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Olfactory orientation of *Cotesia flavipes* Cameron on info chemicals emitted from larvae & excreta of *Chilo auricilius* Dudgeon and *Chilo partellus* Swinhoe

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Abstract
Stalk borer of sugarcane, *Chilo auricilius* Dudgeon and *Chilo partellus* Swinhoe is an important pest of sugarcane, sorghum, maize and other graminaceous plants. They are damage the plants by boring and making tunnel inside the stem and filled by frass. Due to the borers attack the plant lost their productivity. In sugarcane crop the millable quality is severely destroyed by the *C. auricilius* and other borers. *Cotesia flavipes* Cameron is a gregarious larval parasitoid of stalk borer of sugarcane, *Chilo auricilius* Dudgeon, *Chilo partellus* Swinhoe and other borers. In present studies, kairomonal effects of n- hexane based excreta and larval body wash extract of *C. auricilius* and *C. partellus* on *Cotesia flavipes* (Cameron) orientation behaviour in Y-tube olfactometer bioassay (Choice & no choice) have studied under laboratory condition to understand the role of larvae and their excreta on parasitization behaviour of *C. flavipes*. In bioassay the larval body wash of *C. auricilius* attracted significantly higher number of *C. flavipes* (40) than *C. partellus* (32). In case of excreta extract, the excreta extract of *C. partellus* attracted significantly higher number of *C. flavipes* (36) than *C. auricilius* (31). It is indicates the short range odors emanating from the larvae and excreta may play role in attracting and guiding the *C. flavipes* to location of host in plant. The higher number of wasp attraction towards the larval body wash represents the larval odour is an important component for increasing the parasitization efficiency of parasitoids.

Keywords: Kairomone, sugarcane, *Chilo auricilius, Chilo partellus, Cotesia flavipes,* olfactometer

Introduction
Sugarcane, (*Saccharum officinarum* L.) is important cash crop cultivated in India. It is cultivated in about 5.0 million hectare cultivable land in tropical and subtropical states of India with a national average cane yield of about 67.4 t/ha that is much lower than global average cane yield, 70.77 t/ha (FAO, 2013) [1]. There is a number of biotic and abiotic factors responsible for low cane yield. Under the biotic factors the borers are major once. In the category of borer, stalk borer, internode borer and top borer are the major ones. Stalk borer and internode borer are more serious as they damage the standing cane from July to November. Stalk borer may cause 31.8 per cent loss to cane yield and 5.3 to 20.4 per cent to sucrose (Singh *et al.*, 1973) [2].

For the management of cane bores, biological controls measures such as release of larval parasitoids, *Cotesia flavipes* Cameron @ 500 gravid females/ha at weekly interval from July to November are in practice. *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) is a gregarious Indo-Australian larval parasitoid of stalk borer of sugarcane, *Chilo auricilius* Dudgeon and is amenable for mass production on the larvae of *C. auricilius* and *Chilo paetellus* Swinhoe and second most used parasitoid against cane borers of sugarcane (Singh, 2006) [3]; (Tanwar and Varma, 2001) [4].

Efficacy of parasitoids can be enhanced by having the idea of relationship among the host plants, pests and parasitoids (tritrophic relationship) that is influenced by some volatile chemicals emanated from the surface of plant leaves and insect pests (Semiochemicals) (Vinson, 1976) [5].

In sugarcane based agro-ecosystem, the tritrophic relationships (Figure 1) among the plant, insect host and parasitoids. The larvae and pupae are concealed in tunnel made by the larvae inside the cane and are not easily visualized by the parasitoid. Thus the parasitization of concealed larvae is must be guided possibly by chemical cues interactions because the herbivore-induced plant volatiles and larval excreta are important as host finding cues for larval parasitoids (Potting 1996) [6].

Utility of the kairomones extracted from the mass reared host insects and their byproducts enhance the activity of egg and larval parasitoids would be a practical approach. To achieve effective utility of kairomonal materials in laboratory and in field condition were need to be assayed to determine the kairomonal activity.

In present study, some sources of kairomones ie., n-hexane based extracts of larvae and excreta of *C. auricilius* & *C. partellus* were used to study the olfactory orientation of *C. flavipes* in the laboratory through Y- tube olfactometer for better understanding the interaction between pest and parasitoid. This information will apply in future to increasing the efficiency of *C. flavipes* and achieve the goal of higher parasitization rate.

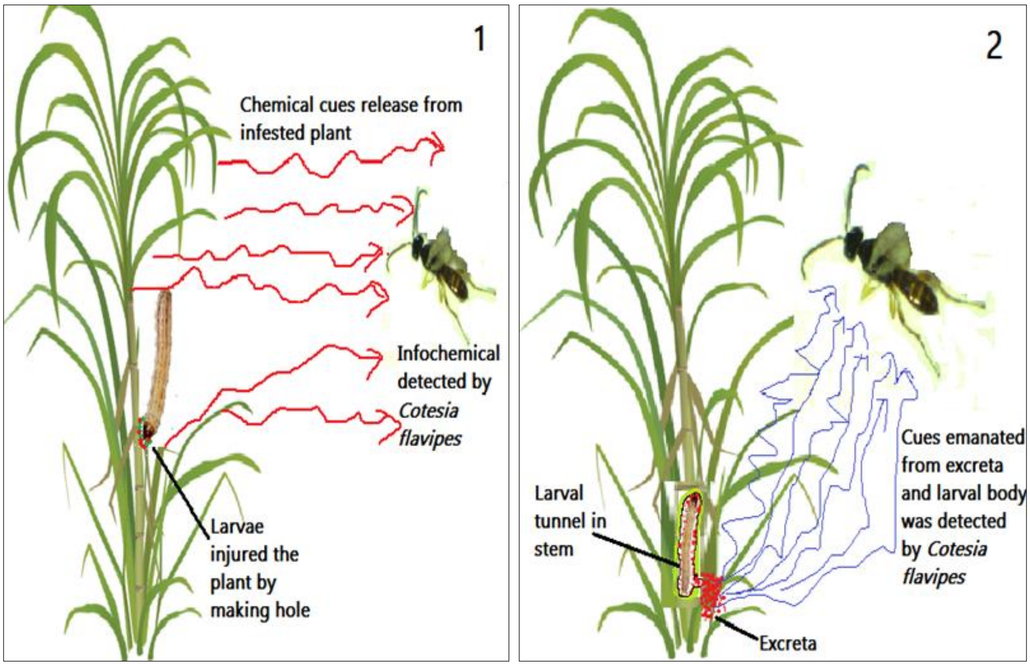

Fig 1: Tritrophic interaction between sugarcane plant, *C. auricilius* larvae and *C. flavipes* in sugarcane ecosystem.

Method and Materials

In order to understand the kairomonal interaction between insect pest and parasitoids, the parasitoid (*Cotesia flavipes* Cameron) are taken to study the effect of kairomones (body wash and frass) on orientation behaviour of parasitoids.

C. flavipes was multiplied in the laboratory on the stalk borer larvae and utilized in this study.

Host insect culture

Stalk borer of sugarcane was multiplied in the laboratory on artificial diet/natural food at 27 ± 1 ^{0}C and 70 % RH in the BOD incubator. The artificial diet developed by Verma *et al.* (1975) [7] was used for rearing the *C. auricilius* and field collected larvae (nucleus culture) of sorghum stem borer, *Chilo partellus* Swinhoe were mass reared on cut stalk of sorghum in wide glass tubes at 27 ± 1 ^{0}C and 70 % RH in the BOD incubator.

Parasitoids culture

Field collected nucleus culture of *C. flavipes* was multiplied in the laboratory on the *C. auricilius* larvae at 27 ± 1 ^{0}C and 70 % RH in the BOD incubator. Well fed (50% honey solution) 24 hours old female wasps were used for bioassay. The Y- tube olfactometer was used for bioassay study of different extracts.

Preparation of extracts

For bio assay studies, different kairomonal extracts were prepared in the laboratory. The materials from which extracts were drawn are as follows

Materials used for Kairomonal extracts: Stalk borer, *Chilo auricilius* Dudgeon (larvae and frass) and sorghum stem borer, *Chilo partellus* Swinhoe (larvae and frass).

Extraction of Kairomonal extract from factitious and natural host

Kairomonal substances are short range info-chemicals, which help the communication between host and parasitoids. As the *Chilo partellus* Swinhoe is used as laboratory host for mass rearing of *Cotesia flavipes* Cameron. Thus the kairomonal potential of *Chilo auricilius* Dudgeon 3rd instars larvae and excreta was compared with factitious laboratory hosts *C. partellus* (larvae and excreta). Extraction of larvae and excreta of *C. auricilius* and *C. partellus* was done as per the method described by Ananthakrishnan *et al.* (1991) [8].

Larval body wash and excreta extract extraction from *C. auricilius* and *C. partellus* larvae

In one stoppered bottle (125 ml), 1.0 gm quantity of excreta or full grown (3rd -4th instar larvae) laboratory reared larvae of *C. auricilius* or *C. partellus* was taken and 30 ml n-Hexane was added. The bottles were shaken in water bath shaker at 135-140 rpm for 2 hours at 28 ^{o}C followed by 20 minutes shaking at 50°C. These were filtered through Whatman No 1 filter paper and concentrated to 10 ml by shaking the bottle containing filtrate in open water bath at 50°C and stored at -20 ^{o}C in a deep freezer and used for bioassay purpose. The extraction of larval body wash or excreta extract was done individually for both species.

Development of Olfactometer Device

Olfactometer is an important device that used to study the parasitic behavior towards semiochemicals in general. The Y tube olfactometer has been devised to study the attraction of parasitoid, *C. flavipes* towards extracts under dual choice set of experiment. Y-tube olfactometer is used for testing the single semiochemical source/samples for choice and no choice experiments. A 'Y'-tube olfactometer was devised in Biological Control Laboratory, IISR, Lucknow for behaviour study of insects with the suitable modification in Y-tube air flow olfactometer developed by Potting *et al*. (1996) [6].

Bioassay of Kairomone

Bioassay studies of extracts from whole body wash and excreta extract of host insects were carried out under laboratory conditions at 26 ± 1°C and 65 ± 5% R.H. in Y-tube olfactometer.

n- Hexane based filtrates of all types of extracts were taken as 100 % concentration. Two strips measuring 2x1cm^2 were cut from Whatman No. 1 filter paper. One strip was treated with 50 µl filtrate as treatment and one other strip treated with 50 µl n Hexane as control. One of each treated strip was placed in individually in two arms of the vertical positioned Y- tube of olfactometer arm marked as "T" (treatment) and "C" (control). The arms and saline drip pipe from air distribution chamber were connected by twist connector for proper air circulation. Individual one day old well fed female wasps of *C. flavipes* (females separated based on antennal dimorphism as their antennae are smaller than male wasp) were released to stem of Y tube and open end of the stem was plugged with n Hexane treated cotton and movement of insect was watched. In one set of experiment, 5 wasps were released and observed. Two sets of the same experiment were maintained in one replication. Each Y tube olfactometer bioassay was replicated for 5 times. To avoid any asymmetrical bias in the sets of experiment the strip treated with odor source and treated with n Hexane were exchanged after the release of five wasps. The procedure adopted here with suitable modifications is described by Potting *et al.* (1996) [6]. Responds of *C. flavipes* to treatment/treated control was recorded by counting the number of wasps visited the target. If the test female walked towards an odor source and crossed the 'choice line' (2 cm after division of the base tube) and stayed there for more than 10 seconds, it was taken as a choice for the odor source in that arm.

Statistical analysis

Data obtained from Y tube olfactometer bioassay were analyzed by Chi square test of independence (α = 0. 05) in SWAU [9] turner faculty online mathematics analysis.

Result and Discussion

The purpose of present study was to elucidate the sources of the volatile stimuli responsible for the attraction and orientation of parasitoids. The *C. partellus* was taken as a host for comparing with *C. auricilius* the natural host of *C. flavipes*.

Response of *C. flavipes* to larval body wash extract of *C. partellus* and *C. auricilius* in "Y" tube olfactometer bioassay

In bioassay, the larval body wash of *C. auricilius* attracted significantly higher number of *C. flavipes* (40) than *C. partellus* (32).

The result showed that larval body wash extract of *C. partellus* and *C. auricilius* attracted the significantly higher number of released wasps of *C. flavipes* but dual choice preference of *C. flavipes* in larval body wash of *C. partellus* and *C. auricilius* was found non-significant (χ^2 = 3.175, df = 1; p < 0.05) (Table 1 and figure 2). In the light of this conclusion is evident that the hypothesis is proved and there is no difference between observed and expected values and the association between the larval body wash of both insect and dual choice preference by *C. flavipes* was independent. On basis of chi-square interpretation, the dual choice preferences by *C. flavipes* in respect of larval body wash of *C. partellus* and *C. auricilius* was independent, the results of preference may be governed by own effect of responsible larval body wash extract. Result indicates the volatiles emitted from the larval body are responsible for parasitization activity by *C. flavipes* wasp because higher number of wasp attraction was recorded in larval body was extracts of both insects. No work has been done on *C. auricilus* but on other insects, it has been reported that wasps of *C. flavipes* follow the chemical cue since searching the host.

Tumlinson *et al.* (1992) [10] and Godfray (1994) [11] reported that the wasps of *C. flavipes* approaching it's host, female is exposed to host derived chemical cues that may be host specific. According to Obonyo *et al.* (2010) [12] the body wash of *Chilo partellus* Swinhoe larvae increase the parasitization rate of *Cotesia flavipes* Cameron, it is a one of the responsible factor for *C. flavipes* attraction. The host finding behaviour of *C. flavipes* in T-tube olfactometer was done by Ramani and Rao (2003) [13] and they reported the *C. flavipes* females were attracted strongly to the arm containing the larval and excreta washings of *Chilo partellus*.

Table 1: Kairomonal effect of larvae body wash extract of *C. partellus* and *C. auricilius* larvae on behaviour of *C. flavipes* in dual choice Y tube olfactometer bioassay.

Two-way Contingency Table	Dual choice test		Total χ^2	Total
	Choice	No choice		
Chilo partellus larvae body wash extract	32 *36* (0.44)	18 *14* (1.14)	1.58	50
Chilo auricilius larvae body wash extract	40 *36* (0.44)	10 *14* (1.14)	1.58	50
Total	72	28	3.175	100

χ^2 = 3.175, df = 1, χ^2/df = 3.17, $P_{\alpha=0.05,1}$ (χ^2 > 3.175) = 3.84, Expected values are displayed in *italics*. Individual χ^2values are displayed in (parentheses).

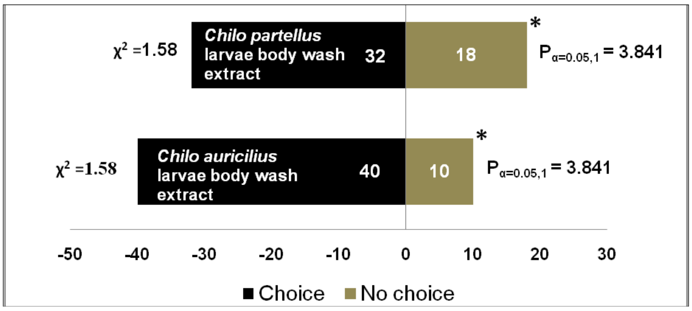

Fig 2: Kairomonal effect of larvae body wash extract of *C. partellus* and *C. auricilius* larvae on behaviour of *C. flavipes* in dual choice Y tube Olfactometer bioassay. Asterisks indicate the dual choice preferences with in test is independent (chi-square, P<0.05)

Response of *Cotesia flavipes* Cameron to excreta extracts of *Chilo partellus* Swinhoe and *Chilo auricilius* Dudgeon in "Y" tube olfactometer bioassay

In bioassay study the excreta extract of *C. partellus* attracted significantly higher number of *C. flavipes* (36) than *C. auricilius* (31).

The result showed that excreta extract of *C. partellus* and *C. auricilius* attracted the significantly higher number of released wasps of *C. flavipes* but dual choice preference of *C. flavipes* in excreta extract of *C. partellus* and *C. auricilius* was found non-significant ($\chi^2 = 1.131$, df = 1; $p < 0.05$) (Table 2 and figure 3). In the light of this conclusion is evident that the hypothesis is proved and there is no difference between observed and expected values and the association between the excreta extract of both insect and dual choice preference by *C. flavipes* was independent. On basis of chi-square interpretation, the dual choice preferences by *C. flavipes* in respect of excreta extract of *C. partellus* and *C. auricilius* was not associated, the results of preference may be governed by own effect of responsible excreta extract.

Table 2: Kairomonal effect of frass extracts of *C. partellus* and *C. auricilius* larvae on behaviour of *C. flavipes* in dual choice Y tube Olfactometer bioassay

Two-way Contingency Table	Dual choice test		Total χ^2	Total
	Choice	No choice		
Chilo partellus larvae excreta extract	36 *33.5* (0.19)	14 *16.5* (0.38)	0.57	50
Chilo auricilius larvae excreta extract	31 *33.5* (0.19)	19 *16.5* (0.38)	0.57	50
Total	67	33	1.131	100
$\chi^2 = 1.131$, df = 1, χ^2/df = 1.13, $P_{\alpha = 0.05,1}$ ($\chi^2 > 1.131$) = 3.84, Expected values are displayed in *italics*. Individual χ^2values are displayed in (parentheses).				

No work has been done on *C. auricilus* but on other insects, it has been reported that wasps of *C. flavipes* follow the chemical cue since searching the host. The results showed that the excreta may play an important role as short range chemical cues during the host search by *C. flavipes*. In year 2003, Ramani and Rao [13] conducted a experiment to study the host finding behaviour of *C. flavipes* in T-tube olfactometer and they reported the *C. flavipes* females attracted strongly to the arm containing the excreta of *Chilo partellus* Swinhoe. The attraction of parasitoids towards the excreta extract represent the cues emitted from excreta is responsible for attracting and guiding the location of host in plant. White *et al.*, (2004) [14], Van Leerdam *et al.*, (1985) [15], Mohyuddin, (1971) [16], Potting, (1996) [6], Steinberg *et al.*, (1992) [17], Srikanth *et al.*, (2000) [18], Ngi-Song and Overholt, (1997) [19] reported that the excreta of *C. partellus* larvae is a short range host location cue for *Cotesia flavipes* Cameron.

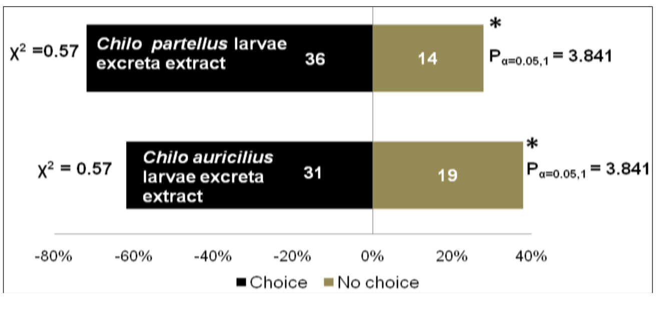

Fig 3: Kairomonal effect of frass extracts of *C. partellus* and *C. auricilius* larvae on behaviour of *C. flavipes* in dual choice Y tube Olfactometer bioassay. Asterisks indicate the dual choice preferences with in test is independent (chi-square, P<0.05)

Conclusion

The results indicate the odors emanating from the larvae and especially excreta may play important role in attracting and guiding the *C. flavipes* to location of host in plant. The highest number of parasitoid visit on larval body washes represents the larval odor is an important component for increasing the parasitization efficiency of parasitoids.

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