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Identification of pheromone components in Indian population of *Galleria mellonella* L. (Pyralidae: Lepidoptera)

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Abstract
In contrary to the short range attraction of male released pheromone in lepidoptera the Greater wax moth *Galleria mellonella* male attracts the female with pheromone along with acoustic signals over long distances. In the laboratory experiments conducted, the pheromone components in the solvent extracted fore wing glands of calling *G. mellonella* male was identified by using GC-MS. The pheromone compounds *viz*., undecanal as the major pheromone compound nonanol, undecanol as the minor pheromone compounds.

Keywords: Greater wax moth, pheromone, GC-MS, undecanal, nonanol, undecanol

Introduction
The domesticated honey bees are affected by many pests and diseases which are responsible for reduction in bee population. Among various natural enemies, the infestation by greater wax moths are potential threat to bee keeping. Wax moths have the potential to be quite problematic for beekeepers around the world, particularly in areas with warm climates.
The larvae of *G. mellonella* feeds on the bee comb, pollen, larval skin and exuviae in both in storage combs and live honey bee colonies [1]. The larva tunnel into the middle of the comb and feeds on the wax, honey, pollen and brood of the honey bee colonies creating webbing. The entire comb is filled with mass of webbing and excreta of the larva called as Galleriasis. Weakened colonies will abscond [8]. Wax moth infestation is seen in weakend colonies, colonies affected by diseases, queenless colonies, colonies affected by pesticide poisoning and colonies with poor maintenance. In India infestation starts at the month of March and reaches its peak during August and starts to decline till February. In two surveys conducted during 2016 may-june wax moth infestation was 80.6% and 72.2% in 12 districts of Tamilnadu and the average was 76.4%. Infestation was almost 100% in coimbatorte, Karur and Erode districts [14]. The adult and larvae also transfers serious diseases to honey bees i.e. foul brood diseases [15]. The faecal matter contains paenibacillus larvae [2].
There were several methods used in management of Greater wax moth include chemical, physical, Biological methods and by using semiochemicals. Both chemical and non-chemical management strategies applied to reduce the losses associated with greater wax moths infestation are limited by various challenges due to the extremely delicate nature of the hive environment. Fumigants Paradichlorobenzene, phlestoxin Ethylene bromide, sulphur dioxide, phosphine and carbon disulphide used in control of greater wax moths are lethal too all life stages of honey bee that makes the honey toxic and unmarketable [9, 10].
Several biorational management tools *viz*., sex pheromones, bait traps, gamma ray induced male sterile technique, light traps [6, 10] utilization of entomoparasitoids *viz Trichogramma* spp. *Bracon hebetor* [9] and *Apanteles galleriae* [7] were proven biocontrol agents, including the predatory role of imported red fire ants, *Solenopsis invicta* and *S. germinita* against wax moth eggs and lavae [9]. But use of Bio control agents were not effective in apiaries as under controlled condition.
Hence in the present context exploitation of pheromone and Kairamone would be an appropriate eco-friendly tool in managing wax moth infestation [3, 22]. Unlike other insects, Greater wax moth has a pair forming system where male releases pheromone along with acoustic signals from fore wing glands which attracts virigin females [17]. Thus using pheromone traps will trap females prevents egg laying. Females of greater wax moths lays about 200-300 eggs during its life time where both male and female wax moths never feed as

Their mouthparts are functionless. Moreover the hatchability of eggs were almost 99-100%. So trapping female will reduce the population of wax moths. Present work was undertaken to characterize the pheromone components in the Indian strains of *G. mellonella* reared under laboratory conditions.

Materials and Methods

The experiment was conducted at the Apiary of Tami Nadu Agricultural University, Coimbatore, Tamil Nadu, India during May 2022.

Rearing of Greater wax moth

The test insects were mass reared in netted insect rearing cages (55x45x50cm size) located at Apiary of Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India from November 2021 to July 2022. Insects were reared both in artificial and natural diet. The wax moth eggs were released onto honey-harvested, aged combs for faster multiplication of target pests. The wax moth larvae were mass reared at an ambient temperature of 27±1 ℃ After emergence, the adult moths were transferred to the plastic containers and allowed for mating. Pieces of paper were kept inside that mating chamber for the purpose of egg laying by moths and for easy collection (Ellis *et al*., 2013). The eggs thus laid on paper pieces were collected and utilized for further multiplication. The old combs were freeze before using it as a feed to avoid the presence of its parasitoids and any other insects and its life stages. Diets were changed and boxes were cleaned every fortnights. Pupa were separated based on taxonomic keys and some to be left for mating purposes female pupa consists of a cloven sternum which represents the bursa copulatrix on its eighth abdominal segment whereas in male it is absent instead it consiusts of two rounded knobs which represents the phallomeres on its ninth abdominal segment [11]. Kwadha *et al*., 2017 confirmed that at larval stage sex specific characters are not morphologically visible so separation of male and female is not possible in larval stage.

Mass culturing on artificial diet

Artificial diet formulated by desai *et al*., 2019 has been partially modified to suit the local conditions by addition of wax, streptomycin sulphate and vitamin E and greater wax moth was mass reared.

Table 1: Modified artificial diet

Wheat flour	100 g
Corn flour	100 g
Glycerine(Glycerol)	175 ml
Honey	175 ml
Milk powder	100g
Wheat bran	100g
Baking yeast powder	50 g
Vitamin E	2 tablets
Streptomycin sulphate	0.5 g

The ingredients were measured separately and dry ingredients were sterilized by heat. The dried ingredients were mixed with liquid ingredients. Vitamin E was added to increase the vitality and adult longevity. Strepromycin sulphate would eliminate contamination by pathogens. Bee wax in the synthetic diet will in increase the growth of larvae.

Assessment of active calling period of greater wax moth

Male and female adults of Greater wax moth were kept in separate test tubes and observed for calling phase during 6.00 PM to 6.00 Am for one week. In adult, Male and females were identified by labial palps. Female possess projecting labial palp which gives a beak like appearance. In Males it will be curved sharply upwards and hooked inwards (snubbed nose) [4, 11, 16] Observations were done at a dark room as Wax moths were nocturnal. Males first spread their wings at 45°in absence of a unmated female. When more than one number of male and female were released in test tube they males first spread their Wings and fan their wings continuously, females in response to fanning will fan their wings too and mating will happen [6, 19]. Even though the wing fanning will continue throughout the entire scotophase the mating behaviour was effective only at the beginning of scotophase. (1-4 hours) (Romel *et al*., 1992) (Hence the Pheromone extraction was done between 6.00 to 10.00PM

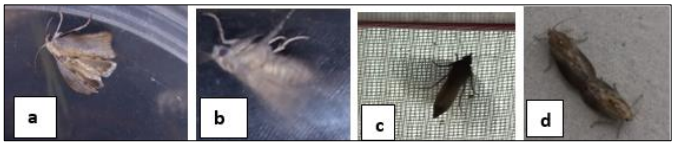

Fig 1: a) Spreading wings at 45° b) Wing fanning of males c) wing fanning of females d) mating

Solvent extraction of wing glands

Solvent extraction was done by slight modification in the method done by svensson *et al.*, 2014. Pheromone extraction was done at 1-3 hr of scotophase as in male greater wax moth pheromone is released from fore wing gland [17]. 20 pairs of forewings is cut with fine scissors under 100 X red light intensity and dipped in 1ml of n-hexane, kept in fridge for overnight for dissolving of pheromone compounds. The extracted compound is then filtered in GC-MS vial using syringe filter. The filtered extract is then analysed by using GC-MS at bio catalyst lab, Department of agricultural Microbiology, TNAU, Coimbatore

GC-MS analysis

Gland extracts were analysed in PERKINELMER CLARUS SQ8C with following specifications: Injector port temperature was programmed to 220°C and interface as 250 °C Initial oven temperature was programmed to 40 °C for 2 min, then an increase in temperature of 10°C /min run to 250 °C hold for 2 min. The detector temperature was maintained at 320 °C and source line temperature was maintained at 230 °C. Helium was used as carrier gas at a flow rate of 1ml/min. DB-5 MS capillary standard non-polar column of 30 m length, 0.25 mm ID and 0.25 µm film thickness with 5% diphenyl-95% dimethyl polysiloxane as stationary phase was used for the analysis. Using ionization voltage of 70 eV, mass spectral data were recorded. Mass spectrometer has an integral library of compounds which automatically search and matches the spectrum produced by the sample. For the interpretation of sample compounds, the spectrums of the sample were compared with spectral database of National Institute of Standard and Technology (NIST 20).1µl of the sample was injected in injection port for the analysis of pheromone compounds.

Results and discussion

The GCMS analysis of solvent extracted male fore wing glands of *G. mellonella* contains undecanal as a major pheromone compound and nonanol, undecanol as minor

pheromone compounds undecanal was identified at peak five with retention time of 9.29 nonanol was identified at peak four with retention time of 7.32 undecanol was identified at peak was identified at peak six with retention time of 10.21. Undecanoic acid was also identified at peak seven with retention time of 11.87 which is in confirmation with previous finding that the pheromone compounds of Greater Wax moth include aldehyde, alcohol and acid of nonane and undecane [18]. The ratio of undecanal, nonanol and undecanol were almost equal. The ratio of identified pheromone compounds was calculated by

Area of compound X ÷ Total area under curve X 100

In the study undecanal was found as the major pheromone compound. Earlier research revealed that the alcohol forms of minor compounds were not effective as a single compound however when combined with major pheromone compounds aids in increased attraction [5, 6, 13, 17]. Undecanoic acid dominates but its role need to be verified [18]. Lebedeva *et al*., 2002 also recorded nonanol and udecanol as minor pheromone compound but behavioural assays were needed in further confirmation.

Table 2: In the study undecanal was found as the major pheromone compound

Name of the pheromone compound	Peak no	Retention time	Area under curve (%)
Undecanal	5	9.29	0.501
Nonanol	4	7.32	0.443
Undecanol	6	10.21	5.67
Undecanoic acid	7	11.87	8.118

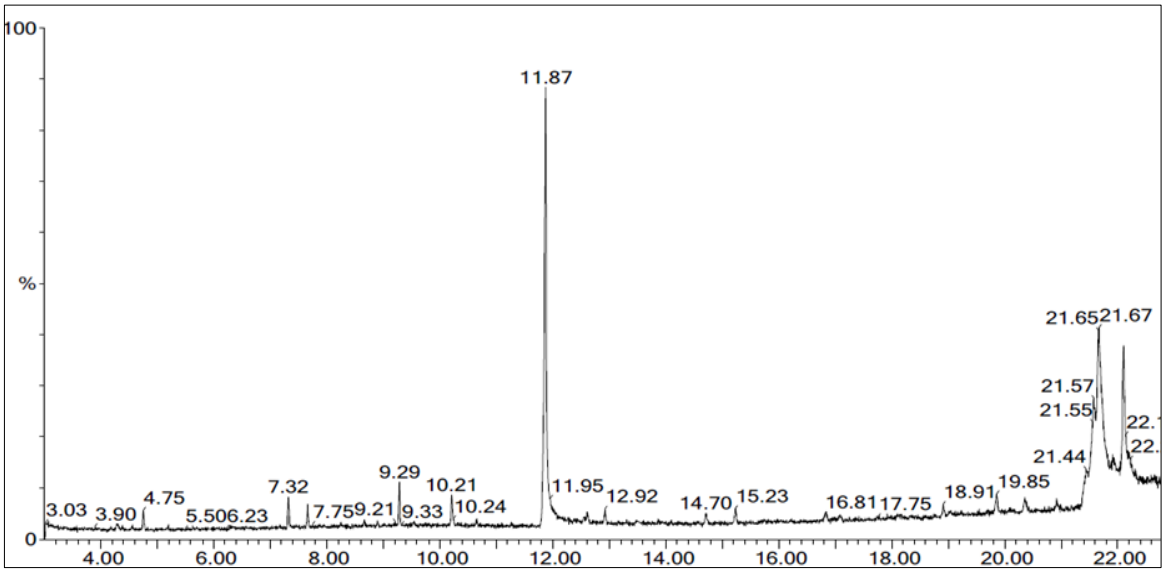

Fig 2: In the study undecanal was found as the major pheromone compound

Conclusion

The pheromone compounds *viz*., undecanal as the major pheromone compound nonanol, undecanol as the minor pheromone compounds were idenfied using GC-MS.

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