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Identification and characterisation of female released sex pheromone components of jute semilooper, *Anomis sabulifera* Guenee (Lepidoptera: Noctuidae)

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Abstract

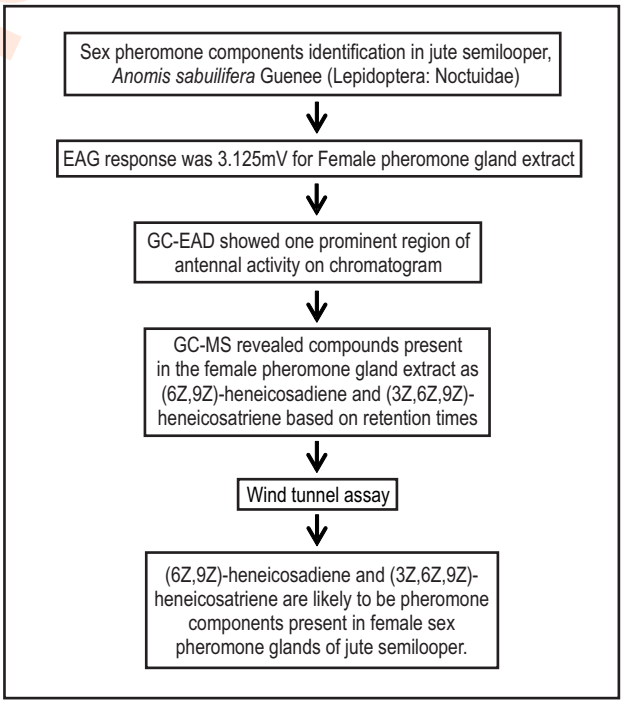
Aim: Identification and characterization of female released sex pheromone components of jute semilooper, *Anomis sabulifera* Guenee (Lepidoptera: Noctuidae) from female pheromone gland extracts.

Methodology: Electroantennogram (EAG) was carried for studying the antennal response; Gas Chromatography coupled with Electro antenna Detector (GC-EAD) was conducted for studying the antennal response of eluted compounds from female pheromone gland extract; Gas Chromatography and Mas Spectrophotometry (GC-MS) was conducted for characterization or getting complete profile of compounds present in the female pheromone gland extract based on retention times. Wind tunnel assay was conducted for studying the behavioural responses of eluted compounds from the female pheromone gland extract.

Results: GC-MS profile of female pheromone gland extract revealed that the GC-EAD active region constituted (6Z,9Z)-heneicosadiene, (3Z,6Z,9Z)-heneicosatriene as active compounds. Preliminary wind tunnel studies for olfactory and behavioural responses showed blend of (6Z,9Z)-heneicosadiene (3 parts) + (3Z,6Z,9Z)-heneicosatriene (1 part) enticed 60% male adults.

Interpretation: (6Z,9Z)-heneicosadiene and (3Z,6Z,9Z)-heneicosatriene are likely to be active pheromone components present in female sex pheromone glands. Blending of these two compounds in precise ratio can enhance the effectiveness of pheromone and can be used as effective strategy in jute IPM.

Key words: *Anomis sabulifera*, Jute semilooper, Noctuidae, Sex pheromone, (6Z,9Z)-heneicosadiene, (3Z,6Z,9Z)-heneicosatriene



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Introduction

Jute, *Corchorus olitorius* (Malvaceae) is an important commercial and cheapest bast fibre crop that is cultivated in Indo-Gangetic plains (Raja, 2012) contributing to more than 80% of total jute production in India (Chapke *et al.,* 2006). India alone contributes 60% of jute and allied fibres production, thus leading the way in protecting the environment. Currently, average productivity of jute in the country is 2.5 t ha^{-1} (AINPJAF 2018-19). Many biotic and abiotic factors are responsible for plateau in productivity and declining the quality of fibres. The infestation of pests has resulted in approximately 20-25% yield loss and also deteriorating the quality of fiber in different jute growing regions of India (Satpathy *et al.,* 2014). These pests in gigantic numbers manifest complete failure of crop causing huge loss to the jute cultivators. Bio-intensive integrated pest management stands as an economic option in the pest combat scenario of jute and allied fibre cultivation (Reddy, 2012).

Biorational strategy as a component of IPM, mediated by use of insect sex pheromones stands as a proxy strategy. It is agreeable and arguable with no apprehensions that pheromones owe few merits on contrast to insecticidal sprays; first pheromones achieve long term pest population suppression. Second, control is enunciated to insect pests that are categorized to be borers, wood dwellers etc. Third, natural enemies are not apprehended by which pest suppression is attained (Nandagopal *et al.,* 2008). For any IPM to be fruitful, it is inevitable to have a strong pest management decision which indirectly depends upon the monitoring pest infestations and can be brought out by using pheromone traps (Yadav *et al.,* 2004). Owing to its crop phenology, accelerated growth rate and difficulty in insecticidal spray applications on account of plant height, sex pheromones usage in jute agro ecosystem have dual advantage firstly by monitoring of major insect pests before causing appreciable damage and secondly for mass trapping the target pest if pest population goes beyond economic threshold level (ETL).

In the present realm of chemical ecology, concerted efforts of chemists, biologists and plant protection experts provide insights of pheromone application technology in conjunction with the concept of using know-how is used for controling insect pests (Ridgway *et al.,* 1990). Use of minute and traceable amounts of species-specific chemical signals in precise blend ratio, manipulate the insect atmosphere which when perceived provoke a behavioural response in opposite sex (Tillman *et al.,* 1999), therefore breaking the mate-finding and mating process has evolved and gained as an elegant strategy in control of insect pests and proved to be a great success (Arn, 1990; Witzgall and Arn, 1997). It is quiet evident that insect sex pheromones are used in varied aspects viz. mass trapping, monitoring and mating disruption (Witzgall, 2001; Islam, 2012). Thus the need for identification, isolation and synthesis of furthermore insect sex pheromones is of utmost importance in providing sustainable and effective control of jute pests and explicitly docks in IPM strategy.

Jute semilooper, *Anomis sabulifera* Guenee (Lepidoptera:Noctuidae) is a potential and major pest of jute, with larvae being the most destructive stage of pest that causes heavy damage by defoliation (Dutta, 1958). The repeated infestation by this pest compromises crop growth and induces profuse branching (Tripathi and Bhattacharya, 1963), thereby resulting in ultimate reduction of fiber yield to the tune of 30.50 - 37.50 % in many varieties of *C. olitorius* (Das and Singh, 1977; Singh and Das, 1979). The pest is also known to feed on both pods and unripe seeds in jute crop cultivated for seed production (Rahman and Khan, 2006; Rahman and Khan, 2012a). The present strategy of controlling this pest is by use of insecticides. The use of sex pheromones which is economical and cost effective stands as an important strategy to manage and minimize the usage of harzadous insecticides currently used in control of *A. sabulifera* (Awasthi, 2007). Presently there are no sex pheromone components isolated, identified, characterized and blended in precise ratios for proving their efficacy as efficient IPM tactic for the management of jute semilooper. Hence, the present study was aimed and conducted to identify, isolate, characterize and blend the pheromone components of jute semilooper, *A. sabulifera* Guenee, a potential pest of jute.

Materials and Methods

Mass rearing of insect: Larval populations of *A. sabulifera* were collected from infested jute crop of farmers' fields at Beraberia and Ratanpur of North 24 Parganas, during mid-June. Larvae were maintained in Petriplates (15 cm diameter and 6 cm height) at 25°C and 80-85% relative humidity and fed on natural diet till pupation at the Biocontrol Laboratory of Crop Protection Division, ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore. Pupae were sexed and placed inside plastic Petriplates (15 cm diameter and 6 cm height). After emergence, male and female moths were kept separately and adults were fed with 10% honey solution. For the extraction of pheromone gland, 2-4 day old virgin females were used whereas for behavioural bioassays, virgin male adults of 2-4 days old were used respectively.

Extraction of sex pheromone: Sex pheromone glands were excised from 2–4 day-old virgin calling females. Glands were forced to extrusion by gently pressuring the tip of the abdomen and were excised using micro-scissors. Fifty to sixty pheromone glands were placed in a 0.5 ml vial (Agilent, Netherlands) containing 5 ml hexane, and were extracted at room temperature for 20 min. The extracts were filtered using syringe filter, and pre-concentrated to 100 µl under a pure nitrogen (N_2) flow and the extracts (N=50) were stored at -20ºC until further use.

Assay of antennal response in male *A. sabulifera* with electroantennogram: Electroantennogram (Syntech, Netherlands) recordings of *A. sabulifera* were made using with Ag-AgCl glass electrodes filled with Beadle–Ephrussi Ringer's solution (Dong *et al.* 2000). The left antennae of male *A. sabulifera* was excised at the base (antennal socket) and inserted

gently into indifferent electrode. The tip of antenna was cut and held inside the recording electrode of EAG. The electrodes were linked with manipulator, amplifier (Syntech, AM-02), oscilloscope and interface board (Syntech, IDAC-02) to process and digitalize the signal. EAG program (Syntech, version 2.6c, 1998) aided in determining the amplified signal. The antennal preparations were continuously delivered with charcoal filtered and humified air @ 500 ml^{-1} min using the airstream system (Syntech, CS-05). A 50 µl each of the standard test compounds dissolved in n-hexane (Sigma–Aldrich, HPLC grade) were smeared onto a filter paper strip (6 cm long and 0.5 cm broad) with Eppendorf pipette and air dried 30 sec for solvent to evaporate before being placed in the Pasteur pipette, whose tip was linked with airstream system (Syntech, CS-05). The airflow of speed 20 ml min^{-1} and pulse duration of 0.5 sec with an exposure interval of 60 sec was dispensed from the airstream system on the antenna and data was recorded accordingly. The test compounds selected in the present study were individual pheromone components isolated from closely related insect species belonging to semilooper category *viz.,* castor semilooper, *Achaea janata* and other insects infesting jute crop viz, hairy caterpillar, *Spilosoma obliqua*. The test compounds used in the EAG recordings were octadecanal, (9Z,12Z)-octadecadienal, (9Z,12Z,15Z)-octadecatrienal, (6Z,9Z)-heneicosadiene, (3Z,6Z,9Z)-heneicosatriene, female pheromone gland extract and hexane as control. Each standard test compound (10µg $µl^{-1}$) was examined with ten antennae from male adults for EAG recordings.

Assay of antennal response in male *A. sabulifera* of eluted compounds from female pheromone gland extracts with Gas Chromatography-Electroanntenogram Detector (GC-EAD): The pheromone gland extract isolated from virgin female adults of *A. sabulifera* were analysed by Gas-Chromatography (Varian 3900 XL) with flame ionization detector (FID) and WCOT (CP-SIL 24 LB/MS) and Varian chromopack capillary column DB-5MS, 30 m x 0.32 mm ID). The effluents from EAD (Syntech, Netherlands) were made to pass onto the antennal setup which comprised of male antenna of *A. sabulifera* in between the Ag/AgCl electrodes. For recording the electrical stimulus of the antenna and amplifying same Syntech AC/DC was used. One microliter of female pheromone gland extract sample was injected using Hamilton precision syringe into splitless mode with helium as carrier gas with flow of 300 ml min^{-1}. The inlet temperature of GC was maintained at 200ºC. The oven temperature program was maintained at 50ºC to 240ºC for 40 min with two ramps, 50ºC for 2 min, then increased at 10ºC min^{-1} to 160ºC, held up for 2 min and again increased at 5ºC min^{-1} upto 240ºC and held for 10 min for complete and orderly elution of compounds present in the extract. The column effluent was analysed with FID at 260ºC. The antennal stimulus both from EAD and FID were recorded via Syntech Intelligent Data Acquisition Controller (IDAC) interface device and was analysed with Syntech GC-EAD software (Syntech, Netherlands).

Identification and characterization of female pheromone gland extracts with Gas chromatography-Mass Spectrometry: For compound identification, pheromone gland extract were analysed using an quadrapole mass spectrometer equipped with a gas chromatography (Varian 3900 XL) with flame ionization detector (FID) and WCOT (CP-SIL 24 LB/MS) and Varian chromopack capillary column 30 m x 0.32 mm ID) and a splitless injector, with helium as carrier gas. Mass spectrometer was maintained at 260ºC. The temperature program was maintained similar to that of GC-EAD. Ionization was achieved by electron impact (70 eV, source temperature 230ºC) and the data were collected with ChemStation software. Data acquisition was upto the tune of 100 Hz (scans/sec) within the mass range of 29-338 amu (atomic mass unit). Identification of eluted compounds were analysed by comparison of spectra with NIST library databases (National Institute of Standards and Technology, 2008), or with published spectra, using retention (Kovat's) indices (published at Pherobase and NIST Chemistry Web Book web sites), and were confirmed by GC co-injection using authentic standards.

Assay of behavioural response of eluted compounds with wind tunnel: The flight orientation of male adult moths of *A. sabulifera* was conducted using a plexiglass wind tunnel (150 x 60 x 60 cm) following the method of Potting *et al.* (1999) with minor modifications. At the upward end, suction fan was installed to draw air into the tunnel @0.5 m sec^{-1}, while the downward end was covered with a nylon mesh. A metal knob for hanging the test sample was placed at a distance of 25 cm from the upwind end, while test insects were released from the downward end of wind tunnel using a plexiglass tube. At least ten male adults of *A. sabulifera* were released into the wind tunnel from the downward end each time using plexiglass tube after anesthetizing them for 2 min in refrigerator. A 100 µl each of the test compound and female pheromone gland extract dissolved in n-hexane (Sigma –Aldrich, HPLC grade) and equal amount of n-hexane serving as control treatment was loaded onto the filter paper, allowed to air dry and then was hanged onto a metal knob.

The enticing ability of each test compound, female pheromone gland extract and flight orientation ability of male adult insects towards the test compound was studied by switching on the air flow 5 min and data was recorded accordingly. Criteria taken into consideration for assessing effectiveness of test compounds were upward movement from source of release to the lure, half way movement in the wind tunnel and lastly aligning on the source of lure. The orientation bioassay was replicated four times with a new set of ten male adults for every replicate against each individual test compound, pheromone gland extract and blend of individual test compounds at precise ratios. Prior to release of male adults in the experimental trial against a new test compound, the wind tunnel was wiped with moist cotton and air was blown for 10 min to prevent cross contamination between the test compounds/ standards.

Statistical analyses: The electroantennogram (mV) recordings obtained by stimulating the antenna from male *A. sabulifera* adults against test standards were analysed by EAG software (EAG 2000, Version 2.7b, Netherlands) and then subjected to one

way ANOVA (SPSS Ver. 16). The effectiveness of eluted compounds and authentic synthetic standards was assessed as percentage of male adult moths attracted to the individual test compounds, their blends and female pheromone gland extract applied on the filter paper in the wind tunnel experiments.

Results and Discussion

The EAG responses elicited by male antenna to the test compounds, *i.e.,* (6Z,9Z)-heneicosadiene, (9Z,12Z)-octadecadienal, (9Z,12Z,15Z)-octadecatrienal, female pheromone gland extract, (3Z,6Z,9Z)-heneicosatriene and octadecanal showed 2.34-, 2.25-, 1.96-, 1.96- and 1.39- folds stimulus in comparison to control *i.e.,* n-hexane. The EAG stimulus of the male antenna of *A. sabulifera* ranged from 3.70mV against (6Z, 9Z)-heneicosadiene to 1.96mV against octadecanal. Though the EAG response elicited by (6Z, 9Z)-heneicosadiene was stronger over other compounds examined no significant difference was noted, except for octadecanal and n-hexane (Table 1; Fig. 1). Gas chromatography coupled with electroantennographic detection analyses of female pheromone gland extract from calling females of *A. sabulifera* (1µl) revealed one prominent region of antennal activity (Fig. 2). The GC-MS profile of female pheromone gland extract revealed the presence of active compounds, *i.e.,* 4-(p-acetoxyphenyl)-2-butanone (retention time 17.168 min), 11-Z-hexadecen-1-ol acetate (retention time 19.348 min), 3-eicosyne (retention time 19.967 min), 9-Z-hexadecanal (retention time 20.417 min), 9Z,12Z,15Z-octadecatrienoic acid (retention time 22.602 min), 9Z,12Z-octadecadienoic acid (retention time 23.190 min), 11-Z-hexadecen-1-ol acetate (retention time 25.022 min), 3Z,6Z,9Z-heneicosatriene (retention time: 25.753 min), 6Z, 9Z-heneicosadiene (retention time: 25.802 min), and 8-hexadecyne (retention time 27.569 min) (Fig. 3). Detailed study of GC-EAD active area revealed one major and one minor peaks corresponding to C_{21} n-alkane compounds.

The GC-MS profile of this particular region considering fragmentation and adduct formation revealed the presence of two prominent and active compounds, the mass spectra of which exhibited molecular ions at m/z 292 and 290. The fragmentation pattern of one compound resembled those of unsaturated compound containing two C=C bond and a C_{21} chain while that of other compound possessed three C=C bond and a C_{21} chain. Based on the retention indices, these compounds were identified as 3Z,6Z,9Z-heneicosatriene (m/z 290; retention times: 25.753 min) (Fig. 4a) and 6Z, 9Z-heneicosadiene (m/z 292; retention times: 25.802 min) (Fig. 4b). Further analysis brought out by the co-injection of synthetic standards in GC-MS reconfirmed the active compounds present in the female pheromone gland extract as 6Z,9Z-heneicosadiene and 3Z,6Z,9Z-heneicosatriene, based on the retention indices obtained. The GC-EAD eluted active compounds and co-injection of synthetic standards synced to a great extent based on retention times when analysed under GC-MS (Fig. 5). Furthermore, the GC-MS profile of female pheromone gland extracts of *A. sabulifera* though showed the presence of other compounds identified, none of these compounds exhibited retention behaviour similar to those of the GC-EAD active components (6Z, 9Z)-heneicosadiene and 3Z,6Z,9Z-heneicosatriene) and were GC-EAD inactive. Though compounds 4-(p-acetoxyphenyl)-2-butanone, (9Z,12Z,15Z)-octadecatrienoic acid, 9Z,12Z-octadecadienoic acid, 11-Z-hexadecen-1-ol acetate and 9-Z-hexadecen-1-ol acetate were identified in the GC-MS profile of female pheromone gland extract the possibility that this compound might form part of pheromone complex is yet to be confirmed.

In the behavioural assays, treatment comprising the blend of female sex pheromone components *viz.,* (6Z,9Z)-heneicosadiene + (3Z,6Z,9Z)-heneicosatriene (3:1) showed 60% source contact where in males landed on the blend (Fig. 6). Interestingly, (6Z,9Z)-heneicosadiene also showed approximately 40% source contact while 3Z,6Z,9Z-heneicosatriene and (9Z,12Z)-octadecadienal never proved to be fruitful in enticing the male adults of *A. sabulifera.* Female pheromone gland extract was the next best treatment in the wind tunnel assay for enticing 50% of *A. sabulifera* male adults. No flight activity or source contact was seen for the control with n-hexane. These results, thus, suggest that 6Z,9Z-heneicosadiene and 3Z,6Z,9Z-heneicosatriene are the main components of female sex pheromone of *A.sabulifera* (Persoons *et al*., 1993b).

The data obtained from GC-EAD and GC-MS analysis, coupled with laboratory and wind tunnel experiments, indicate that (6Z,9Z)-heneicosadiene and (3Z,6Z,9Z)-heneicosatriene are the main components of the female sex pheromone of *A. sabulifera.* Unsaturated hydrocarbons with 18-21 carbon atoms have previously been identified as pheromone components of the female sex pheromone of *A. janata* constituting four components viz. heneicosane, (9Z,12Z)-octadecadienal, (6Z,9Z)-heneicosadiene and (3Z,6Z,9Z)-heneicosatriene in the ratio of (1:1:1:60-70) (Persoons *et al.,* 1993b), the anterior male scent brush extracts likewise comprised two among the four active compounds seen female sex pheromone compounds *viz.,* (3Z,6Z,9Z)-heneicosatriene and (9Z,12Z)-octadecadienal (Jyothi *et al.,* 2005). Similarly, in arctiid moths *Amsacta albistriga*

Table 1: Electroantennogram responses of male antenna of *A. sabulifera* to sex pheromone components

Compounds	Stimulus (mV)
(6Z,9Z) -heneicosadiene	3.70 ± 0.24
(3Z,6Z,9Z)-heneicosatriene	2.20 ± 0.15
(9Z,12Z)-octadecadienal	3.57 ± 0.17
(9Z,12Z,15Z)-octadecatrienal	3.10 ± 0.19
octadecanal	1.96 ± 0.14
Female pheromone gland extract	3.10 ± 0.48
n-hexane	1.58 ± 0.09
CD (P=0.05%)	1.06

*Mean EAG's (absolute values) ± standard errors (mV); repetition times were 5

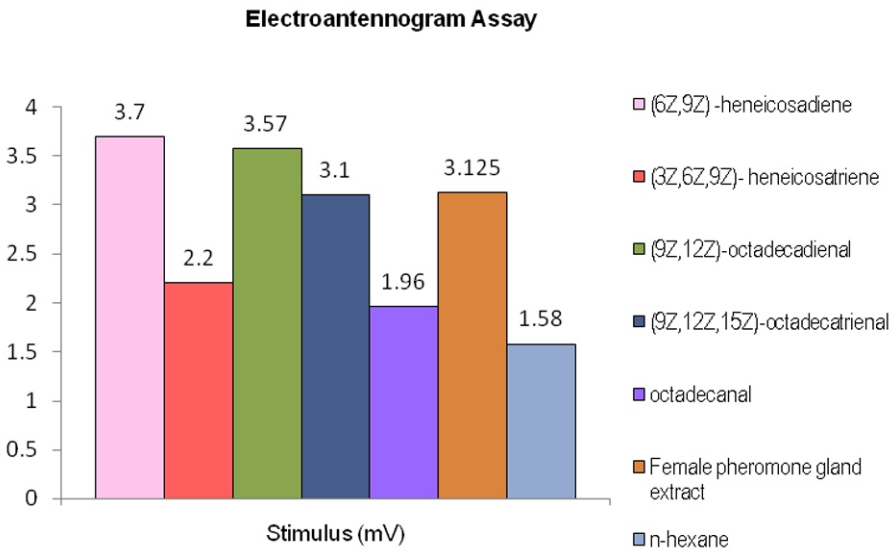

Fig. 1: Antennal response of male *A. sabulifera* in electroantennogram assay to test compounds and female pheromone gland extract.

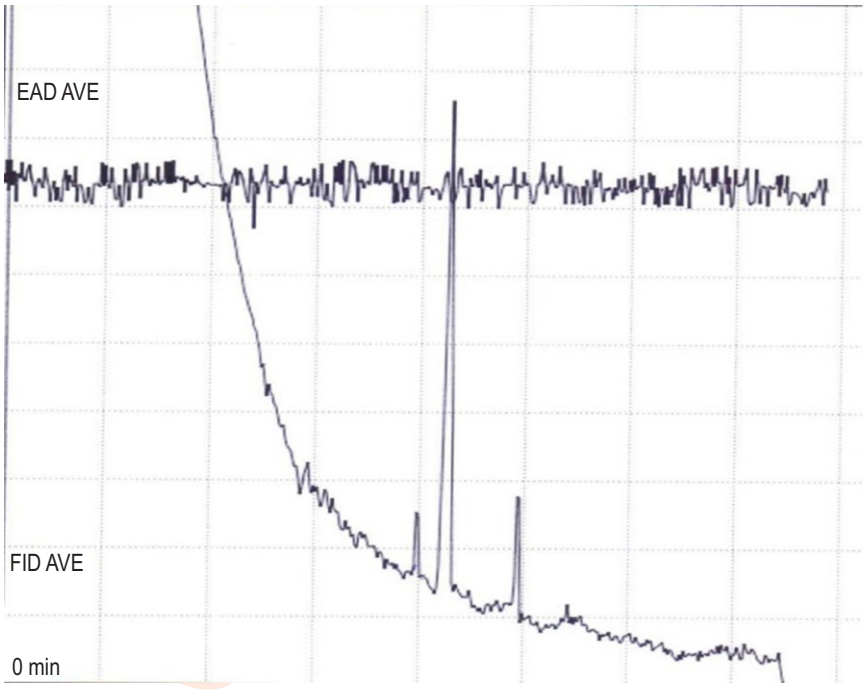

Fig. 2: GC-EAD of female pheromone gland extract of *A. sabulifera* exhibiting antennal activity.

(Lepidoptera: Arctiidae) the female pheromone gland extract comprised of octadecanal, octadecadienal (9Z,12Z,15Z)-octadecatrienal and (3Z,6Z,9Z)-heneicosatriene and hairy caterpillar, *S. obliqua* (Lepidoptera: Arctiidae) comprised five components *viz* (Z3,Z6)-cis-9,10-epoxy-3,6-heneicosadiene, (Z3,Z6)-cis-9,10-epoxy-1,3,6-heneicosatriene, (Z9,Z12)-9,12-octadecadienal, (9Z,12Z,15Z)-octadecatrienal and (3Z,6Z,9Z)-heneicosatriene (Yadav *et al.,* 2001). (3Z,6Z,9Z)-heneicosatriene as component of male produced pheromone from posterior male scent brushes has been reported in *Anticarisa gemmatalis* (Heath

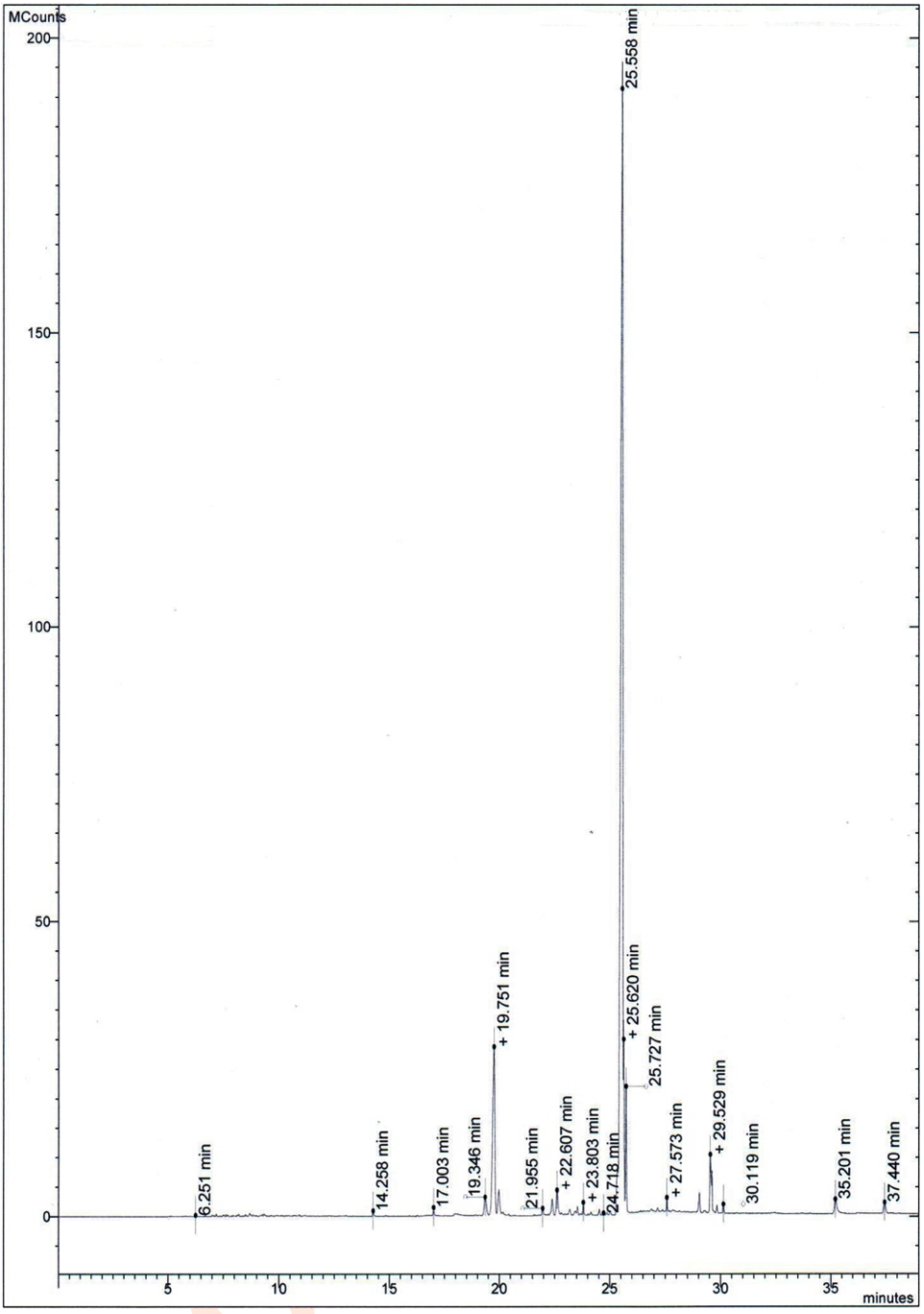

Fig. 3: Total ion chromatogram of female pheromone gland extract of *A. sabulifera.*

et al., 1988). Globally, in recent times female producing sex pheromone components have been identified in various lepidopteran insects *viz.,* (11E)-hexadecenal and (10E, 12E)-hexadecedienal were identified as major sex pheromone components from female pheromone glands of cotton caterpillar, *Diaphaniaindica* (Wakamura *et al.,* 1998). Two active sex pheromone components (9Z)-hexadecenaland (16Z)-hexadecenal were identified from female pheromone glands of sugarcane borer, *Diatraea flavipennella* (Lepidoptera: Pyralidae) (Kalimova *et al.,* 2012); (3E,13Z)-octadecadienal and (3E,13Z)-octadecadienol, respectively, in raspberry crown borer, *Penniseti amarginata* (Lepidoptera: Sesiidae) (Judd *et al.,* 2012).Four sex

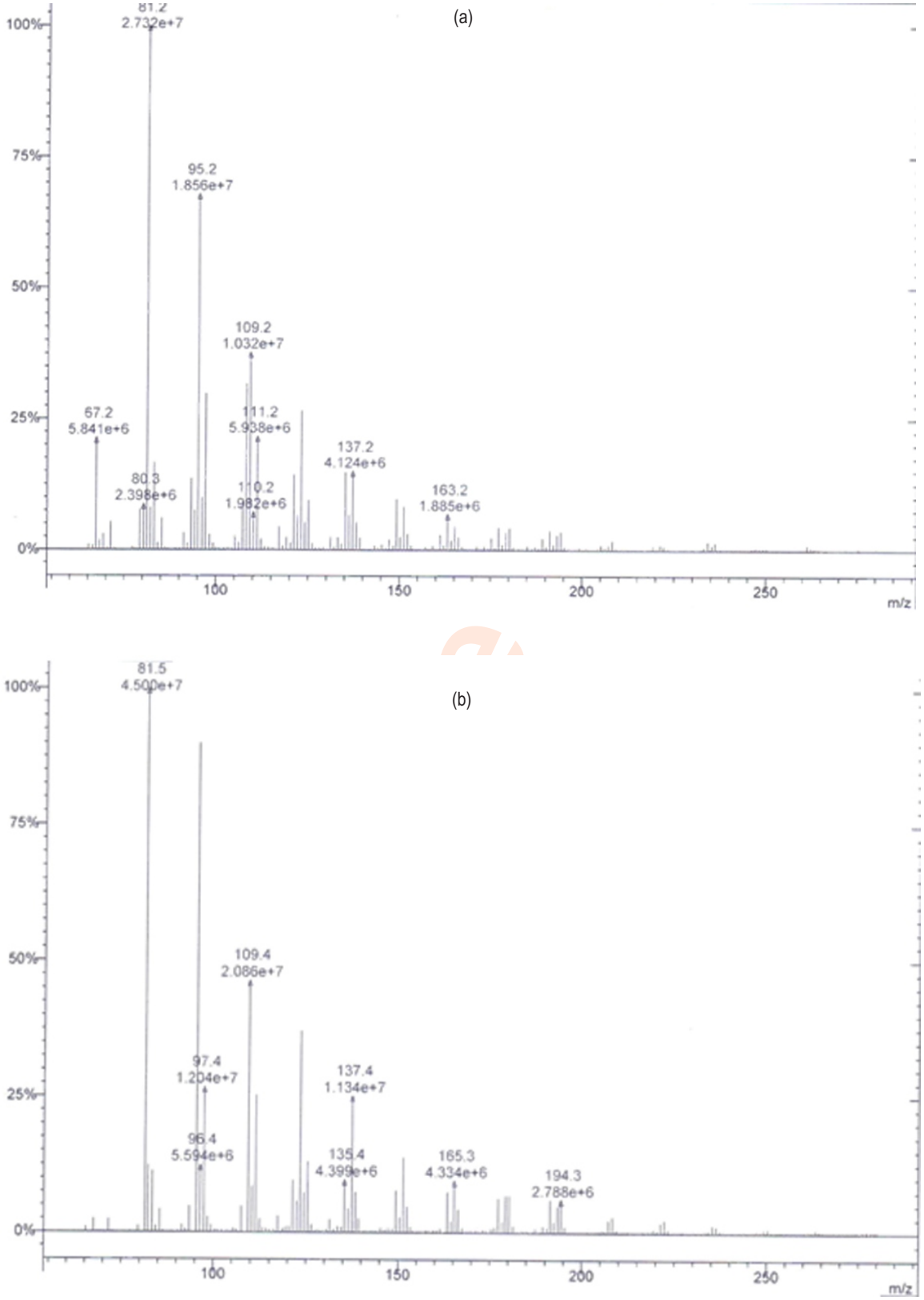

Fig. 4: Mass Spectrum of compound (3Z, 6Z, 9Z)-heneicosatriene (a) and (6Z,9Z)-heneicosadiene (b).

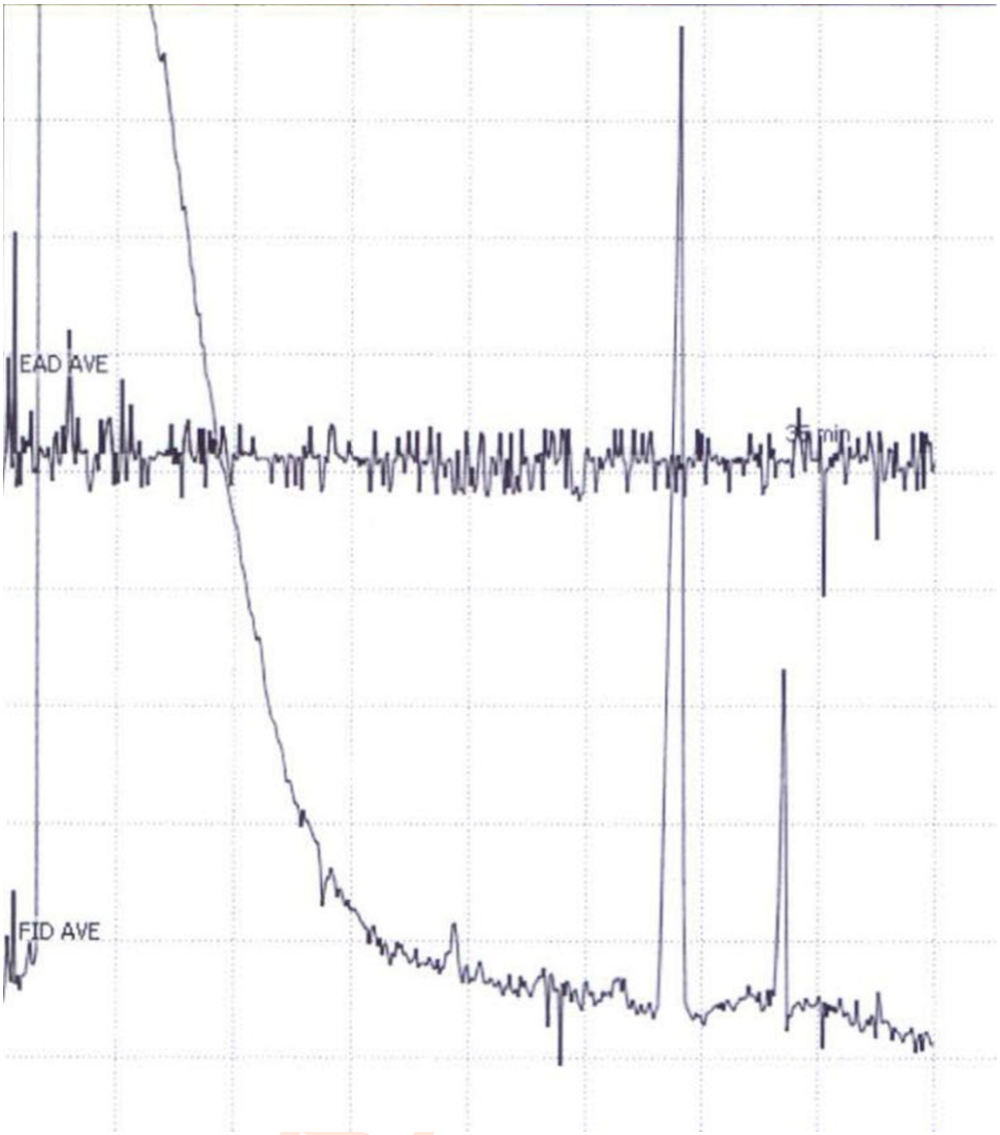

Fig. 5: Response of male antenna of *A. sabulifera* to pheromone blend mixture (6Z,9Z)-heneicosadiene+(3Z,6Z,9Z)-heneicosatriene (3:1).

pheromone components viz. (11E)-tetradecenyl acetate, (11E)-tetradecenol, (11Z)-tetradecenyl acetate and tetradecyl acetate were identified from Chilean fruit leaf roller, *Proeulia auraria* (Lepidoptera: Tortricidae (Reyes-Gracia *et al.,* 2014); (11Z)-hexadecenal, (10E, 12Z)-hexadecadienal and (10E,12E)-hexadecadienal from diurnal hawk moth, *Hemaris affinis* (Lepidoptera: Sphingidae) (Uehara *et al.,* 2015).

Wang *et al.* (2015) reported (7Z)-tetradecenal, (7Z)-tetradecenal and (9Z)-hexadecenal as sex pheromone components produced from female pheromone gland of leafminer, *Holocacista capensis.* Naka *et al.* (2018) reported (3E, 13Z)-octadecadienyl acetate and (3E,13Z)- octadecadien-l-ol as the sex pheromone components of female clearing moth, *Nokoma feralis* (Lepidoptera: Sesiidae). Likewise, (5Z)-tetradecenal and (7Z)-tetradecenal were the active sex pheromone components identified from female pheromone glands of grapewine leaf miner, *Antispila oinophylla* (Wang *et al.,* 2019); (5Z)-dodecenal and (7Z)-tetradecenal as sex pheromone components were identified from female pheromone gland extracts of leafminer, *Holocacista rivillei* (Wang *et al.,* 2019). Similarly, 13-tetradecenyl acetate was identified as female produced sex pheromone component in click beetle, *Melanotus communis* (Coleoptera: Elateridae) (William III *et al.,* 2019). Adult male moths producing sex pheromone to entice female moths in capsule borer, *Conogethes punctiferalis* (Lepidoptera: Pyralidae) was identified as methyl acetophenone or 3-ethyl acetophenone (Stanley *et al.,* 2018).

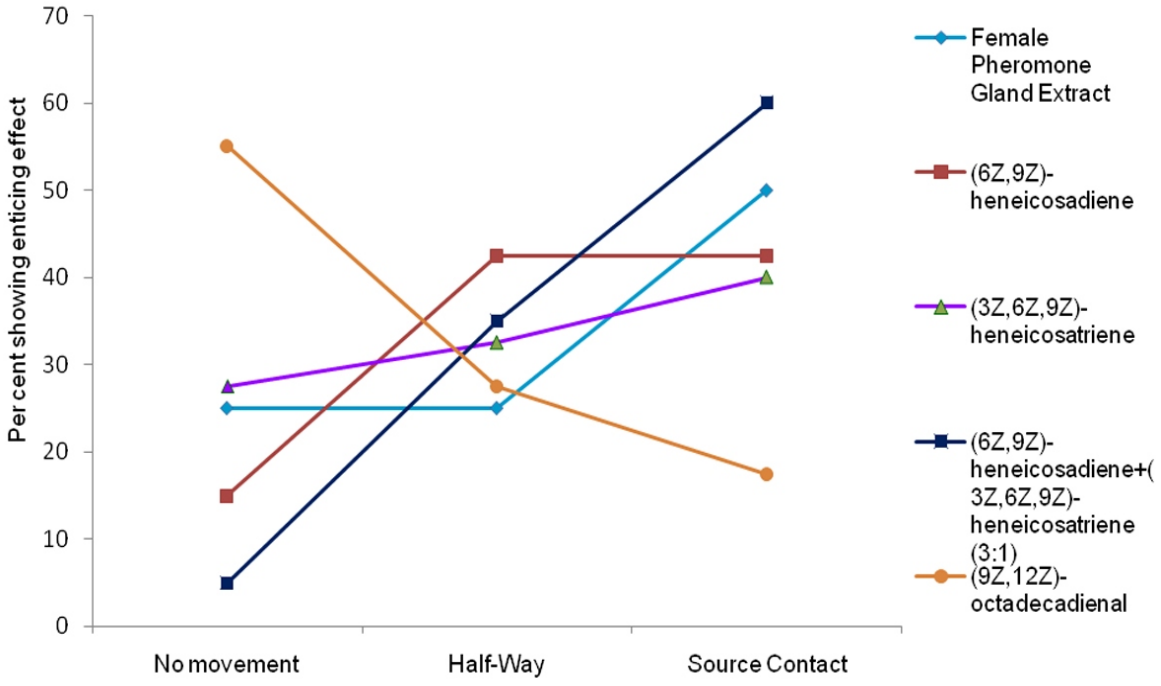

Fig. 6: Flight orientation response of adult *A. sabulifera* males to female sex pheromone gland extract and test compounds in wind tunnel assay

From the wind tunnel experiments, it was quite evident that the male moths showed a typical zig-zag pattern of flight behaviour while being attracted to blend of (6Z,9Z)-heneicosadiene and (3Z,6Z,9Z)-heneicosatriene, which is normally seen during the time of natural mating call (Jyothi *et al.,* 2005; Sheik, 2012). EAG studies showed a quiet significant response of male antenna to the test standards/compounds studied. It is quite inevitable that EAG provide useful information on the selectivity and sensitivity of olfactory receptors in getting stimulated to the standard/compound exposure. In the present study, observations made in EAG synced with the wind tunnel studies to appreciable level. The male antenna *A. sabulifera* elicited a behavioural response to duo standards (6Z,9Z)-heneicosadiene and (3Z,6Z,9Z)-heneicosatriene over other compounds, the same was visualised in the wind tunnel experiment where in blend of (6Z,9Z)-heneicosadiene: (3Z, 6Z, 9Z)- heneicosatriene (3:1) enticed 60% of male moths released in the wind tunnel towards lure, making source contact while 35% making half way mark in the wind tunnel, draws a conclusion that the male antenna of *A. sabulifera* might be actually equipped with receptors for detecting the blend of compounds.

Lures arbitrarily blended in 3:1 ratio (6Z,9Z)-heneicosadiene (3 parts) and (3Z,6Z,9Z)-heneicosatriene (1 part) though provoked a behavioural response in the males of *A. sabulifera,* a precise blend mimicking that of a naturally female released pheromone may enable the highest levels of behavioural response in males. Hence, study in this aspect is to be enunciated coupled with the inclusion of any missing components thereof so as to increase the male traps beyond apprehension when fully deployed under field conditions. The GC-MS profile of female pheromone gland extract eluted eight different components, few of which like 9Z,12Z,15Z-octadecatrienoic acid and 9Z,12Z-octadecadienoic acid are universal in lepidopteran insects and do not prove their uniqueness. Interestingly, 4-(p-acetoxyphenyl)-2-butanone component eluted owes a uniqueness, been a mass trapping compound generally used for trapping fruitflies. Thus, 4-(p-acetoxyphenyl)-2-butanone can bridge the missing component in sex pheromone of *A. sabulifera.* Preliminary field evaluation of pheromone blend (6Z,9Z)-heneicosadiene (3 parts) and (3Z,6Z,9Z)-heneicosatriene (1 part) interestingly attracted fruitflies in the sleeve traps, hence, the blend can be escalated as a substitute for cue lure routinely used mass trapping of fruitflies.

In the present day scenario, jute semilooper control management strategies mainly relied on conventional chemical control methods. But to contemplate and forgo the ill effects of insecticide usage and considering the crop phenology, end product required, resorting on biological control with natural enemies like *Sisyropa formosa* and biorational tactics like pheromones fit as ideal component in jute IPM. The present study proves that (6Z,9Z)-heneicosadiene and (3Z,6Z,9Z)-heneicosatriene are the possible sex pheromone components in jute semilooper. The blending of sex pheromone components identified and any

missing compounds there by, in precise ratio coupled with suitable delivery mechanism when deployed in field level would certainly enhance the effectiveness of the pheromone.

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Add-on Information

Authors' contribution: V.R. Babu: Collection of insects, execution of experiment, manuscript writing; **S. Satoathy:** Execution of experiment and correction of manuscript; **B.V.S. Reddy:** Providing laboratory equipments viz., EAG, GC-EAD, GCMS and wind tunnel.

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