# Role of Kairomones in Ovipositional Preference of Coconut Black-headed Caterpillar Opisina arenosella Walker Lepidoptera: Oecophoridae)

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**Role of Kairomones in Ovipositional Preference of Coconut black headed caterpillar *Opisina arenosella* Walker (Lepidoptera: Oecophoridae)**

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**Abstract**

The oviposition preference assay was conducted to determine the role of host kairomones for oviposition behavior of *Opisina arenosella*. Results revealed that, ovipositing females are significantly attracted for oviposition to coconut leaf and larval frass volatiles than control. The chemicals present in leaf and frass *viz.,* linalool (91.54%), acetophenone (87.58%) and limonene (80.65%) were more attractive among six electrophysiologically active volatiles. These three volatiles can be used to develop a semiochemical based pest management method.

***Key words*** – Coconut black headed caterpillar, Host finding behavior, Plant semiochemicals, Oviposition preference, Frass kairomones

Coconut (*Cocos nucifera* Linn., Palmaceae) is a major plantation crop widely cultivated specially in tropical areas. India is the third largest coconut producer of the world with an area of 2.14 million ha and production of 22684 million nuts. Karnataka is second only to Kerala, in area under coconut (0.52 million ha) and third in production with 5892 million nuts per annum (India stat, 2014).

All parts of the coconut palm are attacked by hundreds of pests round the year *viz*., fronds, nuts, inflorescence, stem and roots. Among the pests that attack the foliage, Coconut black headed caterpillar, *Opisina arenosella* Walker (Lepidoptera: Oecophoridae) is of serious concern. *O. arenosella* is a multivoltine tropical moth having discrete generation cycles with 5 or 6 generations per year (Muralimohan and Srinivasa, 2008). Larvae damage coconut palms in India, Sri Lanka, Bangladesh, Myanmar, Indonesia and Thailand (Perera *et al*., 1989; Srinivasa and Muralimohan, 2008). The female moth lays eggs on underside the coconut leaflets; the larvae feed on parenchymal tissues of coconut leaflets, constructing galleries using faecal matter and silk on the underside of leaflets (Perera, 1987; Ramkumar, 2002). Gregarious feeding by larvae in the leaflets leaving only the upper epidermis gives a scorched appearance to fronds. From a distance, infested orchards, give a burnt-up appearance with drastically reduced green tissue of the canopy. In severely infested orchards reduced nut production, increased premature nut fall and retardation of growth occurs (Lever 1969; Mohandas, 1992). Severe damage on fronds causes >50% crop loss in subsequent year after severe outbreak and it takes four subsequent years for the plams to regain their normal yield (Chandrikamohan *et al*., 2010). Several chemical, biological and cultural methods of control have been developed in the past but practical difficulties have put forward an urgent need to explore an alternative eco-friendly practical approach.

Use of kairomones is a recent trend in developing semiochemical based pest monitoring and management. Owing to its restricted feeding habit it is reasonable to expect *O. arenosella* may use specific host kairomone (host volatiles) to find its oviposition sites. Exploiting the behaviourally active host kairomone as a potential tool for pest monitoring and mass trapping purposes offers a clean and green pest management method. The present investigation on the role of host volatile for oviposition preferences was conducted to identify behaviourally active potential volatile compounds.

Crude volatiles from green leaf and larval frass were extracted by three solvents (HPLC grade) *i.e*., Hexane, Acetone and Dichloromethane (DCM). Undamaged leaflet (3 mm wide) 80g were cut and soaked in a 250ml Borosil glass bottle and 50ml of solvent was added. In another extract freshly collected larval frass (40g) were put in 250 ml glass bottle and 25ml solvent was added. Both the sets were left at room temperature overnight. The elutant was filtered through silica gel and concentrated using a gentle flush of nitrogen. On condensing the sample was stored at -20$^0$ C until used for behavioral assay.

Dual choice oviposition preference assay was conducted in the laboratory using crude extracts and electrophysiologically active six volatile compounds from larval frass and coconut leaf which was identified by Kumara *et al*. (2014) through, GC-MS, EAG and olfactory meter studies *i.e*., 1-Hexanol, Acetophenone, Linalool, Lemonene, 2-Hexanal and Nonanal.

The transparent polystyrene cylinders (10cm dia. and 45 cm ht) were used for the assay. Both sides of the cylinder were covered with two layers of black muslin cloth. Adequate moisture and honey solution was provided in the assay chamber. Odour source was placed on the cloth in one side and other side used as control (or blank). Fifty µl of crude volatile extract was placed on the muslin cloth of odour side and renewed daily. The experimental setup was placed in well ventilated place in the laboratory rack and each odour source kept sufficiently apart to avoid confusion. In each trial a single mated female was placed in the cylinder and allowed to lay eggs. The number of eggs on both sides were counted and removed daily. Observation was taken daily until death of the female and study repeated five times with each odour.

Based on the behavioural response in the above study three compounds *vis*., linalool, acetophenone and lemonene were selected and blended in seven different ratios (as in Table 1) and each blend was tested as above in oviposition preference assays against solvent (Dichloromethane) as control (Table 1). Statistical analysis was done using the SPSS version 21.

In dual choice oviposition assay, females preferred volatile side of the oviposition chamber. Both leaf and frass volatile extract using dichloromethane was preferred (t =11.098, df= 10, p=0.0001 and t=11.910, df=10 p=0.0001). Extracts of frass volatile using hexane was preferred as compared to hexane extract of leaf (t=10.06, df =10, p=0.0001 and t=0.212, df =10, p=0.836). The leaf volatile extracted from acetone was given high preference than control however, the frass volatiles extracted in acetone did not show any preference as compared to control (Figure 01).

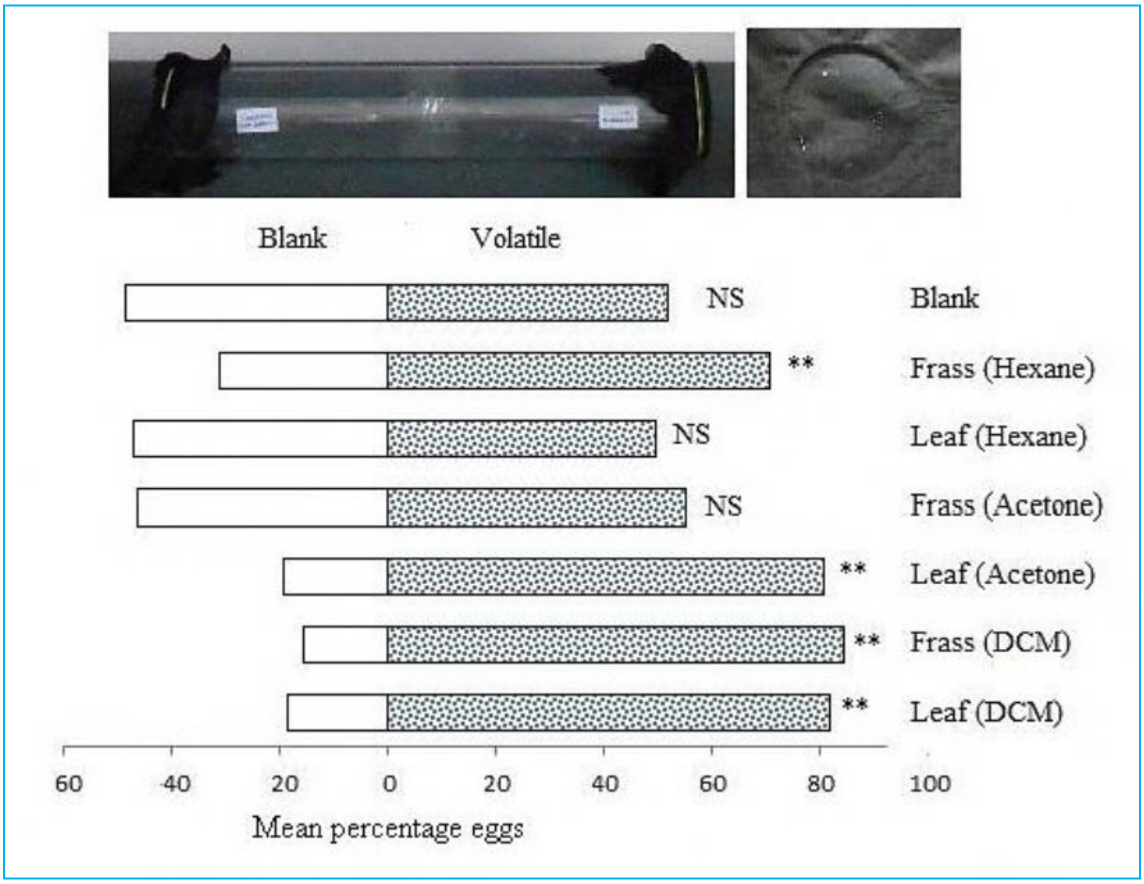


**Fig. 1**. Mean percentage eggs on crude extracts of coconut leaf and *O. arenosella* larval frass volatiles and control based on female dual choice oviposition assay. ** indicates the significant differences in the mean of eggs on volatile side with the control side (p≤0.05).

Dual choice oviposition assay for physiologically active compounds indicated that, females were more attract linalool (t =12.66, df = 8, p = 0.0001), acetophenone (t = 34.74, df

= 8, p= 0.0001) and limonene (t = 38.21, df = 8, p = 0.0001) treated side for egg laying as against control. 1-Hexanol (t=1.99, df =8, p= 0.08) 1-Hexanal (t = 0.83, df = 8, p = 0.429) and Nonanal (t= 1.77, df= 8, p=0.115) were less preferred (Figure 2).

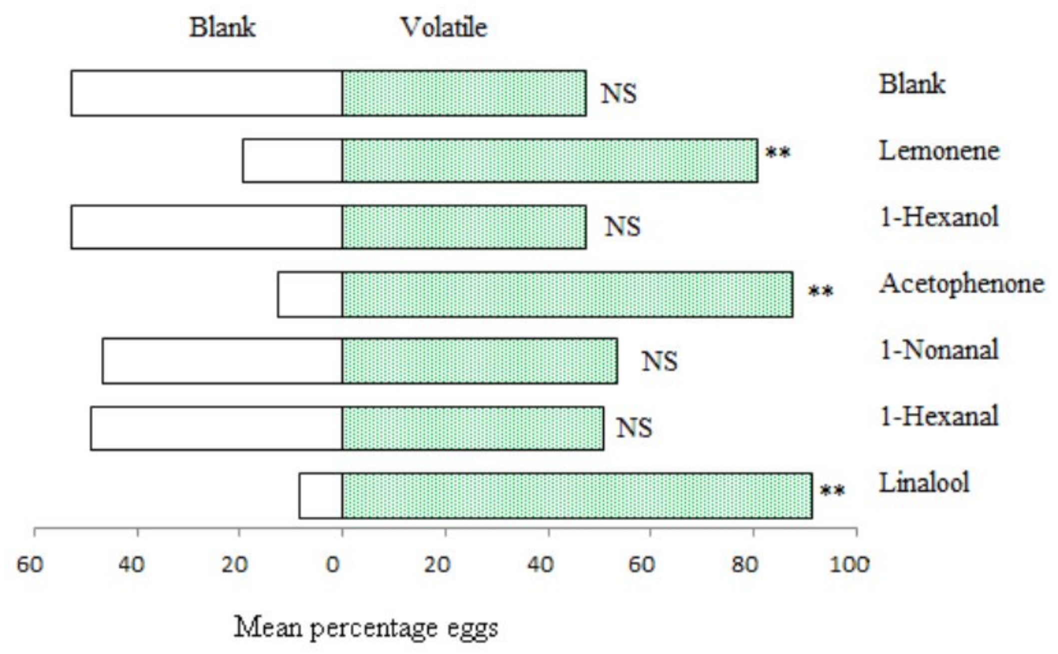


**Fig. 2.** Mean percentage eggs on different synthetic volatile compounds and control based on female dual choice oviposition assay against selected synthetic volatile compounds. ** indicates the significant differences in the mean of eggs on volatile side with the control side ($p \leq 0.05$).

Seven blends were prepared using different ratios of three volatile compounds that resulted in oviposition attraction. Among the blends tried seven, six blends significantly attracted more moths to lay eggs (Table 1). Host volatiles are exploited by herbivores and natural enemies for host plant selection, mating site detection and oviposition site selection. Selection of the host plant and egg laying site is the key factor for larval survival and the progeny fitness. Polyphagous insects rely on the absolute and relative amounts of such common host volatiles during the process of finding oviposition sites (Bruce *et al*., 2005;

Anfora *et al*., 2009). Similarly, field observations and oviposition studies have revealed that *O. arenosella* female usually laid eggs on larval frass of previous generation (Ramkumar, 2002).

**Table. 1**. Mean percentage of eggs laid on different volatile blends and on solvent (DCM)

| Blend | Blend ratio (Acetophenone: Lemonene : Linalool) | Mean±SE on Blend | Mean±SE on Control (DCM) | t value | n | *P |
|---|---|---|---|---|---|---|
| 1 | 1: 1: 1 | 67.85±8.9 | 32.35±9.0 | 2.787 | 5 | 0.024 |
| 2 | 2: 1: 1 | 59.92±3.6 | 40.08±3.6 | 3.858 | 5 | 0.005 |
| 3 | 1: 2: 1 | 64.83±4.1 | 35.17±4.1 | 5.033 | 5 | 0.001 |
| 4 | 1: 1: 2 | 55.63±13.3 | 44.37±13.3 | 0.597 | 5 | 0.567 |
| 5 | 2: 2: 1 | 82.02±4.6 | 17.98±4.6 | 9.709 | 5 | 0.0001 |
| 6 | 1: 2: 2 | 82.28±6.9 | 17.72±6.9 | 6.596 | 5 | 0.0001 |
| 7 | 2: 1: 2 | 85.77±1.97 | 14.23±1.97 | 25.653 | 5 | 0.0001 |

*p≤0.05 is significantly different

Further, it has been demonstrated that in the initial infestation stage a female laid eggs on underside of undamaged coconut leaflets (Muralimohan and Srinivas, 2008). This study confirms the observations of earlier workers and further substantiates that the host volatiles serves as a vital cue to aid the insects in egg laying. The variation of the female preferences may be due to extraction capacity of the solvents used. Further, the results suggest that both larval frass volatiles and coconut leaf volatiles were important cues for finding oviposition sites. Similar studies with other insects have identified the volatiles mediating oviposition selection (Beck *et al*., 2014; Revadi *et al*., 2015; Megido *et al*., 2014). *O. arenosella* females were attracted to volatiles present on the coconut leaflets and larval frass for oviposition. Hence, linalool, limonene and acetophenone are the potential kairomones mediating the oviposition behavior of *O. arenosella* and these can be used to develop semiochemical based management methods.

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