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Olfactory orientation of *Cotesia flavipes* Cameron on infochemicals emitted from *Chilo auricilius* Dudgeon infested sugarcane plant

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Abstract

Sugarcane is ravaged by a number of borer pests but the stalk borer, *Chilo auricilius* Dudgeon is most devastating one and acts as main limiting factor in obtaining high cane yield. The cane borers are effectively managed by larval parasitoid wasp, *Cotesia flavipes* Cameron. For effective management of borer pest by *C. flavipes* needs better understanding the tritrophic interaction between host, plant and parasitoids. So the efficacy of parasitoids can be enhanced by having the idea of relationship among the host plants, pests and parasitoids that is influenced by some volatile chemicals emanated from the plant. In present studies, the olfactory orientation of *C. flavipes* in n-hexane based extracts of leaves, healthy cane part and infested cane part extracts of a plant infested by *Chilo auricilius* Dudgeon of four sugarcane varieties viz., Co0238, CoJ64, CoLk8102 & CoLk94184 was studied in "Y-tube" olfactometer bioassay by choice and no choice experiment. In comparison the extract of all plant parts of four sugarcane varieties, the leaf extract of Co0238 was attracted highest number of *C. flavipes* (38) followed by the leaf extract of CoJ64 (35), infested cane part extract of CoLk94184 (35) and infested cane part extract of CoLk8102 (33). The overall trends of wasp attraction were observed in leaf extracts of sugarcane varieties. Among the leaf extracts the Co0238 attracted highest number of *C. flavipes* (38) followed by CoJ64 (35), CoLk8102 (32) and CoLk94184 (31). It represents the leaves of plant is one of the major responsible component for guiding the parasitoids to attraction toward the plants. Findings indicate that the sugarcane plant damaged by stalk borer, *Chilo auricilius* Dudgeon produces volatile infochemicals that attracted and guiding the parasitoid, *C. flavipes* towards the infested plant. It indicates the long range odors emanating from the infested plants may play role in attracting and guiding the *C. flavipes* to locating the *C. auricilius* infested plant.

Keywords: Synomone, infochemicals, sugarcane, *Chilo auricilius*, *Cotesia flavipes*, olfactometer.

1. Introduction

Sugarcane, (*Saccharum officinarum* L.) is one of the commercial field crops cultivated in India. Sugarcane 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^[5]). There is a number of biotic and abiotic factors responsible for low cane yield. Under biotic factors, insect pests are the major constraints in obtaining higher cane yield. Being a long duration crop, sugarcane provides a congenial environment for the development of a number of insect pest and their bio-agents. Sugarcane is attacked by about 125 insect pests (Patil *et al.*, 2004^[6]) but out of them about 20 insect pests including borers, sucking pests, subterranean and defoliating pests causes economic losses to the crop. 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)^[9]. For the management of cane borers, 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. Biological control by *C. flavipes* offers feasible, eco-friendly, effective and sustainable management of borer pests of sugarcane.

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 partellus* Swinhoe and second most used parasitoid against cane borers of sugarcane (Singh, 2006)^[8]; (Tanwar and Varma, 2001)^[13]. Efficacy of parasitoids can be enhanced by having the idea of relationship among the host plants, pests and parasitoids (tritrophic relationship) (Figure 1) that is influenced by some

volatile chemicals emanated from the surface of plant leaves and insect pests (Vinson, 1976)^[18].

In sugarcane based agro-ecosystem, the study of tritrophic relationships (Figure 1) among the plant, insect host and parasitoids are most important because 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 are important as host finding cues for larval parasitoids (Potting, 1996)^[7].

In present study, some sources of synomones such as n-hexane extracts of sugarcane leaves, healthy and infested portion of the cane infested by sugarcane stalk borer in four sugarcane varieties wise Co0238, CoJ64, CoLk8102, CoLk94184, were used for bioassay purpose for olfactory response of *C. flavipes* in the laboratory through Y-tube olfactometer.

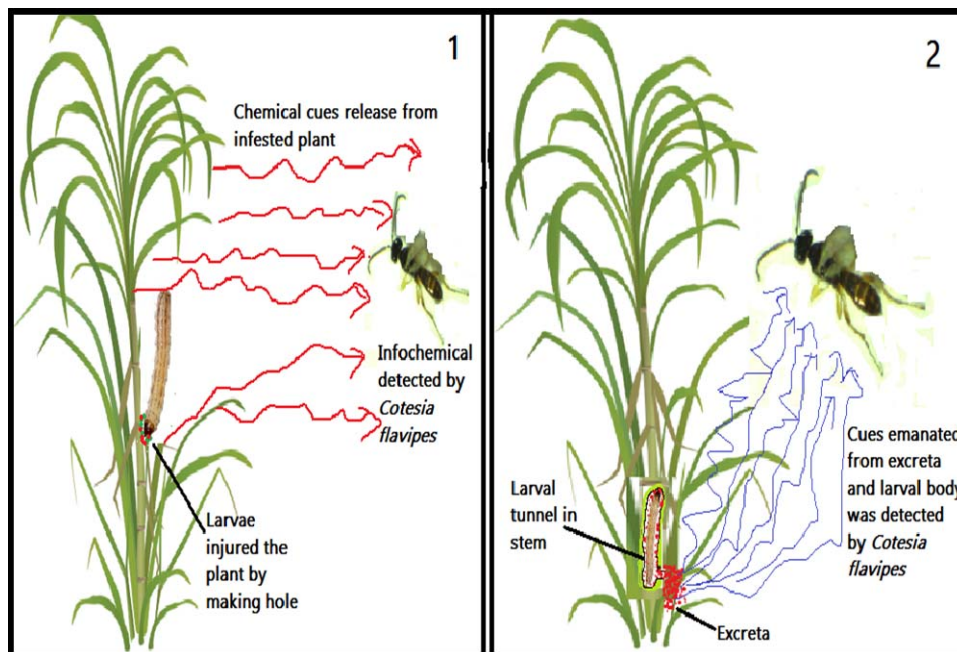


Fig 1: Tritrophic interaction between sugarcane plant, *C. auricilius* larvae and *C. flavipes* in sugarcane ecosystem

Method and Materials

In order to understand the Synomonal tritrophic interaction between insect pest, plant and parasitoids, the parasitoids (*Cotesia flavipes* Cameron) are taken to study the effect of synomones (extracts of plant parts of plant infested by stalk borer, *Chilo auricilius* Dudgeon) on parasitoids of sugarcane stalk borer.

Four sugarcane varieties viz., Co0238, CoJ64, CoLk8102 and CoLk94184 were planted at experimental farm of ICAR-Indian Institute of Sugarcane Research Lucknow in February, 2014 and 2015. A good crop was raised by giving all agronomic practices on time.

Insect Culture

Stalk borer of sugarcane was multiplied in the laboratory on artificial diet/natural food (cane bits) at 27 ± 1 °C and 70 % RH in the BOD incubator. The artificial diet developed by Verma *et al.* (1975)^[16] was used for rearing the *C. auricilius*. Field collected nucleus culture of *C. flavipes* was multiplied in the laboratory on the *C. auricilius* larvae at 27 ± 1 °C and 70 % RH in the BOD incubator. Well fed (50% honey solution) 24 hours old 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 synomonal extracts were prepared in the laboratory. The materials from which extracts were drawn are as follows

Materials used for synomonal extracts: Leaves, healthy and infested part of the sugarcane plant damaged by stalk borer of all four sugarcane varieties.

Extraction of synomones from sugarcane plant

Sample Preparation

Cut sets of all four sugarcane varieties viz., Co0238, CoLk8102, CoJ64, CoLk94184 were planted in plots measuring 6m x 4.5 m at IISR, Research Farm in the month of February, 2015. Before extraction of synomones, 5 month old healthy sugarcane plants (5 from each variety) were inoculated with 3rd instar stalk borer larvae @ 9 larvae / plant. Each cane was drilled to make up ward vertical tunnel (3-4 cm) at three places at the distance of 10 cm in upper half of the cane and three larvae were pushed in each tunnel. As and when insects start feeding on plants, the plants release immediately info-chemical signals for parasitoids. Plant samples were collected 5 days after larval feeding. Infested plants were cut and brought to the laboratory. From top 4th to 6th leaves were cut for extraction process because these leaves actively release a good quantity of long range volatile organic compounds and info-chemicals. Leaves and cane were washed thoroughly in distilled water and soaked excess water was wiped out with tissue paper. After the cleaning the cane was split gently in two parts from the base with the help of sterilized knife. Infested and healthy parts of cane were cut in 3 cm long and 0.25 cm thick pieces separately in sterilized petridishes. Leaves of stalk borer infested plants were also washed in distilled water and cut in to small pieces (1.5 cm²) separately in sterilized petridishes.

In the case of infested cane, the excreta, exuviae of larvae, head capsules after moultings etc. were cleaned gently by n-hexane (99% AR) before preparing the sample and rest of the method was the same as in the case of healthy cane parts.

Extraction of healthy and infested portions of the cane infested by *C. auricilius*

Ten gram of cut pieces of infested cane part samples was taken in one conical flask (500 ml) containing 130 ml n-hexane (99% AR) and kept for overnight. In next day the extract was filtered through Whatman No. 1 filter paper in clean and sterilized conical flask (250 ml). These extract are stored in deep freezer at -20 °C for bioassay study. Same processes are applied in extraction of healthy cane part extract from *C. auricilius* infested plant from all varieties of sugarcane.

Extraction of leaves of plant infested by *C. auricilius*

Ten gram of cut pieces of leaf samples was taken in one conical flask (500 ml) containing 130 ml n-hexane (99% AR) and kept for overnight. In next day the extract was filtered through Whatman No. 1 filter paper in clean and sterilized conical flask (250 ml). These extract are stored in deep freezer at -20 °C for bioassay study.

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 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 (1996) [7].

Bioassay of Synomone

Bioassay studies of extracts from different parts of host plant were carried out under laboratory conditions at 26 ± 1 °C and $65 \pm 5\%$ R.H. in Y-tube olfactometer.

Bioassay of Synomonal extracts for larval parasitoid, *C. flavipes*

n-Hexane based filtrates of all types of extracts were taken as 100 % concentration. Two strips measuring 2cm x1cm 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 (1996) [7]. 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 ($\alpha = 0.05$) in SWAU [11] turner faculty online mathematics analysis.

Result and Discussion

Response of *C. flavipes* in different plant parts extract of four sugarcane variety in "Y-tube" olfactometer bioassay

The purpose of the present research was to elucidate the sources of the volatile stimuli responsible for this attraction. This was achieved by testing the attractiveness of leaves, healthy cane part and infested cane part extracts of a plant infested by *Chilo auricilius* Dudgeon of four sugarcane varieties (Co0238, CoJ64, CoLk8102 and CoLk94184). The dual choice experiment was performed in the laboratory to understand the orientation of parasitoid under natural conditions. Present study is mainly targeted to attractive behavior of parasitoid to plant synomone.

In comparison the extract of all plant parts of four sugarcane varieties, the leaf extract of Co0238 was attracted highest number of *C. flavipes* (38) (Figure 2 ; Table 1) followed by the leaf extract of CoJ64 (35) and infested cane part extract of CoLk94184 (35). The lowest number of *C. flavipes* was attracted in infested cane part extract of CoJ64 (20) and healthy cane part extract of CoLk8102 (20).

The bioassay study of infested cane part extract of CoJ64 and healthy cane part extract of CoLk8102 attracted the less number of parasitoids (20) as against n-hexane control (no choice) (30) and this result clearly represents the dual choice variable (Choice and No choice) are associated ($\chi^2 = 9.26$, $df = 1$; $p > 0.05$).

Attractiveness of leaf, infested cane part and healthy cane part extract of each individual variety was compared. In the case of Co0238, the leaf extract was attracted more number of *C. flavipes* (38), followed by infested cane part extract (31) and healthy cane part extracts (29). The plant part extract of CoJ64, the leaf extract was attracted highest number of *C. flavipes* (35) followed by healthy cane part extract (30) and infested cane part extract (20). The plant parts extract of CoLk8102, the infested cane part extract was attracted more number of *C. flavipes* (33) followed by leaf extract (32) and cane part extracts (20). The plant part extract of CoLk94184, the infested cane part extract attracted highest number of *C. flavipes* (35) followed by healthy cane part extract (32) and leaf extract (31).

Among the leaf extracts of all four sugarcane varieties, the leaf extract of Co0238 attracted highest number of *C. flavipes* (38) followed by CoJ64 (35), CoLk8102 (32) and CoLk94184 (31).

Among the infested cane part extracts of four sugarcane varieties, CoLk94184 was attracted highest number of *C. flavipes* (35) followed by CoLk8102 (33), Co0238 (31), and CoJ64 (20).

Among the healthy cane part extracts of four sugarcane varieties, CoLk94184 was attracted highest number of *C. flavipes* (32) followed by CoJ64 (30), Co0238 (29) and CoLk8102 (20).

The overall trends of wasp attraction were observed in leaf extracts at sugarcane varieties. It represents the leaves of plant is responsible major component for guiding the parasitoids to attraction toward the plants.

C. flavipes were more attracted to odors from the extracts of leaves, healthy and infested cane part of all sugarcane variety as compared with n-hexane control. The dual choice preferences by *C. flavipes* in leaf, healthy and infested cane part extract of four sugarcane varieties was found significant ($\chi^2 = 27.827$, $df = 11$; $p > 0.05$). In the light of data, it is evident that there is significant difference between observed frequency and expected frequencies and the association between the all plant part (leaf, healthy and infested cane part) extracts of four sugarcane variety and dual choice preference by *C. flavipes* was dependent. On basis of chi square interpretation, the dual choice preferences by *C. flavipes* is associated with the effect of all varietal leaf, healthy and infested cane part extract. It indicates that preference is govern by effect of all plant part (leaf, healthy and infested cane part) extract of four sugarcane variety.

Findings indicate that the sugarcane plant damaged by stalk borer, *Chilo auricilius* Dudgeon produces volatile infochemicals that attract the parasitoid, *Cotesia flavipes* Cameron. A large amount of work has been reported in other crops not in sugarcane.

Plants have evolved several characteristics (physical and bio-

chemical) to defend them against herbivore attack (Tallamy and Raupp, 1991) [12]. An indirect defense mechanism of plants by releasing the volatile chemical signals is to promote the effectiveness of natural enemies of the herbivore (Dicke and Sabelis, 1988) [3]. This may be achieved by mediating an interaction between the herbivore and its natural enemies.

It is known that *C. flavipes* can discriminate the volatile chemicals emitted from un-infested and infested plants (Potting, 1996) [7]. When at the time of feeding by herbivores, it work as trigger to induce the volatile synomonal chemical signal and the damage by herbivores can increase the emission of plant volatiles enormously (Dicke *et al.*, 1990) [2]; (Turlings *et al.*, 1990) [14] and these plant volatiles can be specific indicators of herbivore identity (Dicke *et al.*, 1990) [2] [4]. If these chemicals attract natural enemies whose activities are favorable to the plant, they are herbivore-induced synomones (Vet and Dicke, 1992) [17]. The herbivore-induced synomonal chemical signal is not restricted to the infested plant parts, but occurs systemically throughout the plant (Dicke *et al.*, 1990) [2]; (Turlings and Tumlinson, 1992) [15]. Potting (1996) [7] reported the volatile emanating from the larvae infested plant part are more attractive to *C. flavipes* as compare the other plant parts. Bekele *et al.* (2003) [1] and Steinberg *et al.* (1992) [10] also reported that *Cotesia spp.* is highly attracted to the herbivore infested plant or plant parts of maize.

Table 1: Synomonal effect of different plant parts extract of four sugarcane varieties on *C. flavipes* behaviour in dual choice experiment on Y-tube olfactometer

Variety	Extract of different plant parts	Dual choice test		Total χ^2	Total
		Choice	No choice		
Co0238	Healthy leaf extract from <i>C. auricilius</i> infested sugarcane plant	38 30.50 (1.84)	12 19.50 (2.88)	4.72	50
	Infested cane part extract from <i>C. auricilius</i> infested plant	31 30.50 (0.01)	19 19.50 (0.01)	0.02	50
	Healthy cane part extract from <i>C. auricilius</i> infested plant	29 30.50 (0.07)	21 19.50 (0.12)	0.19	50
CoJ64	Healthy leaf extract from <i>C. auricilius</i> infested sugarcane plant	35 30.50 (0.66)	15 19.50 (1.04)	1.7	50
	Infested cane part extract from <i>C. auricilius</i> infested plant	20 30.50 (3.61)	30 19.50 (5.65)	9.26	50
	Healthy cane part extract from <i>C. auricilius</i> infested plant	30 30.50 (0.01)	20 19.50 (0.01)	0.02	50
CoLk8102	Healthy leaf extract from <i>C. auricilius</i> infested sugarcane plant	32 30.50 (0.07)	18 19.50 (0.12)	0.19	50
	Infested cane part extract from <i>C. auricilius</i> infested plant	33 30.50 (0.20)	17 19.50 (0.32)	0.52	50
	Healthy cane part extract from <i>C. auricilius</i> infested plant	20 30.50 (3.61)	30 19.50 (5.65)	9.26	50
CoLk94184	Healthy leaf extract from <i>C. auricilius</i> infested sugarcane plant	31 30.50 (0.01)	19 19.50 (0.01)	0.02	50
	Infested cane part extract from <i>C. auricilius</i> infested plant	35 30.50 (0.66)	15 19.50 (1.04)	1.7	50
	Healthy cane part extract from <i>C. auricilius</i> infested plant	32 30.50 (0.07)	18 19.50 (0.12)	0.19	50
Total		366	234	27.827	600

$\chi^2 = 27.827$, $df = 11$, $\chi^2/df = 2.52$, $P_{\alpha=0.05,11} (\chi^2 < 27.827) = 19.675$, $P_{\alpha=0.05,1} = 3.841$. Expected values are displayed in *italics* Individual χ^2 values are displayed in (parentheses)

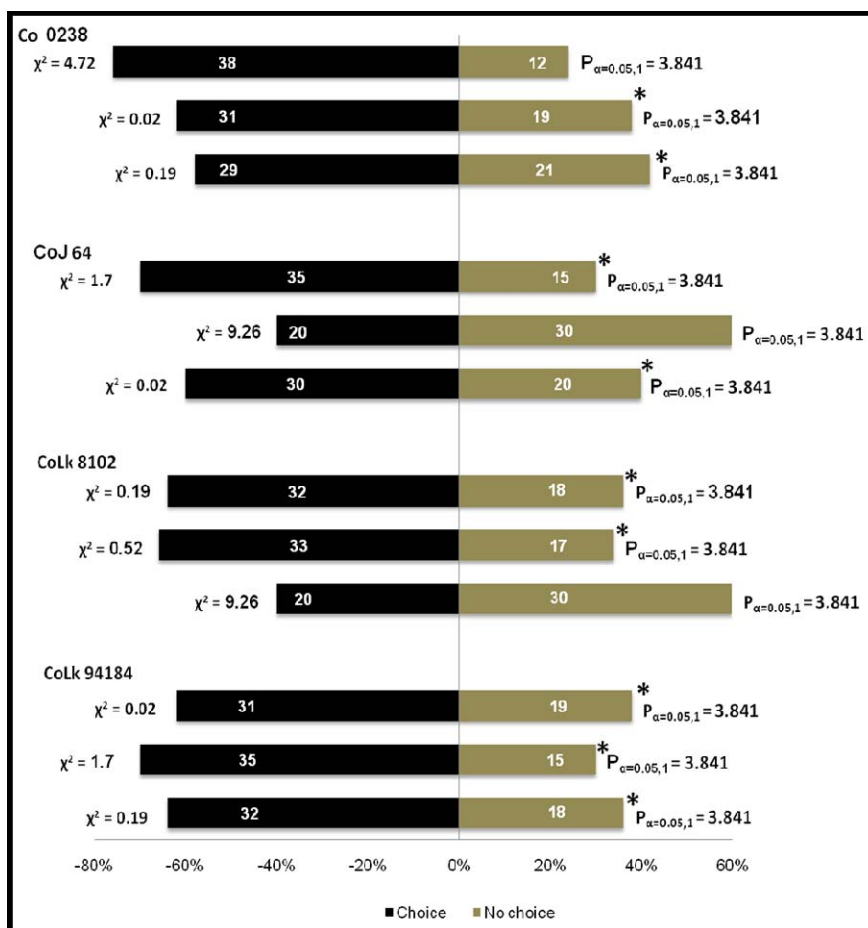


Fig 2: Symnomonal effect of different plant parts extract of four sugarcane varieties on *C. flavipes* behaviour in dual choice experiment on Y-tube olfactometer. Asterisks indicate the dual choice preferences with in test is independent (chi-square, $P < 0.05$)

Conclusion

The leaves of plant are one of the major responsible components for guiding the parasitoids to attraction toward the plants. The sugarcane plant damaged by stalk borer, *Chilo auricilius* Dudgeon produces volatile info chemicals that attracted and guiding the parasitoid, *C. flavipes* towards the infested plant.

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