 minutes. Most of the aphids remained in the test chamber, only 2-3 number of aphids moved towards the Arm- B (Extract) and returned back within 5 minutes showing repulsive effect (Graph 6).This showed the presence of alarm pheromones. (B)The effect of volatile extract from female aphid species on one species against the other species was also tested by using adsorbent method.

Experiment conducted on the effect of volatile extract of female A.nerii against female A. gossypii

In this experiment, 100 aphids were released in the test chamber. 100 out of 51 aphids within 6hrs moved towards the Arm-A (Control).Most of the aphids remain in the test chamber, only 2-3 number of aphids moved towards the Arm-B (Extract) and returned back within 5 minutes (Graph 7). It showed repulsive effect due to the presence of alarm pheromones.

Experiment conducted on the effect of volatile extract of female A.nerii against A.craccivora

In this experiment, 100 aphids were released in the test chamber. Maximum number of aphids moved towards the Arm-A (Control) i.e. 51 aphids within 6 hrs. Most of the aphids remain in the test chamber, only 0-1 number of aphids moved towards the Arm-B (Extract) and returned back within 5 minutes (Graph 8). It showed repulsive effect due to the presence of alarm pheromones. Above experiment showed the repulsive effect due to the volatile extract. (C)Later the effect of volatile extract from the female aphid species using adsorbent method was also observed on its bio-control agents (Ladybird beetle larvae) and natural enemies (Ants).

Experiments conducted on the effect of volatile extract of female A.nerii against Bio-control agents (Coccinella septempunctata Linn)

In this experiment, 10 larvae of ladybird beetle were released into the test chamber. All the 10 larvae of ladybird beetle grubs remained stationary in the test chamber for 30 minutes then they started moving towards the arms. After around 45 minutes, three of them moved into the Arm-B containing extract. At 6hrs duration, maximum number of ladybird beetle grubs moved into the Arm-B (Extract) i.e. approximately 2-3. Only few i.e. 0-1 numbers of grubs moved towards Arm-A (Control). Here in case of ladybird beetles, attraction is seen towards the Arm-B containing extract (Graph 9). The reason that ladybird beetle grubs are predators to aphids can be accounted to the strong EBF factor that is causing the attraction.

***Experiments conducted on the effect of volatile extract of female A.nerii against Ants* (*Camponotus compressus* Fabricus)**

In this experiment, 10 numbers of ants were released in the test chamber. They showed unusual behavior, all the ants moved at a fast rate inside the Y-Tube including Test Chamber, Arm-A and Arm-B. After 15 minutes, all the ants aggregated in the Arm-B containing extract, within 2 minutes they moved back to the Test Chamber but approximately1-3 ants remained in the Arm- B. After 1hr, all ants had aggregated in the Test Chamber. No ants moved towards the Arm-A (Control). So, we can conclude that Ants are attracted towards the volatile extract (Graph 10). This attraction is centric to the mutually interactive relationship between Aphid and Ants. Hence, the outcome of behavioral studies showing the effect of volatile extracts of female *A.nerii* on Aphids, Coccinellid and Ants has been documented (Table 3). This showed that *Aphis nerii* showed repulsion effect towards its own species and one against the other species. In case of bio-control agents and natural enemies, the volatile extract of female showed the kairomonal effect. Based on the data collected from both the aphid species and its natural enemies, the repulsive index (RI) and attractive index (AI) were calculated in (Table 2 & Table 4). χ^2 (Chi-square) goodness- of –fit confirmed that the aphid showed repulsion against its own species and other species whereas the natural enemies were attracted towards the volatile extract of female aphid species. Hence, the goodness–of–fit results provide strong evidence for the presence of alarm pheromones.

Table 3

Outcome of Behavioral bioassay showed the effects of volatile extracts of female A.neriii (Boyer de Fonscolombe) on Aphids, Coccinellid (Ladybird beetle grubs) and Ants

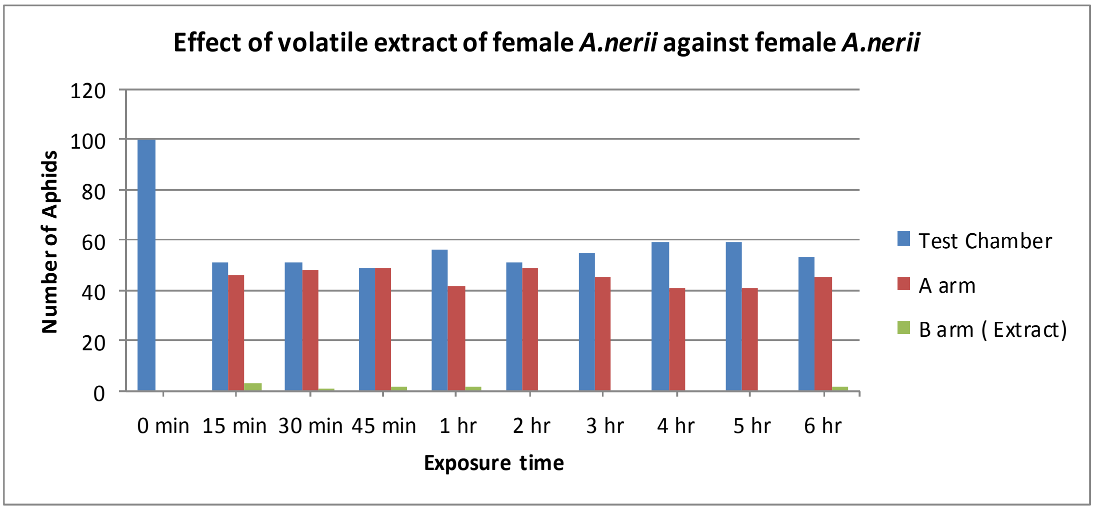
Sr.No.	Volatile extracts of female *A.nerii*	Repulsion Effects	Attraction effects	Remark
	Effects of Volatile extracts from female aphid species against its own species			Repulsion Effect (Semiochemicals)
1.	Volatile extracts from female *A.nerii* against female *A.nerii*	√	×	
	Effects of Volatile extracts from female aphid species on one species against the other species			
2.	Volatiles extracts from female *A.nerii* against female *A.gossypii*	√	×	
3.	Volatile extracts from female *A.nerii* against female *A.craccivora*	√	×	
	Volatile extracts from female aphid species on its bio-control agents and natural enemies			Kairomonal Effect
4.	Volatile extract from female *A.nerii* against *Coccinella septempunctata*	×	√	
5.	Volatile extract from female *A.nerii* against *Camponotus compressus*	×	√	

***√ = Yes,* X = No**

A) *To observe the effect of volatile extracts from female aphid against its own species*

Graph 6

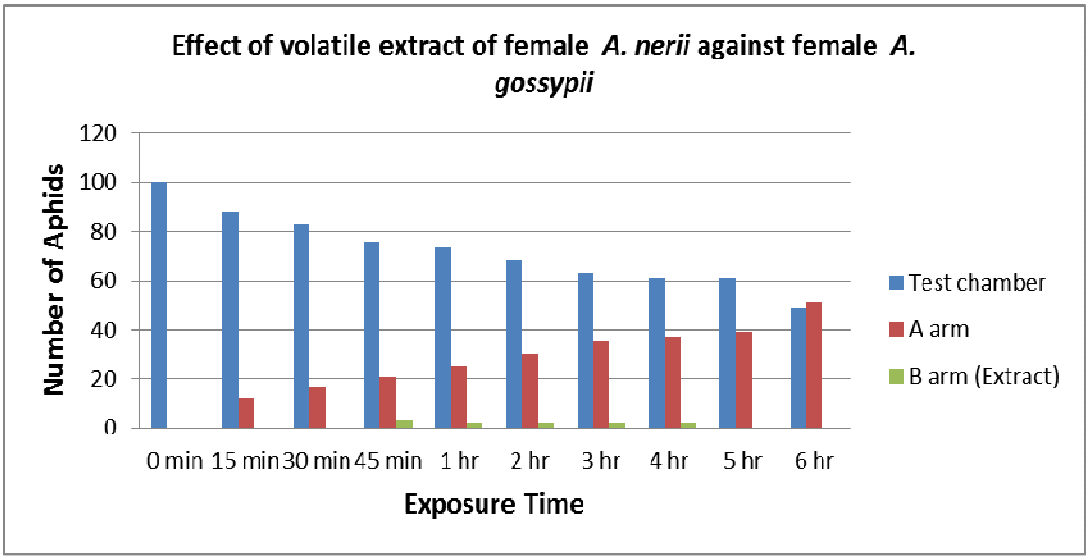
Effect of volatile extracts from female A.nerii against female A.nerii



B) To observe the effect of volatile extracts from female aphid on one species against the another

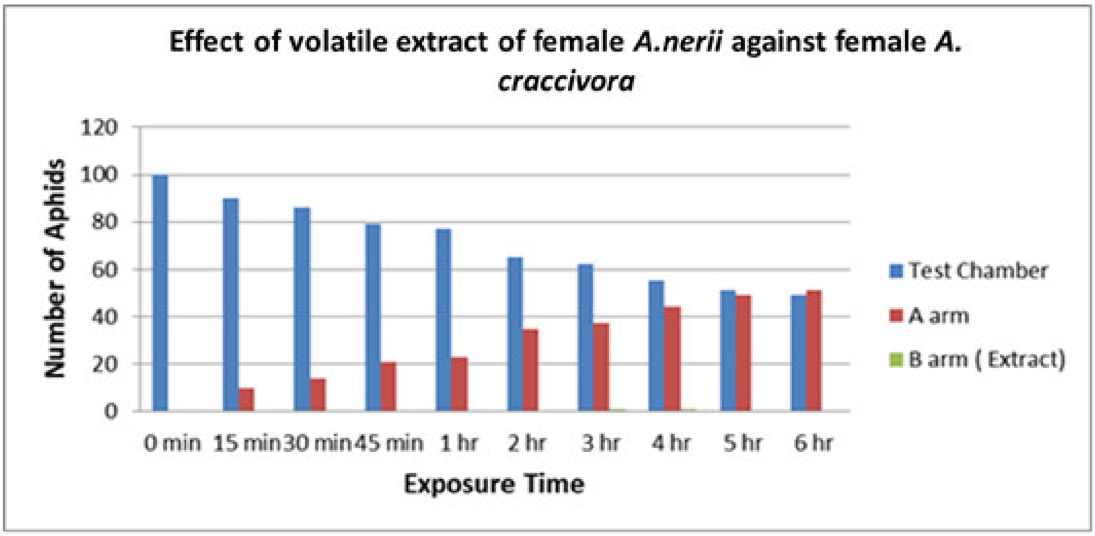
Graph 7

Effect of volatile extracts from female A.nerii against female A.gossypii



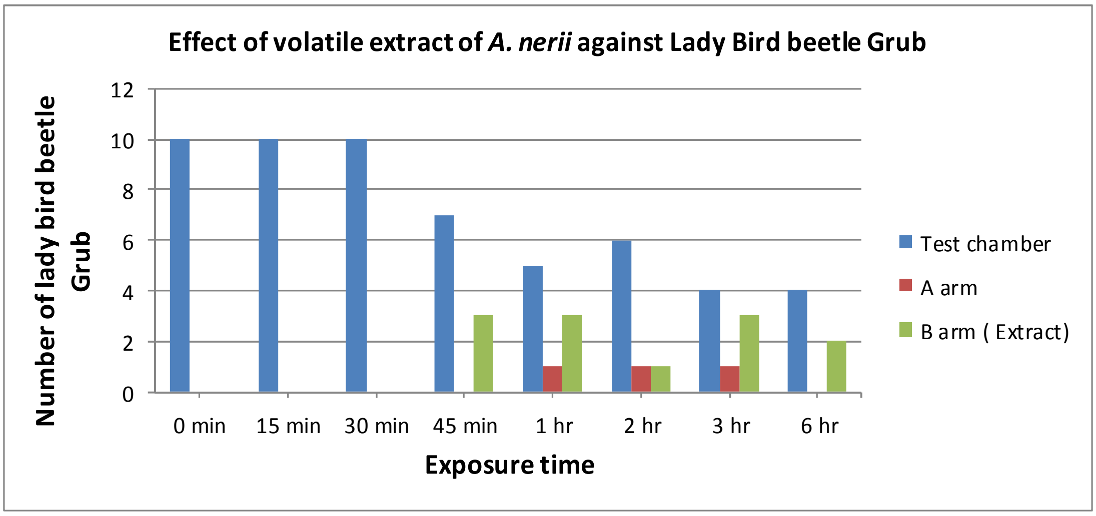
Graph 8

Effect of volatile extracts from female A.nerii against female A.craccivora



C) To observe the effect of volatile extract from female aphid species on the bio- control agents (Ladybird beetle grub) and natural enemies (Ants)

Graph 9
Effect of volatile extract of female A. nerii against Ladybird beetle Grub



Graph 10
Effect of volatile extract of female Aphis nerii against Ants

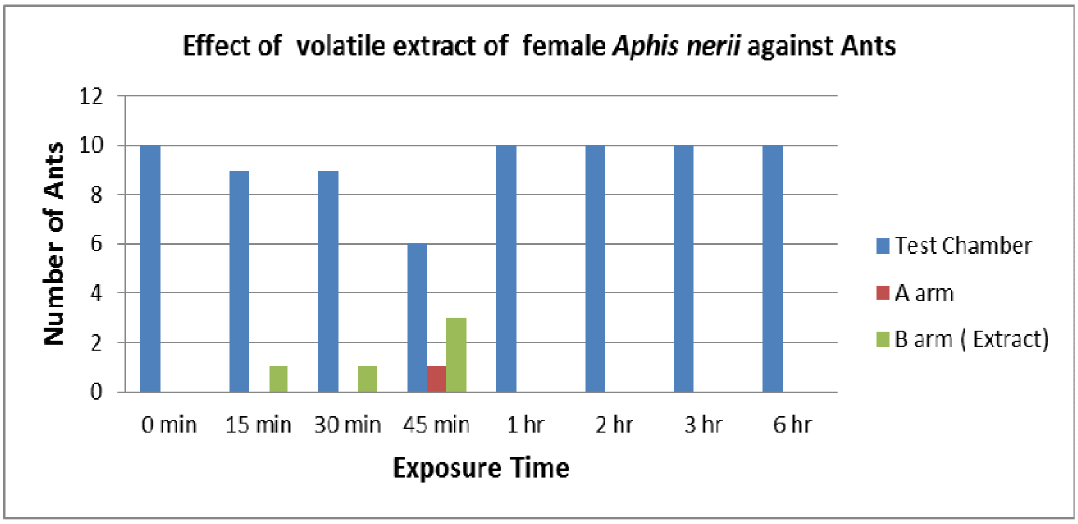


Table 4
Bioassay study using female volatile extract of aphid, Aphis nerii (*Boyer da fonscolombe*) against its own, other species and natural enemies for chemical communication

Adult released in Experiment Setup				**Percentage Repellency (%)**	**Repulsive Index (RI)**	**X^2 value & Significance to response**
Species	**Number of insect released per trial**	**Number of insect responded**				
A.nerii (Extract) against its own species	100	E	1	97.53%	95.19	1.404[a] (*S = 0.496)
		C	40.6			
A.nerii (Extract) against *A. gossypii*	100	E	1.1	95.88%	92.08	1.897[a] (*S= 0.387)
		C	26.7			
A.nerii (Extract) against *A.crassivora*	100	E	0.2	99.29%	98.60	1.385[a] (*S=0.500)
		C	28.4			
Bio- control agent (Coccinellid beetle) and Natural enemies (Ants)						
Species	**Number of insect released per trial**	**Number of insect responded**		**Percentage Attraction (%)**	**Attractive index (AI)**	**X^2 value & Significance to response**
Aphis nerii (Extract) against *Coccinella septempunctata*	10	E	1.5	75%	0.116	0.533[a] (*S= 0.766)
		C	0.375			
Aphis nerii (Extract) against *Camponotus compressors*	10	E	0.625	80%	0.050	0.500[a] (*S=0.779)
		C	0.125			

S= Significance, df = 2

The result of behavior studies were an encouragement for further fractionation and identification of volatile from n-hexane solvent extract by using GC-MS.

(b)Chemical Analysis of aphid alarm pheromone

The collected air born volatiles in n-hexane were identified as mixture of sesquiterpenes (E-β-farnesene) in which the major volatiles which are identified from both confinement and adsorbent methods are:

***Analysis of Aphis gossypii* (Glover)**

During ***Confinement method*** (AG -1), the chromatogram shows total 14 peaks, including low as well as high peaks. The high peaks are mainly counted during analysis. A graph was plotted using Concentration of volatile vs Retention time. The isolated compounds are mainly the Hydrocarbon like 1-Hexene-5 methyl (7.37), Naphthalene (10.84), Squalene (15.18), Phenols (24.83) and Farnesol isomer A (27.30) (Graph 11).

In ***Adsorbent method*** (AG-2), the chromatogram shows total 15 peaks, including low as well as high peaks. The isolated compounds were Hydrocarbons (7.41), Napthalene (10.64), Squalene (14.65), Phenol (24.76) and Farnesol isomer A (27.28) (Graph 12).

***Analysis of Aphis nerii* (Boyer de Fonscolombe)**

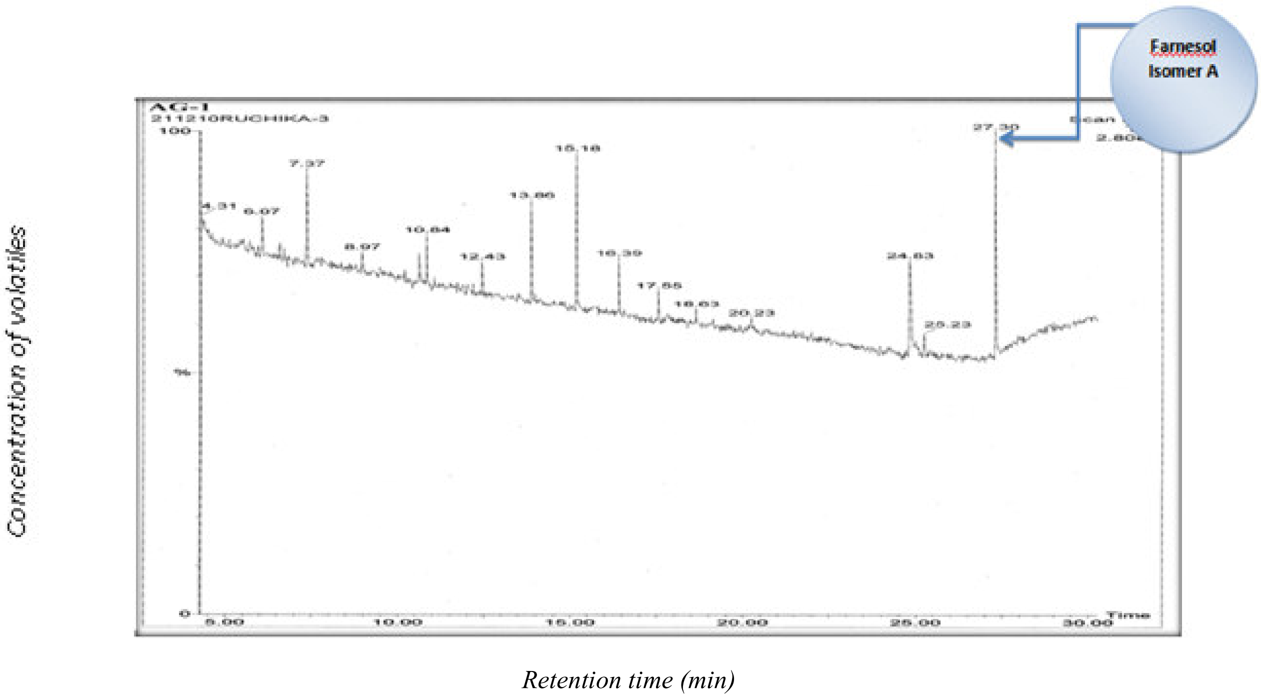
During ***Confinement method*** (AN-1), the chromatogram shows the total 6 peaks, including very low as well as high peaks. The isolated compounds of volatile compounds of *Aphis nerii* are mainly Hydrocarbons (7.74), Napthelene (10.64), Phenol-3-pentadecyl (24.81), Farnesol isomer A (27.30) and Pentatricosane (30.02) (Graph 13).

In ***Adsobent method*** (AN-2), the chromatogram shows the total 12 peaks, including low as well as high peaks. The isolated compounds were mainly Hydrocarbon (7.37), Naphthalene (10.84), 1-Hexene-2-methyl (12.43), 1-Hexene-5-methyl (13.86), Squalene (15.17), Phenol (20.12), Farnesol isomer A (27.32) (Graph 14).

The collected volatiles from the group of aphids in sufficient quantities could be identified by Mass Spectrophotometry (MS) by matching reference and retention time. The identified structure of chemical compound was matched with the mass spectral library. Here, Farnesol isomer A (farnesene) compound was identified from both the species of aphids. The volatile extract of *Aphis gossypii* using adsorbent method showed the presence of Farnesol isomer A at the retention time 27 minutes 28 seconds. Whereas the volatile extract of *Aphis nerii* showed the presence of Farnesol isomer A at the retention time 27 minutes 32 seconds. The mass spectral graph of this compound is also mentioned in (Figure 1a; Figure 2a). The Farnesol isomer A is a natural organic compound which is an acyclic sesquiterpene alcohol (Figure 1b; Figure 2b). It is a pheromone of several other insects. Mainly the mixtures of sesquiterpenes are components of the alarm pheromone such as farnesene compound. In 2008, it has been reported that farnesene compound (mixture of sesquiterpenes) acts as an alarm pheromone of aphids[17]. Aphids have capability to produce an alarm pheromone with sesquiterpene, (E)-β-farnesene as the primary component. It was released in response to physical stress including attack by natural enemies[18]. This pheromone acts to warn related individuals of predation[19]. These alarm pheromones also act as a kairomonal cue for aphid natural enemies. Hence the present research concluded that mixtures of sesquiterpenes (farnesene compound) act as an alarm pheromones.

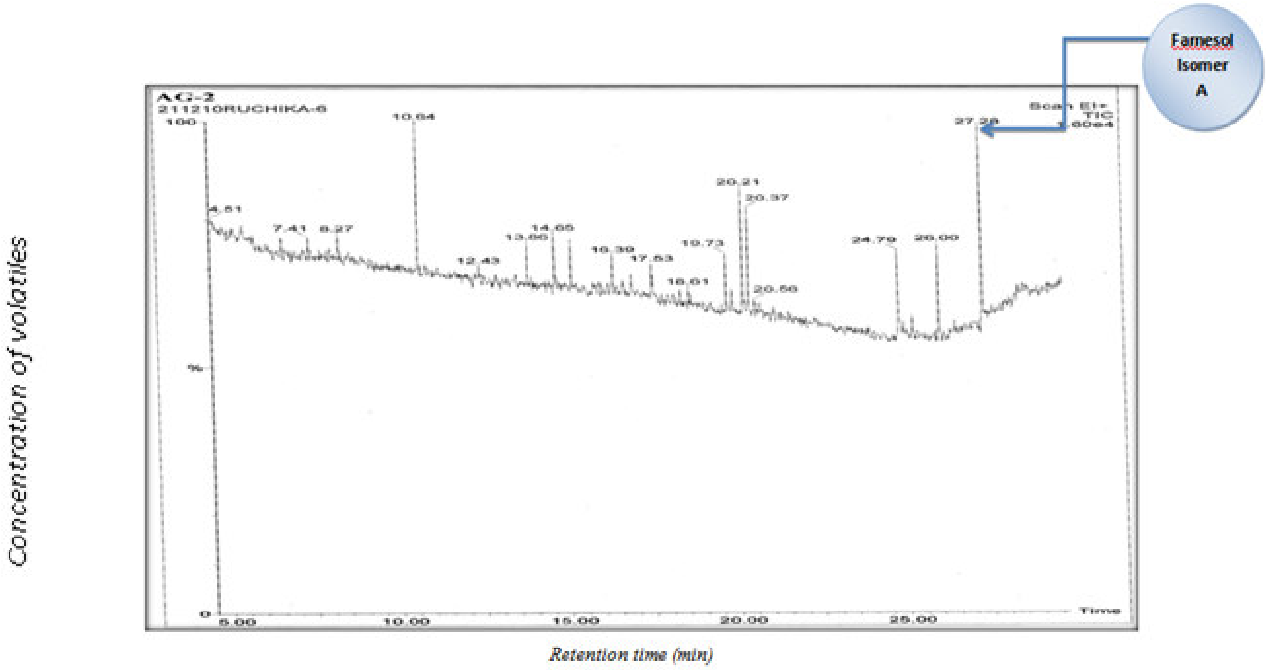
Graph 11

The inset chromatogram revealed by GC using TIC (total ion current) of an alarm pheromone isolated from Aphis gossypii **(Glover)** ***using n- hexane as solvent by using Confinement method.***



Graph 12

The inset chromatogram revealed by GC using TIC (total ion count) of an alarm pheromone isolated from Aphis gossypii* (Glover) *using n- hexane as a solvent by using Adsorbent method.



Identification of Pheromone compound (Mass- spectrophotometry) – Farnesene compound (As alarm pheromone)

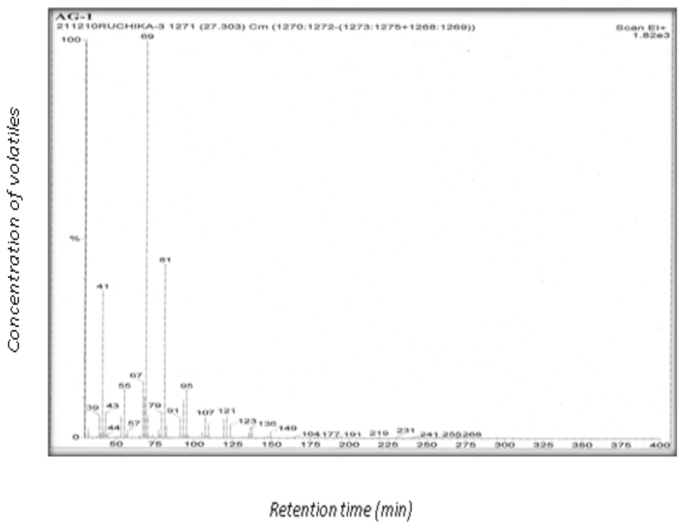


Figure 1(a)
Representation of Mass Spectrum of the farnesene

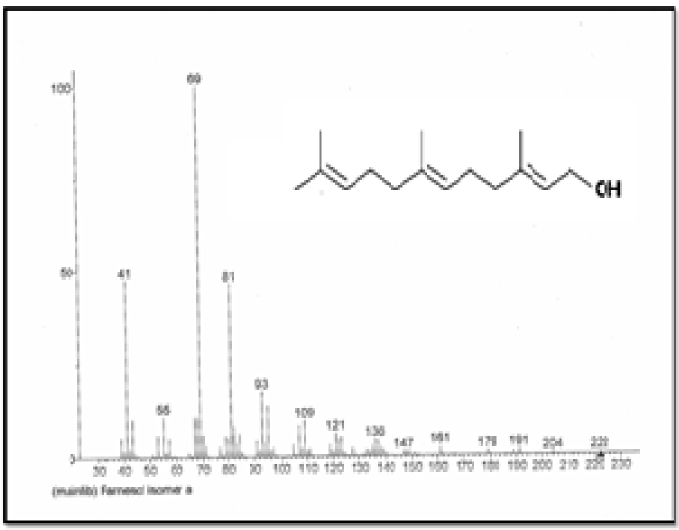
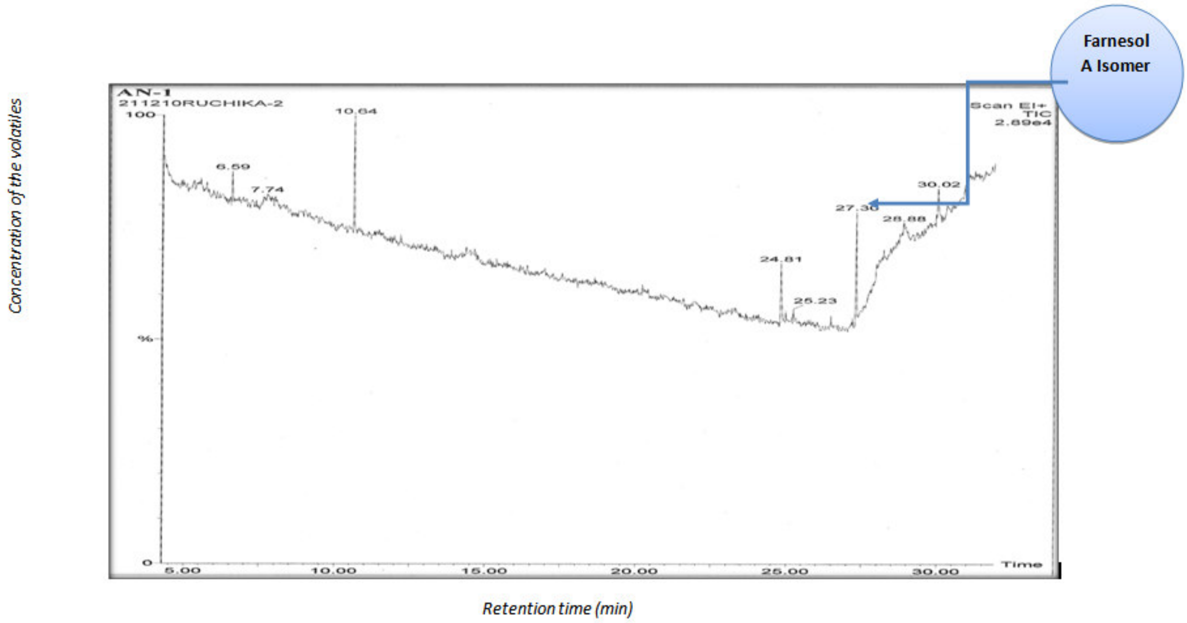


Figure 1(b)
Structure of Farnesol isomer A through mass spectral library.

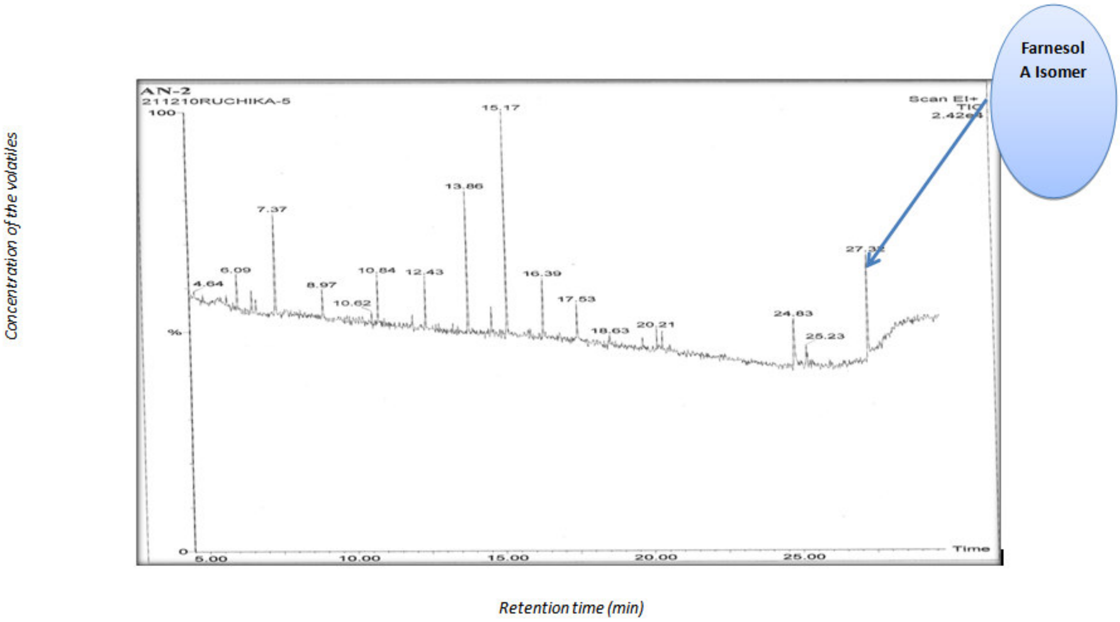
Graph 13

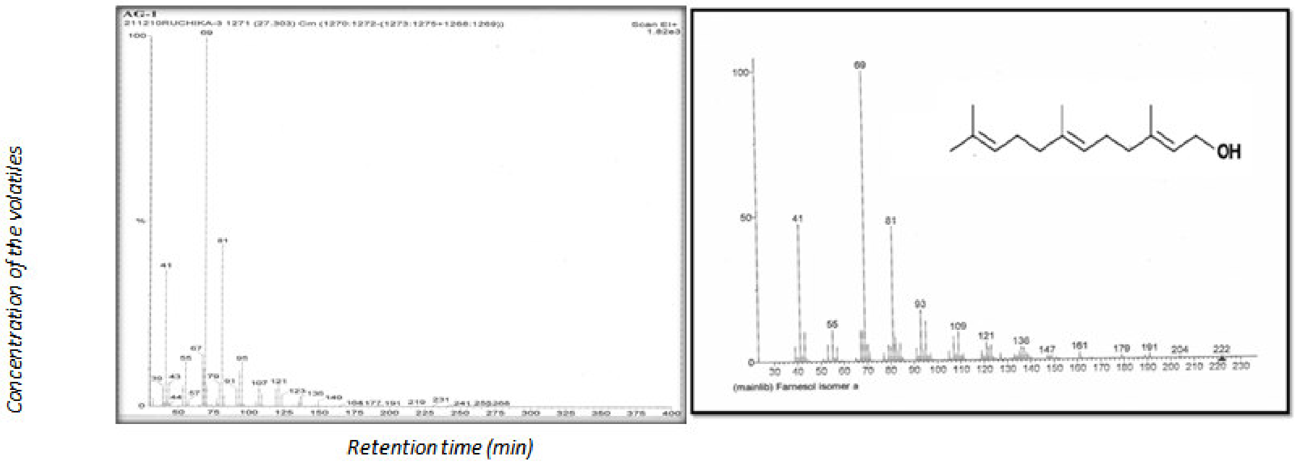
The inset chromatogram revealed by GC using TIC (total ion current) of an alarm pheromone isolated from Aphis nerii **(Boyer de Fonscolombe)** ***using n- hexane as the solvent by using Confinement method***



Graph 14

The inset chromatogram revealed by GC using TIC (total ion current) of an alarm pheromone isolated from Aphis nerii **(Boyer de Fonscolombe)** ***using n- hexane as the solvent using Adsorbent method***





(A) (B)

Figure 2

Identification of Pheromone compound (Mass- spectrophotometry) – Farnesene compound (As alarm pheromone) (A): Representation of Mass Spectrum of the farnesene. (B): Structure of Farnesol isomer A through mass spectral library.

DISCUSSION

Aphids (Aphididae: Homoptera) are valuable study systems for investigating the roles of genetic variation and phenotypic plasticity on population, chemical ecological, and evolutionary dynamics[20]. Worldwide, *Aphis gossypii* (Glover) is known to attack a large number of plant species, including crops (Cereals, Pulses, and Oilseeds), Vegetables, Fruits, Ornamental plants, Weeds and wild plants[21]. *Aphis nerii* along with its principal host plant (Oleander) is thought to be Mediterranean in origin. *Aphis nerii* has since become a common invasive species in warm temperate and tropical regions of the world[22]. It is a widely distributed species of insect prevalent throughout India. A list of 48 species of aphids attacking 62 Medicinal, 23 Ornamental, 20 Vegetables, 11 Fruits, 8 Pulses, 7 Cereals, 6 Oil-seed plants etc. were recorded at Eastern Uttar Pradesh[23]. In Vadodara agroecosystem, approx.30 host plant species from 16 different families were recorded[24]. During 2013, it was reported that 10-90% yield loss in India to the economically important crops depending upon severity of damage and crop stage was due to aphid[25]. Natarajan, (2007)[26] at Central Institute for Cotton Research, Coimbatore emphasized on the management of agriculturally important sucking pests of cotton. Hence this information suggested that management interventions should be focused against reproduction of adult females to prevent the multiplication and spread of the pest. Therefore behavioural bioassays were done by using adult females to determine an effective management scheme by finding the evidence of alarm pheromones from females. The present research gave encouraging results which showed responsiveness against its own species and one against the other species. Laboratory bioassay reported presence of alarm pheromones. Further the volatile extract was isolated and identified. The extract contains the mixture of sesquiterpenes, farnesol isomer A compound which is an alarm pheromone in aphids. In 2008, Gembloux Agricultural University, Belgium reported mixture of sesquiterpenes mainly farnesene as an aphid alarm pheromone[19]. Nault et al in (1973)[27] also identified the components of aphid alarm pheromone, sesquiterpene hydrocarbon (E) -β-farnesene (EBF), it has been given considerable attention both as an aphid repellent and as a coccinellid attractant. (E)-β-farnesene has one naturally occurring isomer. Vos et al (2010)[28] found (*E*)-β-farnesene (EBF) as the predominant constituent of the alarm pheromone in *Myzus persicae* (Sulzur) and many other aphid species. In 1982 at Rothamsted Experimental Station (UK), scientists reported that when attacked or irritated, aphids can produce defensive secretions from their cornicles[29]. In addition to their mechanical defensive action, these secretions generally release volatile alarm pheromones, causing other aphids in the area to disperse. This alarm pheromone acts as repellent to warn or disperse the insect species from its own species. The sesquiterpene, (E)-β-farnesene (EBF), is the principal component of the alarm pheromone of many aphid species. When an aphid is attacked, it releases EBF in a range of concentrations, depending on the stress that is encountered, as well as the specific species, lineage, and developmental stage of the aphid itself. In case of natural enemies such as a ladybird beetles (which is a biocontrol agent) and ants, this volatile extract of pheromone shows a Kairomonal effect. It is reported that upon predator attack, individual aphids may release a small droplet from their abdominal cornicles containing an alarm pheromone[30] to warn the colony of this danger[31]. Based on the laboratory bioassay, we came to know that the associated natural enemies were attracted towards the EBF factor (Sesquiterpene). EBF may also attract some species of aphid predators[32]. EBF leads aphids to undertake predator avoidance behaviors and to produce more winged offspring. Kunert et al. (2010)[33] concluded the predator avoidance behavior; (E)-β-farnesene, from plants does not serve as a direct defense against aphids. The alarm pheromones act as a Kairomone for natural enemies. Hence, present observations conclude that the ladybird beetles larvae/grubs are highly attracted towards the volatile extract of aphid alarm pheromone containing the E -β-farnesene compound. In 2008, it was also reported that in four-arm olfactometer, the males and females of ladybird beetles are highly attracted towards the E-β-farnesene component[34]. The cornical droplets may be attractive to natural enemies and results in an increased risk of predation for the signaler, thereby selecting the prudent alarm signal. In 2000, investigations using the olfactory cues of the multicoloured Asian ladybird beetles, *Harmonia axyndis* (Pallas) were used to locate the pea aphids[35]. Apart from this, ladybird beetles were considered as the predators for aphids. The beetle larvae are considered to be beneficial insects, as bio-control agents. By feeding, they bring down the population of aphids. The chemical ecology of insect and its tritrophic interaction was found out in 2004. It concluded that the natural enemies such as ants get attracted towards the alarm pheromones [36]. Ants and aphids have a strong mutual interaction between them. In 2005, the Ecological Society of Japan worked on aphid-ant interaction[37]. Ants play a major role for infestation of aphids. For all these interactions, EBF is the main component or we can say a game planner. This paper has come out with some very positive and important research in the field of integrated pest management. This is the first such work on the Isolation of aphids within Gujarat of Zoology department of the M.S. University of Baroda, Vadodara, Gujarat. Outside India, researchers touched on many aspects of alternative control of insect pests like Verheggen in 2008 at Gembloux Agricultural University, Belgium worked on the production of alarm pheromone in aphids and perception by ants and natural enemies. Department of Insecticide and Fungicides, AFRC Institute of Arable Crops Research, U.K. worked on the chemical ecology of aphid[38]. In 2011, Department of Evolutionary Neuroethology, Max-Planck-Institute for Chemical Ecology, Germany reported orchid flowers (smell like aphids) mimic aphid alarm pheromones to attract hoverflies for pollination[39]. Hence these types of Semiochemical (Both Pheromones and Kairomones) can be used as repellent to warn or disperse the population of its own species and other species, which can reduce the population of aphids in the fields. It can be used as attractant for bio-control agents and other natural enemies. Therefore the results from behavioural bioassay and isolation of semiochemical compounds encourage for the further proper identification, isolation and synthesis of alarm pheromone in future by collaborating with various R&D sectors. This will be helpful for development of ecofriendly control methods leading to an ultimate contribution to minimize the load of pesticides from agricultural fields.

CONCLUSION

Aphids are the major pests of economically important crops. Since, they have a high reproductive rate, their ability to hide in cracks and crevices of plants and propensity to spread quickly proved that aphid cause severe damage to crops. Many insects were associated with them. Various control tactics are being used in agricultural fields of Vadodara but it is difficult to control them. So, the control of aphids becomes a challenge with no signs of perfect solution. Our study emphasized on the knowledge of the biology and chemical ecology of aphids led to information about the presence of pheromones of aphids. The present study results showed that by finding the evidence of the presence of pheromone and various Kariomones in *Aphis gossypii* and *Aphis nerii* can be further characterized and synthesized for the development of pheromone traps for both the species of aphids which add an alternative control against pesticides.

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