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Electrophysiological response of *Riptortus pedestris* to *Dolichos lablab* Volatiles

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Abstract
The present study and analysis was done to know the possible compounds to which *Riptortus pedestris* was showing response. Through electrophysiological studies, the plant volatiles were identified as 1,3-dimethylbenzene, (E,Z)2,4-heptadienal, 2-formylpyrrole, Hexadecanoic acid, 2-formylpyrrole, 2,3-butanediol, Ethyl heptanoate, Undecane, 1-(4-methylphenyl)-ethanone, 1-phenylpentane-1-ol, (E,E)-1-(piperidin-1-yl)-dodeca-2,4-dien-1-one, 2,3-benzopyrrole, where-as Methyl-3-hydroxy-2-methylbutanoate, Ethylhexadeconate, (Z, Z, Z, E)-3, 6, 9, 17-tricosatitraene were known to be possible aggregation pheromone.Similarly1-(4-methylphenyl)-ethanone, 1-phenylpentane-1-ol, (E, E)-1-(piperidin-1-yl)-dodeca-2, 4-dien-1-one, 2,3-benzopyrrole were reported as female sex pherpmone in several species of same family. In this study the response of this insect to these identified compounds is established.

Keywords: *Riptortus pedestris*, electro-antennogram, gas chromatography-electroantenogram detector (GC-EAD)

Introduction
Dolichos lablab Family-Fabaceae, Raceme. It is an annual or short lived (110-120 days) pulse The primary cause attributed to lowering of field bean yield can be due to the heavy infestation by an array of Insect pest complex. As many as 55 species of insects and a species of mite feeding on the crop from the seedling to the harvesting stage of the crop in Karnataka [6]. The pod borers were recorded as a major drawback for the low productivity in Indian condition which sometimes incur the production loss to an extent of 54% in Indian beans [10]. The major yield loss was inflicted by the pod feeders which accounts both the pod borers and pod bugs respectively. From a range of sucking pests, leaf footed coreid bug, *Riptortus pedestris* (Fabricius) and lablab bug, *Coptosoma cribraria* (Fabricius) occur commonly, more frequently and found in large numbers throughout the cropping period especially during winter days [14].The adults and late staged nymphs of bugs infest later stages of crop growth by congregating on tender vines, early pods and sucking sap resulting in fading of vines, shoots and destroying economic part i.e. pods [1, 11].
Plants release a variety of volatile organic compounds that play a major and multiple roles in interactions with other plants and animals [5, 8, 12]. Insect herbivores exploit these volatiles through olfaction to locate their host plants at a distance, for feeding, mating and egg-laying [2, 3, 9, 13, 17, 18, 20]. The Plant volatile compounds are products and bi-products of diverse metabolic pathways, but many are derived from the Isoprenoid or terpenoid metabolic pathways [4, 15]. The constitutive headspace of undamaged plants varies with genotype, phenological stage, and environmental conditions. It is conceivable that insects use the volatile signals or messages that correlate with this variation to distinguish the most suitable hosts [2, 7, 21]. Knowledge of the chemicals and mechanisms that mediate host plant location and identification by insect herbivores is essential for our understanding of plant-insect relationships, and also will lead to the advancement in development of novel tools for insect pest management [19]. In the present study, attempts were made for identification and possible use of the compounds for pest management in *Dolichos lablab*, Family-Fabaceae,

Materials and Methods

Collection of insects and plant samples

Adult and nymphs of *Riptortus pedestris* feeding on *Dolichos lablab* plant species were collected from Attur farm of ICAR-NBAIR (13.0968ºN, 77.5666ºE), Insects were collected by using net with minimal disturbance and stored in plastic jars. Male, female and nymphs were separated and stored separately. Various parts of the plants were also collected and stored in a polythene bag and brought to laboratory for feeding pest and volatile collection.

Plant volatile collection

Freshly collected plants of *D. lablab* were confined in a 2-L glass jar that was closed with a ground-glass fitting later packed firmly using sticky tape. The cut end of the plant branch was placed in a 50-ml beaker of water. A charcoal-filtered pressurised airstream was pulled over the plant material from the bottom to the top of the jar to carry the bursting volatiles, and 50-mg Porapak- Q (80/100 mesh; Alltech, Deerfield, IL, USA) which was caught between plugs of glass-wool in a 4×40 mm glass tube. Before use, traps were rinsed sequentially with 3 ml methanol, ether, and redistilled hexane, after 15 min treatments in ultrasonic baths in ether and hexane, respectively. The air flow was maintained at150 ml/min, exchanging the headspace in the jar 4.5 times/h. Collections were done for 4 hours at 20–22 °C and 10–30 lux light. The charcoal filter for incoming air and the Super Q trap for out-coming air were connected with glass fittings to the jar. All glassware was heated to 200 °C for 10 h to remove any traces of other volatiles.

After volatile collections, trapped volatiles were extracted immediately with 2 ml each of Hexane, DCM, Methanol and Acetone (redistilled; LabScan, Malmö, Sweden),then Sample volumes were reduced to 50–60 µl, at ambient temperature in Francke-vials with an elongated tip (5 cm× 2 mm d.). Samples were stored in micro-centrifuge tubes at −18 °C [2].

Gas chromatography electroantennogram detector (GCEAD)

For GC–EAD, 2µl aliquot of each of the plant foliage volatile extracts, collected using Porapak-Q technique, were injected into a Hewlett-Packard (HP) 5890 Series II gas chromatograph equipped with an HP-1 column (30 m × 0.25 mm ID × 0.25 µm film thickness', Agilent, Palo Alto, California, USA). Helium gas was used as the carrier gas at 1 ml/min and the volatiles were analysed in the Split-less mode at an injector temperature of 280 °C and a 5 min delay using split valve of. The oven temperature was held at 35 °C for 3 minutes, then programmed at 10 °C/min to 280 °C and maintained at this temperature for 10 minutes. The column effluent was split into 1:1 after addition of make-up helium gas for simultaneous detection by flame ionization detector (FID) and EAD. For EAD detection, silver-coated wires in drawn-out glass capillaries (1.5 mm I.D.), were filled with KCl saline solution that served as reference and recording electrodes.

Antennal preparations were first made by putting the adult mated *R. pedestris* female or male in refrigerator using a glass vial to reduce their movement and activity. Then the base of the head and distal end of antenna were cut with the help of a scalpel. The base of the head was then connected to the reference electrode while the tip of the antenna was connected to the recording electrode, where electrodes were filled with 1N KCl saline solution. The analog signal was detected through a probe (INR-II, Syntech, Hilversum, Germany), captured and processed with a data acquisition controller (IDAC–4, Syntech, Germany), and later analyzed with software (EAG 2000, Syntech) on a personal computer. Each plant volatiles were analyzed using fresh male or female insect antennae. Each of the crude headspace field bean volatiles was tested.

Results and Discussion

1. EAG response of matured *R. pedestris* towards *D.lablab* plant volatiles.

The extracted Volatiles from Porapak-Q using various polar/nonpolar solvents produced the electrophysiological response as follows

Table 1: EAG response of matured *R. pedestris* towards *D. lablab* plant volatiles, air and solvents

Stimulus	Solvent used and response in milivolts		
Air		0.3	
	Hexane extract	DCM extract	Methanol
Solvent(pure)	0.35	0.4	0.5
Field bean leaf	0.4	0.5	1.0
Field bean pod	0.4	0.55	0.6

EAG-Response of *R. pedsetris*

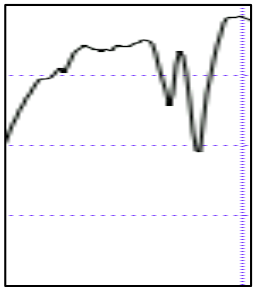

Fig 1: EAG response towards normal air.

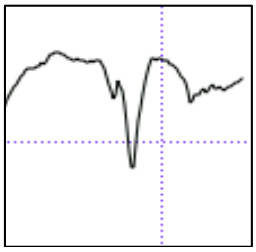

Fig 2: EAG response towards Hexane.

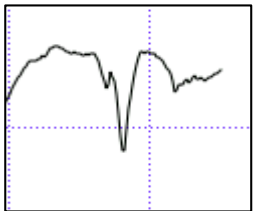

Fig 3: EAG response towards Dichloromethane.

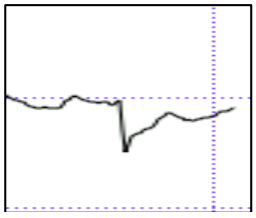

Fig 4: EAG response towards Methanol

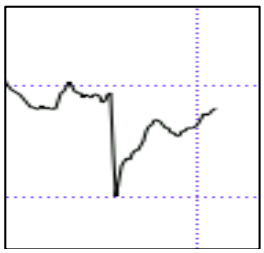

Fig 5: EAG response towards Field bean leaf volatiles extracted in Dichloromethane.

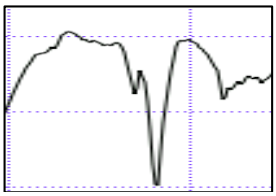

Fig 6: EAG response towards Field bean leaf volatiles extracted in Hexane.

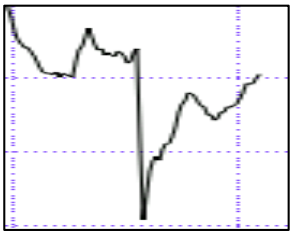

Fig 7: EAG response towards Fieldbean leaf volatiles extracted in Methanol.

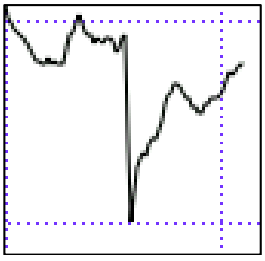

Fig 8: EAG response towards Field bean pod volatiles extracted in Dichloromethane

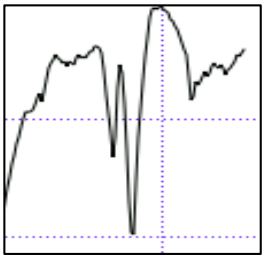

Fig 9: EAG response towards Field bean pod volatiles extracted in Hexane.

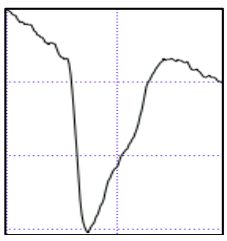

Fig 10: EAG response towards Field bean pod volatiles extracted in Methanol.

2. GC- EAD response of *R. pedestris* towards *D. lablab* plant volatiles

Table 2: Electrophysiologically active compounds identified from extracts from plant parts.

Solvent used	Plant part	Retention time	Compound identified using kovats index
Hexane	Field bean pod	6.8	1,3-dimethylbenzene and (E,Z) 2,4-heptadienal
		18.2	2-formylpyrrole
Acetone	Field bean pod	17.8	Hexadecanoic acid
Dichloromethane	Field bean pod	5.0	2-formylpyrrole and 2,3-butanediol
		6.4	Ethyl heptanoate and Undecane

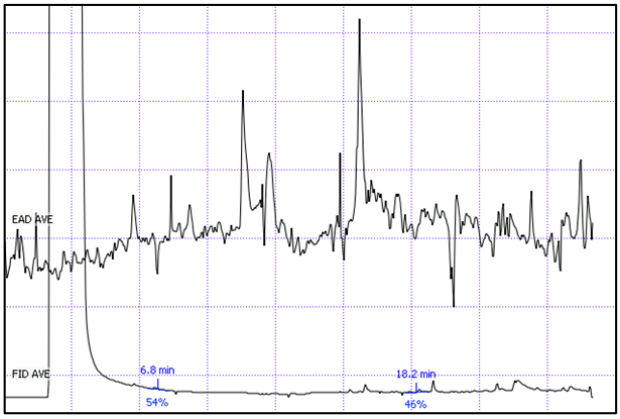

Fig 11: Electrophysiological response of *R.pedestris*on Field bean pod air entrapment hexane extract.

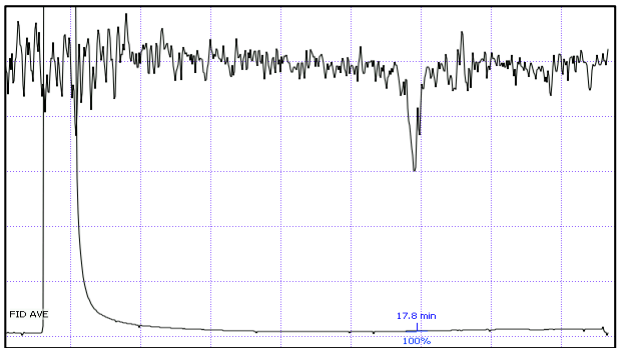

Fig 12: Electrophysiological response of *R.pedestris*on Field bean pod air entrapment acetone extract.

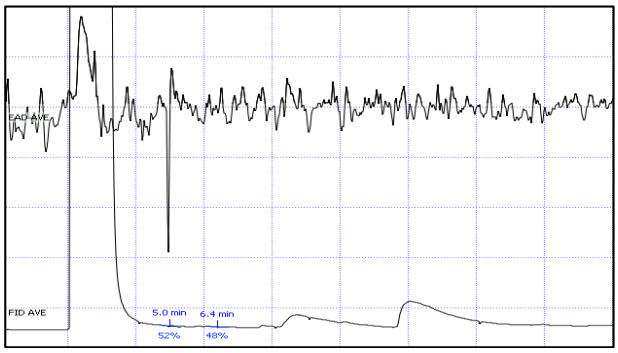

Fig 13: Electro physiological response of *R.pedestris*on Field bean pod air entrapment Dichloromethane extract.

3. GC-EAD response of 1st instars nymphs of *R pedestris*towards aggregating pheromone.

Table 3: Electrophysiologically active aggregation pheromone identified from 1st instar nymphs of *R. pedestris*

Solvent used	compound	Retention time	Compound identified by using kovats index
Hexane	Aggregation pheromone of *R.pedestris*	11.7	Methyl-3-hydroxy-2-methylbutanoate
		20.7	Ethylhexadeconate
		23.7	(Z,Z,Z,E)-3,6,9,17-tricosatitraene

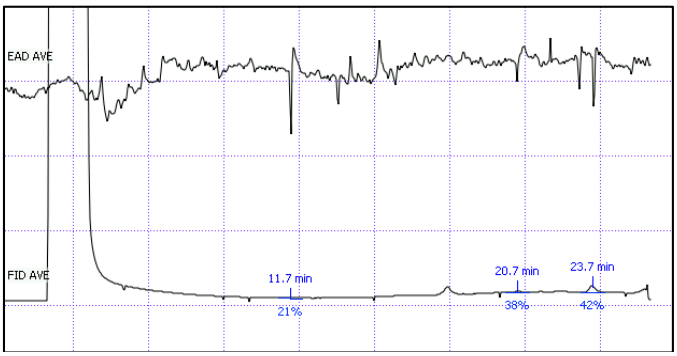

Fig 14: Electro physiological response of 1st instars nymphs of *R. pedestris*.

4. GC-EAD response of male *R.pedestris*towards sex pheromone released by Female.

Solvent used	compound	Retention time	Compound detected using kovats
Dichloromethane	Female sex pheromone of *R. pedestris*	9.2	1-(4-methylphenyl)-ethanone
			1-phenylpentane-1-ol
		22.2	(E, E)-1-(piperidin-1-yl)-dodeca-2,4-dien-1-one
		23.5	2,3-benzopyrrole

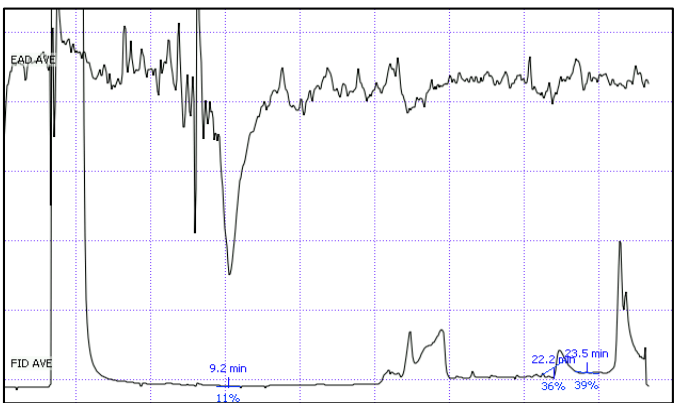

Fig 15: Electro physiological response of male *R. pedestris* towards sex pheromone released by Female.

Volatile of plants that hosts herbivorous insects play a major role in attracting or deterring coreid bugs and help them in completing their life cycle and ultimately leads in yield reduction. In the present study, preliminary observations in the field indicated that insect population increase during winter and declined during summer. 1st instar nymphs of coreid bugs would feed on leaf sap in groups and adult male and female feed on pods. These bugs would mate as soon as they emerge out as adults and due to multiple mating their population explodes. Mating was done during late mornings and female often lay eggs on pods.

D. lablab plant produces number of volatiles to which *R. pedestris* would show some response. In this experiment we collected information about the possible volatiles using GC-EAD, Kovats calculator to which *R. pedestris* would show electrophysiological response. This work is carried out to know the possible compounds to which the insect is giving response and use of these compounds for the possible development of the mass trapping system.

Through electrophysiological and analyticial studies, the possible plant volatiles identified were 1,3-dimethylbenzene, (E,Z)2,4-heptadienal, 2-formylpyrrole, Hexadecanoic acid, 2-formylpyrrole, 2,3-butanediol, Ethyl heptanoate, Undecane, 1-(4-methylphenyl)-ethanone, 1-phenylpentane-1-ol, (E,E)-1-(piperidin-1-yl)-dodeca-2,4-dien-1-one, 2,3-benzopyrrole. The possible aggregation pheromone were Methyl-3-hydroxy-2-methylbutanoate, Ethylhexadeconate, (Z, Z, Z, E)-3, 6, 9, 17-tricosatitraene.

The possible female sex pheromone were identified as 1-(4-methylphenyl)-ethanone, 1-phenylpentane-1-ol, (E, E)-1-(piperidin-1-yl)-dodeca-2, 4-dien-1-one, 2,3-benzopyrrole.

Identification of all these volatiles need to be confirmed through FTIR and NMR studies. Synthesized compounds need to be formulated and studied at field conditions to document their role as attractants, Insect Kairomones etc. Further, use of these compounds as a mean of mass trapping is needed to be conducted and the efforts to understand the mechanism involved in the aggregation process of this insect is under studies.The results concluded that *R. pedestris* were host specific. They can search the host (*Dolichos lablab*) by olfaction. They can sense the volatiles bursting from the host plant and these information's were gathered based on results of EAG and GC-EAD.

Conclusion

From the study, we identified the electrophysiological compounds of *D. lablab* for *Riptortus pedestris* and along with volatiles released by females during mating period and by nymphs during 1st instar. Pest showed clear response towards Kairomones and pheromones in a precise way. This pawed the way in understanding insect behaviour for different kinds of volatiles i.e., in semiochemical communication with the host plant and with its own species.

Electrophysiological results were used to gather information about possible kairomonal compounds of *D.lablab* and pheromones of *R. pedestris*.

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