Evidence of female-produced sex pheromone in red pumpkin beetle, *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae)

The red pumpkin beetle, *Aulacophora foveicollis* Lucas is a serious pest and causes severe damage in pumpkin, *Cucurbita moschata* Duch. Ex Poir and other cucurbits. Both larval and adult stages are injurious to the crops. The former feeds on roots and stems of the plants, while the latter feeds on foliage, petals and fruits[1]. Red pumpkin beetle is mainly being controlled by chemicals, and thus ecology-based pest management (EBPM) will be an eco-friendly strategy to manage this injurious pest. It was found that the females produced sex pheromone. This may be a potential tool for managing this pest using sex-pheromone trap. Thus the use of chemical pesticides may be reduced. Moreover, no information on sex communication in this species is available so far.

Field-collected adults of red pumpkin beetle were reared in the laboratory at 12 h L: 12 h D, 25 ± 1°C and 80–85% RH condition. Following the hatching of eggs, the neonate grubs were reared on tender pumpkin slices to get virgin male and female to study the reproductive biology (Table 1) and sex communication of this insect. Laboratory bioassay was carried out using the Y-tube olfactometer[2] and pheromone extraction chamber to study the reproductive biology of red pumpkin beetle.

An all-glass Y-tube olfactometer (Figure 1) was used to study the behavioural bio-assay in the virgin adults[2]. The central main part of the olfactometer was a Y-tube consisting of two arms fitted to broad tubes serving as a test chamber (B) in which the materials to be tested were kept. The middle portion of the Y-tube was fitted with a broad conical chamber called release chamber (A), where the insects to be tested for responsiveness were released. Air was blown from the other end of the Y-tube using a motor (C) (Neikkei, 2000, 230 V, 50–60 Hz). In the test chambers, inward glass projections were provided to place meshes, so that the adults were allowed only to reach the test chamber, but were not able to touch the confined material. The entire olfactometer was washed thoroughly with soap solution before each experiment.

Studies conducted in a dark room with live adults in the olfactometer to avoid any visual contact, indicated the presence of pheromonal attraction between sexes. After this confirmation, the sex that attracted its opposite sex was confined in a multicapillary volatile collection apparatus (Figure 2) for collection of volatiles[3]. The apparatus consists of an air-loading chamber (A) (size 42 cm × 36 cm) through which air was blown and which opened at five exits. The exits were closed with wide-mouthed, small chambers for confining the adults, with long capillary tubes at the ends (30 cm). Air from the motor (Neikkei, 2000, 230 V, 50–60 Hz) is filtered by passing through activated charcoal and then into the air-loading chamber. From this chamber, air was passed through multi-resting chambers in which the adults were confined (B). The volatiles produced by the adults

Table 1. Biology of red pumpkin beetle, *Aulacophora foveicollis* (Lucas)

Biology	Days ± SE
Incubation period	11.70 ± 0.23
Larval period	12.25 ± 0.14
Pupal period	11.25 ± 0.09
Male longevity	62.70 ± 2.40
Female longevity	63.05 ± 1.68
Sex ratio	1.5 : 1
Fertility percentage of egg	86.14 ± 1.24
Pre-mating period	02.25 ± 0.12
Mating period	12.15 ± 0.47
Oviposition period	08.70 ± 0.55
Post-oviposition period	25.70 ± 1.62

SE, Standard error.

Figure 1. Y-tube olfactometer.

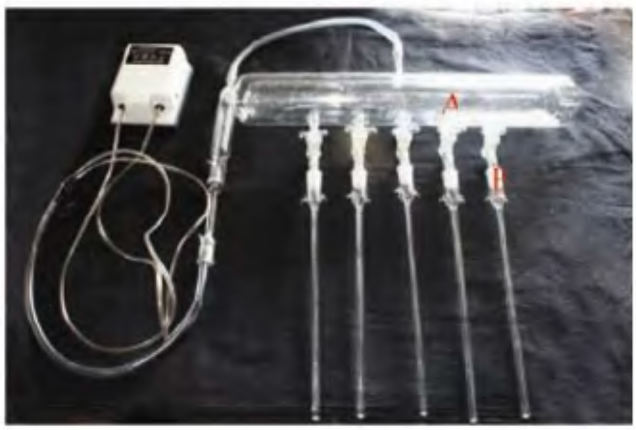


Figure 2. Multicapillary volatile collection apparatus.

Table 2. Bioassay study using live adults and volatiles for pheromonal communication

Adult released in release chamber			Preference				
Sex	Number	Number responded	Percentage	For sex	Choice combination	χ^2 value and significance	Remarks
Male	51	31	60.78	F	M vs F	8.24*	Females attracted males
Female	51	4	07.84	M	M vs F	43.82 NS	Females are not attracted by males
Male	34	23	67.65	FV	FV vs S	3.69*	Males are attracted by female volatile
Female	37	2	05.41	FV	FV vs S	33.25 NS	Females are not attracted by female volatile
Female	36	3	08.33	MV	MV vs S	30.39 NS	Females are not attracted by male volatile
Male	35	1	02.86	MV	MV vs S	33.14 NS	Males are not attracted by male volatile
Male	31	23	74.19	FV	MV vs FV	2.09*	Males are attracted by female volatile
Female	22	1	04.55	MV	MV vs FV	20.14 NS	Females are not attracted by male volatile

*

Table 3. Attractive index and responsiveness of different sexes of *Aulacophora foveicollis*

Sex of the adult in release chamber	Combinations in each arm of test chamber	Responsiveness to		Attractive index
		Test material	Percentage	
Male	Live female	Live female	71.66 R	0.72[a]
Male	Female volatile vs solvent	Female volatile	67.38 R	0.67[a]
Female	Male volatile vs solvent	Male volatile	7.86 NR	0.08[b]
Female	Live male	Live male	7.53 NR	0.07[b]
Female	Live female	Live female	0.57 NR	0.06[b]
Female	Female volatile vs solvent	Female volatile	5.00 NR	0.05[b]
Male	Male volatile vs solvent	Male volatile	2.86 NR	0.03[b]
Male	Live male	Live male	0.00 NR	0.00[b]

R, Responded; NR, Not responded; *CD (0.05) = 0.099.

settled on the inner walls of these capillary tubes after evaporation.

Collection of volatiles was done during the peak pheromone-producing hours (i.e. the time during premating and mating behaviours, studied through observation of their mating habits in the dark room), preferably during the early hours of the scotophase. Mating activity was predominant during early morning hours (i.e. 2–7 am). Then the confined adults were removed and the resting chambers were cleaned. The deposited chemicals were collected by washing the capillary tubes several times with *n*-hexane after removing from the device. Bioassay using live adults and their volatiles collected from the sexes was carried out using the olfactometer. The response of each sex of live adults to the opposite sex and the same sex and also to their volatiles was studied by counting the number of adults entering into each arm. The solvent was used as control for volatiles and the empty arm as control for live adults.

Based on the data colleted as described above, the attractive index (AI) was worked out as below[4].

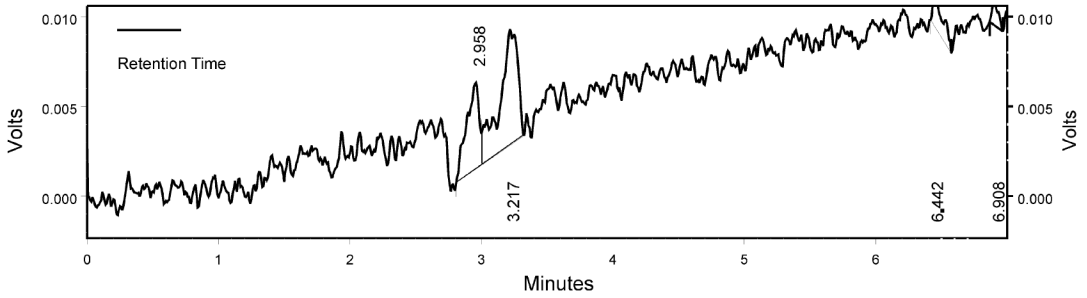
$$\mathrm{AI} = \frac{(\text{No. of insects responded to test material} - \text{No. of insects responded to control})}{(\text{No. of insects released} - \text{No. of insects responded to control})}.$$

A study on the response of sexes to their respective sex was conducted in the laboratory. In the first trial, 51 males were released into the release chamber. Thirty-one males entered into the arm containing females. This indicated that the females attracted the males. In the second set of experiments, having male and female beetles in the test arms, 51 females were released in the release chamber. Only four females were attracted by the male and so it was concluded that the males did not attract the females.

The volatiles were collected from the females based on the outcome of this study. The response of the male and female adults to the female volatiles was estimated. The female volatile was kept in one arm and the solvent *n*-hexane was kept in another arm. In the first set of experiments, 34 males were released in release chamber in five groups. Twenty-three males were attracted towards the arm containing the female volatile. In the next set of experiments, 37 females were released into the release chamber in five groups. Only two females moved to the arm containing the volatile female. This clearly indicated that the female volatile did not attract the females. From the present study, it is found that the female volatile attracted only the males and failed to attract the females (Table 2).

The above experiment was also conducted with male volatile. In the first set of experiments, 36 females were released into the release chamber in five groups, and only three females were attracted by the male volatile. Thus the male volatile did not attract the females. In another set of experiments, 35 males were released into the release chamber in five groups. Among them, only one male entered the arm containing the male volatile. This shows that the male volatile did not attract the males (Table 2).

Another study was conducted keeping both male and female volatiles, simultaneously, to evaluate the responsiveness to different sexes of red pumpkin beetle. Out of 31 males released in four groups,



UV (210 nm)

Pk #	Retention time	Area	Area (%)	Height
1	2.958	32,257	29.598	4807
2	3.217	62,443	57.297	6482
3	6.442	9742	8.939	1594
4	6.908	4540	4.166	1483
Total		108,982	100.00	14,366

Figure 3. HPLC analysis of volatile collected from 12-day-old virgin female.

23 responded to the female volatile. This shows that the female volatile alone attracted the males. In another set of experiment, 22 females were released into the release chamber in three groups. Only one female entered into the arm containing the male volatile. So it is concluded that the male volatile does not attract the females (Table 2).

Statistical analysis using χ^2 goodness-of-fit also confirmed that the male beetles were attracted to female beetles and female volatiles[5]. The formula used for analysis is: $X^2 = \sum(O - E)^2/E$.

The responsiveness of the adult beetles was converted to AI and subjected to statistical analysis (Table 3). The maximum value of 0.72 was recorded for the live females which attracted the males. The AI value for female volatile was 0.67, which was equivalent to live females. Hence further investigations needed to isolate, identify and characterize the compounds of female volatile will be indicative of the pheromone produced by the females of *A. foveicollis*.

HPLC analysis of the volatiles of females revealed that the highest peaks were observed in all samples collected from females of different ages (7 to 14 days old) between the retention time 3.22 and 3.27 min. The peaks were monitored by UV absorbance at 210 nm. The mobile phase was 0.05% 2-propanol in hexane and the flow rate was 1 ml/min. The silica column was used[6] and the retention time was kept up to 7 min. The highest peak was noted in the volatile collected from 12-day-old females (Figure 3).

Studies regarding sex communication in this serious pest of pumpkin indicated that the females produced sex pheromones. Attraction of males to females has been well established. A similar phenomenon was also seen in other coleopterans like banded cucumber beetle, *Diabrotica balteata*[7] and Colarado potato beetle, *Leptinotarsa decemlineata*[8]. Such pheromonal attractions were tested in wind tunnels and using olfactometer[9].

In this study, it is found that the male beetle is attracted towards the female. Such an attraction of male towards female of Japanese beetle, *Popillia japonica* has been reported[10]. Similarly, females of soybean beetle, *Anomala rufocuprea* played an important role in mating by producing pheromone[11]. Females of elaterid beetle, *Limonius californicus* attracted males[12]. In this study, the volatiles from females were collected with *n*-hexane. The pheromonal extracts of *A. flavipes* was also collected by using *n*-hexane[13]. In the olfactometer study, males of *A. flavipes* moved towards the female pheromone[13]. In our study also, the males were attracted to the females and the volatiles collected from the females.

This study confirms the evidence regarding presence of female sex pheromone in red pumpkin beetle. Standardization of HPLC to obtain the peaks has been done in this study. This will help in further fractionating the global extract into splits. Thus it may be possible to isolate, identify, characterize and synthesize the pheromone to develop pheromone trap for managing this destructive pest of pumpkin in an eco-friendly way.

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