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Studies on the presence of Semiochemicals E-β-Farnesene (EBF) from *Aphis craccivora* (Koch), Gujarat, India

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Abstract
The Aphids, *Aphis craccivora* (Koch) is a serious pest in agricultural fields of Vadodara. In an attempt to find the solutions to reduce the infestations by this pest, study was carried out on the aphid semiochemicals. The alarm pheromone of aphids was isolated by fractionation of crude pheromone extract obtained by aeration of virgin females. The pheromone was identified as mixture of sesquiterpenes compounds (*E-β-farnesene*), by using GC-MS. To see the effectiveness of extract, behavioural bioassays (*using Y-tube olfactometer*) were conducted under laboratory conditions. The results showed that the presence of semiochemicals as alarm pheromones in the female volatile extract which could repel its own species and other species while it attracts the predators and other associated natural enemies. The repulsive index (RI = 97.08; 92.25 and 81.73 *for A. craccivora* and *A. gossypii & A. nerii*) and attractive index (AI = 0.054, 0.025 for *C. septempunctata* and *C. compressus*) were calculated. The responsiveness (χ^2 values – goodness of fit) against extract {*for finding significant values* S*= 0.009; 0.030, 0.001 & S*= 0.024; 0.035} was calculated to provide strong evidence for the presence of alarm pheromones. Hence the information contained in this paper pave way to the identification of proper management practices to maximize lure methods during effective reproductive period.

Keywords: *Aphis craccivora*, Semiochemicals, GC- MS, Y- tube olfactometer

1. Introduction
The earlier studies on the populations of aphids, *Aphis craccivora* (Koch) (Hemiptera: Aphididae) reveal its economic impact as a major concern for all economically important crops in the agricultural fields of Vadodara [1]. Cow pea (*Vigna unguiculata* L. Walp) is one of the most important legume crops in the world [2]. It has a large spectrum of uses: dried grain for human consumption (main use) but also leaves, fresh beans, fresh bean pods, cowpea as well used as green manure and fodder. It is a main staple food of Gujarat [3, 1]. The major constraint for cowpea grain production is insect damage [4]. It was reported that *Aphis craccivora* (Koch) is one of the key pests of cowpea [5] affecting 90% of plants according to the field study. *Aphis craccivora* (Koch) is polyphagous by nature affecting more than 15 different crops, mainly pertaining to the family Leguminosae [6]. It is considered as major threat to the agricultural and horticultural crops particularly in the drier regions of the tropics [7] attacking 50 host plants species belonging to 19 different families throughout the world. It is known to be an important vector of plant viral disease, transmitting over 30 plant viruses [8]. In India, it has been reported from almost all states infesting over 569 plant species [9], aphids has taken leading position among sucking pests. *Aphis craccivora* (Koch) causes yield loss by directly infesting leaves, stems, fruits, roots and also cause damage indirectly by secreting honey dew which causes development of sooty mold as well as attracting ants as transporting agent of the aphids to the different host plants [10]. Both nymphs and adults suck plant sap and cause serious damage right from the seedling to pod bearing stage. Due to heavy infestation, young seedlings succumb to death, whereas the older plants show symptoms such as stunting, crinkling and curling of leaves, delayed flowering, shriveling of pods and finally resulting in yield reduction [11]. Razaq *et al*. [12] reported 10-90% yield loss in India to the economically important crops depending upon severity of damage and crop stage by aphids. During 1987, it has been reported that as high as 100% yield reduction of different bean crops was due to aphid infestation [13]. To protect their crops from 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 [14].The continuous usage of synthetic insecticides caused health hazards, development of pest genotypes resistance to pesticides, resurgence and upset by pests and environmental pollution [15].The use of these insecticides is hazardous to the environment and non-target insects like pollinators and predators [16].Several efforts were made all over the world to devise region specific integrated pest management (IPM) systems. The outbreak of *Aphis craccivora* (Koch) in Indian agricultural fields necessitates the study on the development of bio-rational alternatives to control of *Aphis craccivora* (Koch). Therefore, the present study on the presence of Semiochemicals E-β-Farnesene (EBF) from *Aphis craccivora* (Koch), Gujarat, India was conducted.

2. Materials and Methods

2.1 Collection, Preservation and Identification of Aphids

The present study was conducted during the period from September 2008 to September 2011.The maximum aphid population was observed in the month of January to March. Aphids are polyphagous by nature traversing from Cash crops (Pigeon pea) to oilseeds (Cotton crop) to vegetables (Brinjal) to ornamental plants (Hibiscus). Sample of aphids, *Aphis craccivora* (Koch) were collected from the bean plants from all the agricultural sites of fields of Vadodara (Site I –Channi & Site III- Dabhoi which were 20kms away from Vadodara). Afterwards they 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 [17]. 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.

2.2 Mass multiplication of *Aphis craccivora* (Koch) under laboratory condition

The rearing and breeding of *Aphis craccivora* (Koch) *was* carried at room temperature (20-25ºC, 70-75% R.H.) under a photoperiod of 16L: 8D in the laboratory. The bean twigs, harboring *Aphis craccivora* (Koch) colonies, were collected from the fields and brought to the laboratory. The aphids present on these bean twigs were gently removed with the help of soft camel hair brush and were released on the bean twigs 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 bean twigs for mass rearing. Culture obtained was used for collection of volatiles and behavior bioassays experiments.

2.3 Chemical ecology of Aphids

Aphid olfaction (Proxim al primary rhinaria, distal primary rhinaria and secondary rhinaria), Aphid alarm pheromone (by using two cornicals situated on the upper surface of the abdomen near the tail, aphids produce droplets that emit a repellent odour for conspecifics) [18]. The droplet secreted by the cornicals were found to contain mainly sesquiterpenes ($C_{15}H_{24}$) named (E)-7, 11-dimethyl-3-methylene-1, 6, 10-dodecatriene, or (*E*)-ß-farnesene or trans-ß-farnesene [19]. Aphid sex pheromones were situated on the tibial region of the 3rd hind leg of the aphid.

2.4 Collection of Volatile extract from *Aphis craccivora* (Koch)

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

A) Confinement / Wash Method [20]

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 also used for the behavioural bioassay.

B) Adsorbent Method [21]

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 with an appropriate adsorbent using 10ml of n-hexane. This volatile extract preserved at -20°C in 5ml glass vials with Teflon-liners amber bottles. This solution was then used for bioassay studies.

2.5 Behavioural Bioassay using Y- Tube olfactometer [22]

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 test chamber (Size 20 cm). Air is 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 appropriate equipment (Canon Powershot ISI- 120). Approximately, 100 aphids were released to 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. Readings were taken at every 0min, 15min, 30min, 45min, 1hr, 2hrs, 3 hrs, 4hrs, 5hrs and 6 hrs. 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 spreadsheet layout (Microsoft excel, 2007). Graphs were generated from the spreadsheets.

The repellent activity of volatile extract extracted from *Aphis craccivora* (Koch) species was recorded in terms of percent repellency {Percent Effective Repellency (ER%) = (Nc-NT/Nc) x 100} where Nc 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 = (Nc - N_T)/ (Nc+N_T) x 100 where Nc = 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 percentage effective attractancy and attractive index (AI) for associated insects were calculated. The formulas are given as: Percentage Effective Attractancy (EA %) = ($N_T - N_c / N_T$ x 100) and 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 IBM Statistical Software for $\chi 2$ (Chi square) goodness–of-fit for significance of response.

2.6 Identification of semiochemicals [23], [20]

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 (TCD) were used. The peaks in GC monitor were matched with mass spectral library to identify the compound name and structure.

2.7 Statistical Analysis

The raw data collected from the readings were transferred to an electronic format and converted into spreadsheet layout (Microsoft excel, 2007). Based on the data collected, the repellent activity of volatile extract extracted from *Aphis craccivora* (Koch) species was recorded in terms of percentage effective repellency (ER%) = (Nc- NT/Nc) x 100}. The attractive activity of volatile extract was recorded in terms of percentage effective attractancy (EA %) = ($N_T - N_c / N_T$ x 100). The repulsive index {RI= (Nc - N_T)/ (Nc+N_T) x 100)} for aphid's own species and other species and attractive index {AI = ($N_T - N_c$)/ (N_R-N_C) for other associated natural enemies were recorded using suitable formulas. The collected data analyzed by SPSS-19 IBM Statistical Software for $\chi 2$ (Chi square) goodness–of-fit for significance of response.

3. Results

Earlier studies on assessment of incidence and severity of damage suggested that, plant species belonging to family Malvaceae (17%), Fabaceae (16%), Solanaceae (12%) and Asclepiadaceae (10%) were found as preferred host plants of aphids in Vadodara. There are total 6 types of species of aphids namely *Aphis gossypii*, *Aphis craccivora*, *Aphis nerii*, *Myzus persicae*, *Aphis brassicae*, *Aphis fabae* found in agricultural fields of Vadodara. Most abundant species considered as major pests in agroecosystem of Vadodara include *Aphis gossypii* (Glover); *Aphis craccivora* (Koch) and *Aphis nerii* (Boyer de fonscolombe).

Cowpea aphid, *Aphis craccivora* (Koch) is an important pest of wide range of leguminous crops such as cowpea, groundnut, pigeon pea, chickpea, peas, mungbean and urdbean are pertaining the family Fabaceae. It is a difficult pest to control with insecticides because of its polyphagous nature with very short life cycle and high reproduction rates. So, alternative bio- rational control such as pheromone was the need of urge.

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

Y- Tube consisting of two arms to which fitted a broad tube serving as Test Chamber (T_c).

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)

A) Experiment with *Aphis craccivora* (Koch)

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 setup consisting of female volatile extracted by using confinement method. In this, 20 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.

i) **The effect of volatile extracts of female *A.craccivora* 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. 82 aphids. Most of the aphids remained in the test chamber, only 1-2 number of aphids moved towards the arm B (Extract). This shows that repulsive effect was due to the presence of alarm pheromones (Fig 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.

i) **Experiment conducted on effect of volatile extract of female *A. craccivora* against female *A. gossypii:* -** In this experiment, 100 aphids were released in the test chamber. Out of them, 56 aphids within 5hrs moved towards the arm A. Most of the aphids remain in the test chamber, only 2-5 number of aphids moved towards the extract (arm B) and returned back within 4hrs to the test chamber (Fig 2a).

ii) **Experiment conducted on effect of volatile extract of female *A. craccivora* against *A. nerii:* -**In this experiment, 100 aphids were released in the test chamber. Out of them, maximum number of aphids moved towards the arm A i.e. 28 aphids within 3 hrs. Most of the aphids remain in the test chamber, only 2-4 number of aphid's moved towards the extract (arm B) (Fig 2b). Above experiments confirm that the repulsive effect observed was 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 (multicolored ladybird beetle grubs) and natural enemies (Ants).

i) **Experiments conducted on effect of volatile extract of female *A. craccivora* against Bio-control agents: -** In this experiment, 10 larvae/grubs of ladybird beetle (*Coccinella septempunctata* Linn.) were released in the test chamber. All the 10 grubs of Ladybird beetles remain in the test chamber for 15 minutes, afterwards, around 1 – 1 each grubs moved towards both the arms A and B. Maximum number of ladybird beetle grubs moves towards

arm B (Extract). At 6 hrs, approx. 3 larvae were found in the arm B. Only few numbers of grubs moved towards arm A. In case of ladybird beetles, attraction was observed towards the volatile extract (Fig 3a). The reason was that ladybird beetles grubs are predators on aphids and the strong EBF factor has forced them to attract.

ii) **Experiments conducted on effect of volatile extract of female *A. craccivora* 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 45 minutes, 9 of them are aggregated in the test chamber. At 2hrs, 2 of them were moved towards the arm B (Extract) and remain there in the arm B for 2hrs. After 3 hrs, again all ants are aggregated in test chamber. No ants were moved towards the arm A. So, it can be concluded that ants were attracted towards the volatile extract (Fig 3b). Hence, the outcome of behavioural studies showing the effect of volatile extracts of female *A. craccivora* on aphids, coccinellids and ants was documented (Table 1). This showed that *Aphis craccivora* (Koch) 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 *Aphis craccivora* species and its natural enemies, the repulsive index (RI), attractive index (AI) and significant values (*S) were calculated in (Table 2). The responsiveness, $\chi 2$ (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 1: Outcome of Behavioral bioassay showed the effects of volatile extracts of female *Aphis craccivora* (Koch) on Aphids, Coccinellids (Ladybird Beetle Grubs) and Ants

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

*√ = *Yes*, * *X = No*

A) Effect of volatile extract from female aphid species against its own species

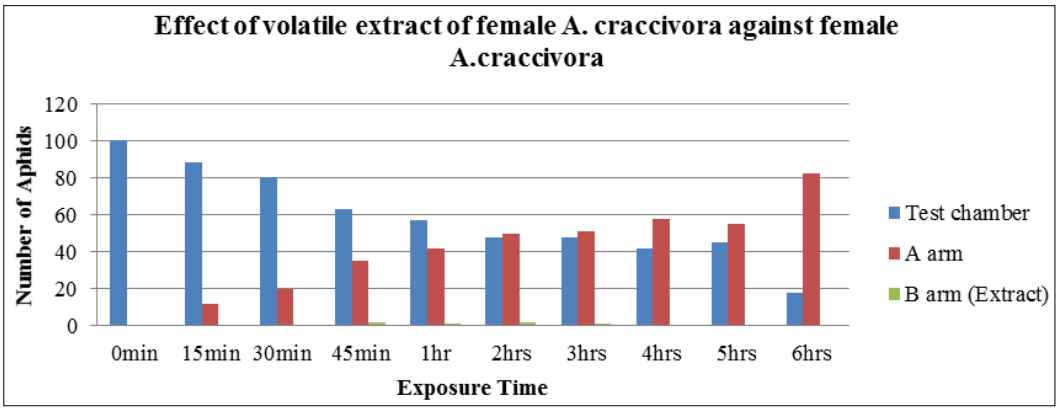
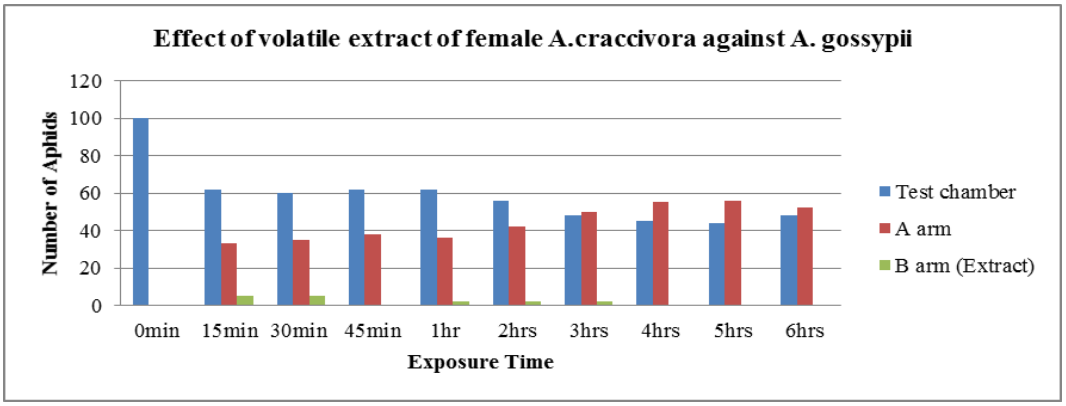
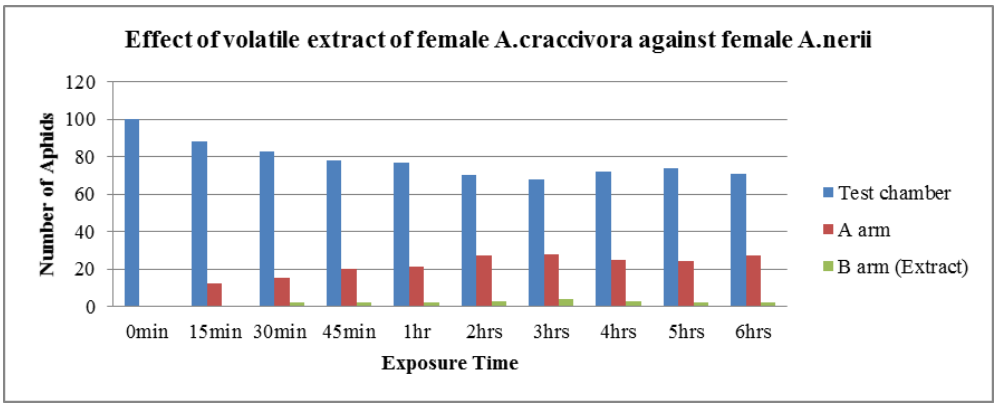


Fig 1: Effect of volatile extracts from female *A. craccivora* against female *A. craccivora*

B) To observe the effect of volatile extract from female aphid species on one species against the other species.



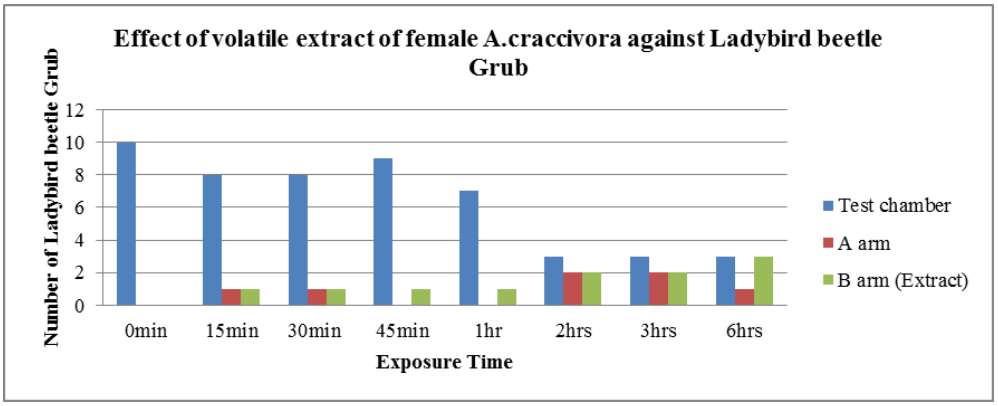
(a) Effect of volatile extracts from female *A. craccivora* against female *A. gossypii*



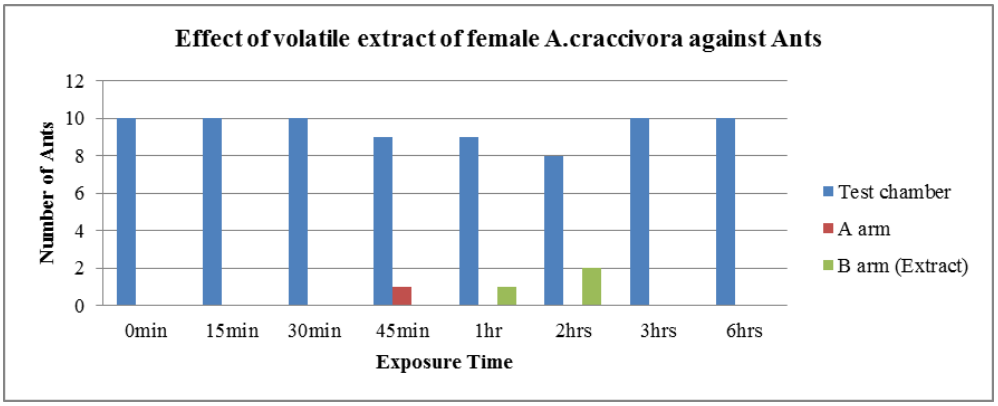
(b) Effect of volatile extracts from female *A. craccivora* against female *A. nerii*

Fig 2: To observe the effect of volatile extract from female aphid species on one species against the other species **(a)** Effect of volatile extracts from female *A. craccivora* against female *A. gossypii* **(b)** Effect of volatile extracts from female *A. craccivora* against female *A. nerii.*

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



(a) Effect of volatile extract of female *A. craccivora* against ladybird beetle grubs



(b) Effect of volatile extract of female *A. craccivora* against Ants

Fig 3: To observe the effect of volatile extract from female aphid species on its bio- control agents (Ladybird beetle grubs) and natural enemies (Ants) **(a)** Effect of volatile extract of female *A. craccivora* against multicoloured ladybird beetle grubs **(b)** Effect of volatile extract of female *A. craccivora* against Ants.

Table 2: Bioassay study using female volatile extract of aphid, *Aphis craccivora* (Koch) against its own, other species and natural enemies for chemical communication.

Adult released in Experiment Setup				Percentage Repellency (%)	Repulsive Index (RI)	χ^2 value & Significance to response
Species	**Number of insect released per trial**	**Number of insect responded**				
Aphis craccivora (Extract) against its own species	100	**E**	0.6	98.52%	97.08	$\chi^2 = 6.857^a$ df = 1 (*S = 0.009)
		C	40.5			
Aphis craccivora (Extract) against *Aphis gossypii*	100	**E**	1.6	95.97%	92.25	$\chi^2 = 4.704^a$ df = 1 (*S = 0.030)
		C	39.7			
Aphis craccivora (Extract) against *Aphis nerii*	100	**E**	2	89.95%	81.73	$\chi^2 = 13.443^a$ df = 2 (*S = 0.001)
		C	19.9			

Bio-control agent (Coccinellid beetles) and Natural enemies (Ants)						
Species	**Number of insect released per trial**	**Number of insect responded**		**Percentage Attraction (%)**	**Attractive Index (AI)**	**χ^2 value & Significance to response**
Aphis craccivora (Extract) against *Coccinella septumpunctata*	10	**E**	1.375	36.36%	0.054	$\chi^2 = 7.445^a$ df = 2 (*S = 0.024)
		C	0.875			
Aphis craccivora (Extract) against *Camponotus compressus*	10	**E**	0.375	76.92%	0.025	$\chi^2 = 4.444^a$ df = 1 (*S = 0.035)
		C	0.125			

**S = Significance; df = 1, 2*

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

3.2. Chemical analysis of aphid alarm pheromones

GC analysis of the volatile blend released by aphids revealed by the presence of several volatile compounds namely hydrocarbons, phenols, fatty acids, monoterpenes and presence of sesquiterpenes (farnesol isomer A which is a farnesene compound which is known as aphid alarm pheromone). The aphid alarm pheromones were identified by using polar and non- polar columns and Gas – chromatography (GC) separation techniques. The Fig was plotted Concentration of volatile vs. Retention time (min).

The collected air borne volatiles in n-hexane were identified as mixtures of sesquiterpenes (E-β- farnesene) in which the major volatiles which are identified by confinement and adsorbent method are:

A) Analysis of *Aphis craccivora* (Koch)

During Confinement method (AC-1), the chromatogram showed the total 6 peaks, including low as well as high peaks. The high peaks were mainly counted during analysis. A Fig was plotted using concentration of volatile vs retention time (min). The isolated compounds of volatile compounds of *Aphis craccivora* (Koch) are mainly Hydrocarbon such as Cyclohexane (5.43), Fatty acids (7.72), 1-Butene-2-ethyl-methyl (10.62), Squalene (12.18), Phenol pentadcyl (24.83), β- (farnesol isomer A) (27.32) and Oleic acid (27.87) (Fig. 1).

In Adsorbent method (AC-2), the chromatogram showed total 12 peaks including low as well as high peaks. The isolated compounds were mainly Hydrocarbon (7.41), 1-Butene-2-ethyl-methyl (10.64), Squalene (14.65), Phenol (20.21), Di- butyl phthalate (24.79), Hexene 1, 3, 5- trimethyl (26.00), Farnesol isomer A (27.28) *etc.* (Fig. 2)

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 structure of chemical compound was to be identified by mass spectrophotometry. The identified structure of chemical compound was matched with the mass spectral library. Here, Farnesol isomer A (farnesene) compound was identified from *Aphis craccivora* species. The volatile extract of *Aphis craccivora* (Koch) using adsorbent method showed the presence of Farnesol isomer A at the retention time 27 minutes 28 seconds in *Aphis craccivora* (Koch). The mass spectral Fig of this compound is also mentioned in (Fig. 3a). The Farnesol isomer is a natural organic compound which is an acyclic sesquiterpene alcohol (Fig. 3b). It is a pheromone of several other insects. Mainly the mixtures of sesquiterpenes are components of the alarm pheromone such as farnesene compound. Verheggen (2008) [24] reported that farnesene compound (mixture of sesquiterpenes) acts as an alarm pheromone of aphids. Pickett *et al* [25] in 1992 also identified farnesene as an alarm pheromone. Aphids have capability to produce an alarm pheromone with Sesquiterpene, (E)-β-farnesene as the primary component. It is released in response to physical stress including attack by natural enemies [26]. This pheromone act to warn related individuals of predation [27]. This could also act as a kairomonal cue for aphid natural enemies. Hence the present research concluded that mixtures of sesquiterpenes (farnesene compounds) act as an alarm pheromones.

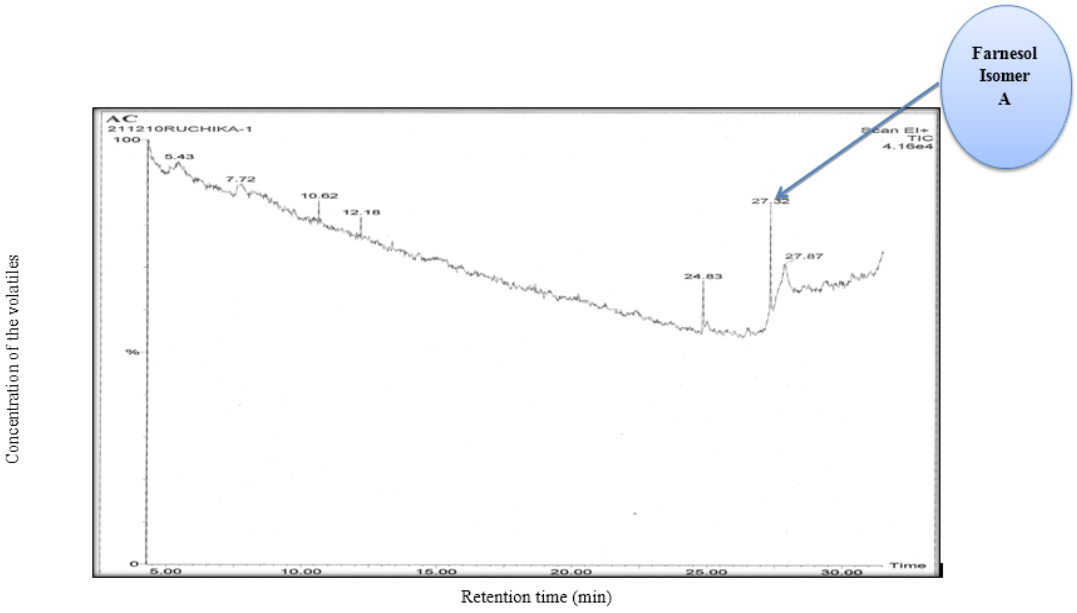


Fig 1: The inset chromatogram revealed by GC using TIC (total ion current) of an alarm pheromone isolated from *Aphis craccivora* (Koch) using n- hexane as solvent by using confinement method.

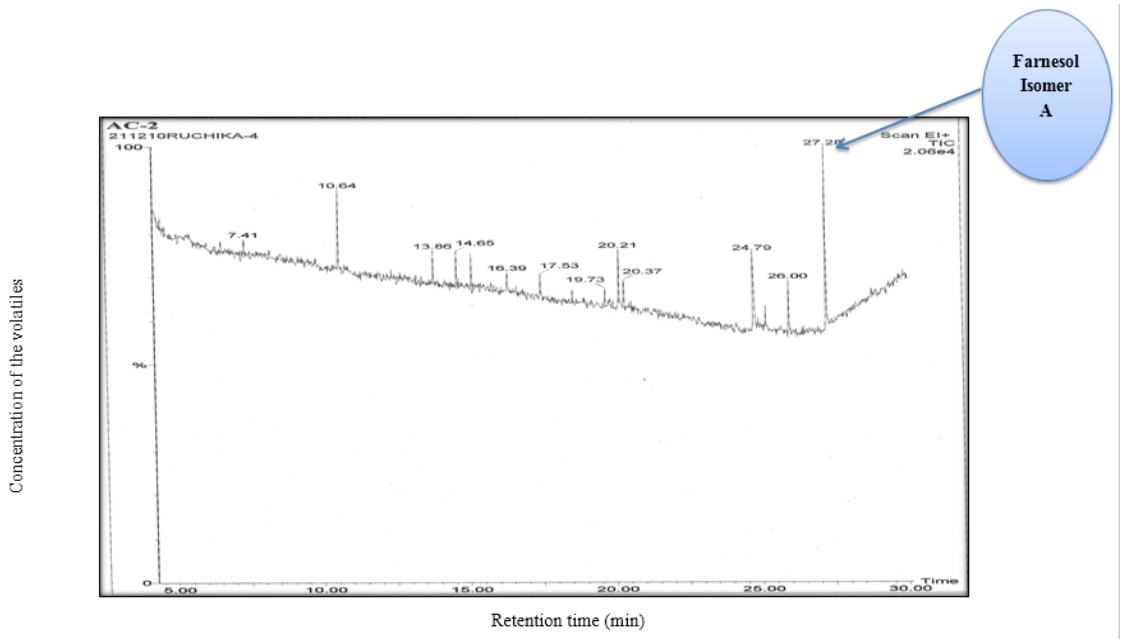
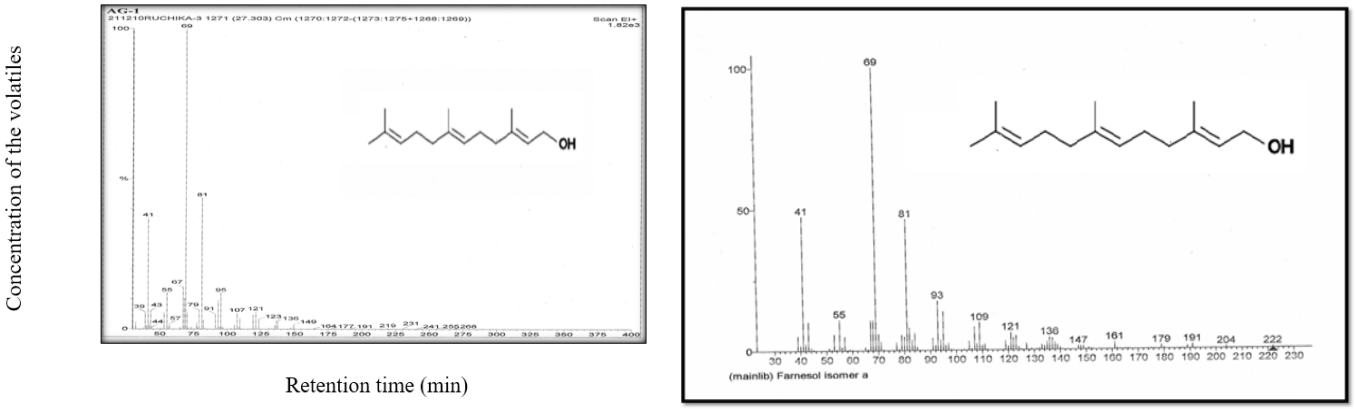


Fig 2: The inset chromatogram revealed by GC using TIC (total ion current) of an alarm pheromone isolated from *Aphis craccivora* (Koch) using n- hexane as solvent by using adsorbent method.



(a) (b)

Fig 3: Identification of Pheromone compound (Mass- spectrophotometry) – Farnesene compound (As alarm pheromone) (a) Representation of MS–Graph of the farnesene. (b) Structure of Farnesol isomer A through mass spectral library.

4. Discussion

Worldwide, *Aphis craccivora* (Koch) is associated with many host plants in the Leguminosae and also in many other plant families so that it attacks about 50 crops in 19 different plant families [28]. The cowpea aphid, *Aphis craccivora* (Koch) (Aphididae: Homoptera) is a widely distributed species of insect prevalent throughout India [29]. 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 in Eastern Uttar Pradesh [30]. Aphids were found to be major pests of the economically important crops of agroecosystems [31].They cause severe damage to many host plants. In Vadodara agro ecosystem, approx. 30 host plant species from 16 different families were recorded [32]. During 2013, it is reported that 10-90% yield loss in India to the economically important crops depending upon severity of damage and crop stage is due to aphid [33]. 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, behavioural bioassay 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 the fruitful results 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 [24]. Nault *et al.* [34] 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.* [35] 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 reported that when attacked or irritated, aphids can produce defensive secretions from their cornicles [36]. In addition to their mechanical defensive action, these secretions generally release volatile alarm pheromones, causing other aphids in the area to disperse. It is also released by aphids as an alarm pheromone upon death to warn away other aphids [37]. This alarm

pheromone acts as repellent to warn or disperse the insect's 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 can release 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 [38] to warn the colony of this danger [35, 39]. Based on the laboratory bioassay, the present study came to know that the natural enemies such as Ants and Ladybird beetles were attracted towards the EBF factor (Sesquiterpene). EBF may also attract some species of aphid predators [40]. EBF leads aphids to undertake predator avoidance behaviors and to produce more winged offspring. Kunert *et al.* [41] concluded the predator avoidance behavior; (E)-b-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 multicoloured ladybird beetles larvae/grubs are highly attracted towards the volatile extract of aphid pheromone that is alarm pheromone containing the β- farnesene compound. In 2008, it is also reported that in four-arm olfactometer, the males and females of ladybird beetles are highly attracted towards the E- β-farnesene component [42]. 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 multi-coloured Asian ladybird beetles, *Harmonia axyridis* (Pallas) were used to locate the pea aphids [43]. 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 [44]. Ants and aphids have a strong mutual interaction between them. 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. In India, the fields of Entomology main work have been on morphology, taxonomy, physiology, ecology and population dynamics but very little work on Isolation of lures. This is the first such work on Isolation of aphids within Gujarat from Zoology department of the M.S. University of Baroda .Vadodara, Gujarat. Natarajan, (2007) [45] at Central Institute for Cotton Research, Coimbatore emphasized on the management of agriculturally important sucking pests of cotton. Outside India, researchers touched on many aspects like Verheggen in 2008 [24] 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 [25]. In 2005, the Ecological Society of Japan worked on aphid-ant interaction [46]. 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 [47]. Hence, these 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 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 semiochemicals compounds encourage for the further proper identification isolation and synthesis of alarm pheromone in future by collaborating with various R&D sectors from aphid species. This will be helpful for development of ecofriendly control methods leading to an ultimate contribution to minimize the load of pesticides from agricultural fields.

5. Conclusion

Aphids are the major threat in agricultural fields of Vadodara. 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 causes severe damage to the economically important crops. Many insects were associated with them. The 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. In an attempt to find the solutions to reduce the infestations by this pest, the present study emphasized on the knowledge of the biology and chemical ecology of aphids which led to information about the presence of pheromones of aphids. Usually aphids secrete both alarm and sex pheromones but it is quite difficult to scan the males in the life cycle because males are found on the secondary host and once in a year. The results revealed that the rate of repulsion of female aphids towards the female volatile extracts which could repel its own species and other species while it attracts the predators and other associated natural enemies. The evidence of the presence of pheromone and various kariomones in *Aphis craccivora* can be further characterized and synthesized for the development of pheromone lures and traps for aphids which add an alternative control against pesticides. It would also useful in providing good scope for the further development of ecofriendly methods for *Aphis craccivora* control.

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