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Microwave Oven Assisted Extraction of Banana pseudostem kairomones as attractant of *Odoiporus longicollis* Olivier (Coleoptera:Curculionidae): Electroantennogram investigations

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ABSTRACT

Microwave oven assisted extract was prepared from banana pseudostem and tested its kairomonal activities on O. longicollis using Electroantennogram technique. In addition, the results were tested with two other methods such as Solvent extraction and Air-entrainment extraction for comparison of its activities. It was found that the Micro oven assisted extract elicited the maximum EAG response in female, whereas, Solvent extract was found higher antennal activity in male only and the Air-entrainment extract elicited poor response both in male and female O. longicollis.

Key words: *Odoiporus longicollis*, Microwave oven assisted extraction, Solvent extraction, Air-entrainment extraction, Kairomones, Electroantennogram.

INTRODUCTION

Semiochemicals are playing very important role in modifying the behaviour of the insects [1]. At present, Coleopterans have been studied a lot using semiochemicals [2, 3 and 4]. Host attractants are referred as kairomones which comes under the category of semiochemicals. The kairomones are multi components of organic compounds such as simple aromatics, terpenes, aliphatic alcohols etc [5]. The kairomones can be used as attractant for insects either male or female/ both. Identification of kairomones has been facilitated by the EAG technique. The pseudostem extract for this investigation was prepared by many methods. Literature survey revealed that no work has been reported so far about the use of pseudostem extract obtained from microwave oven assisted extraction for investigating the weevils. Hence, an attempt was made to prepare the micro oven assisted extraction of banana pseudostem and use the same in electroantennogram

investigation. Further, the results obtained in the present study were compared with the standard methods with a view to knowing the kairomonal activity.

MATERIALS AND METHODS

Insect culture

The *O. longicollis* colony was raised from the field-collected weevils and grubs. In case of adults, they were brought to laboratory and put in a perforated (on the lid for ventilation and in bottom for drainage of water collected from pseudostem) plastic container (10 L) and fed with banana pseudostem of locally available cultivar Nendran. The pseudostem was changed as and when required. In case of grubs, each grub was separately put in small perforated plastic containers (height 7.0 cm and dia. 4.0 cm, TARSONS, Kolcutta) with a small piece of banana leaf sheath. The leaf sheath was changed until pupation. Emergence was noted on the container for age old culture. The adults were maintained in the laboratory at 12 h L: 12 h D, 25 ± 2°C and 65-70 % RH conditions. Weevils were sexed on the basis of rostrum characteristics to use them in different experiments

(i) *Microwave Oven Assisted Extraction*

A 100g of banana leaf sheath (pieces) was put in a 500 ml flask containing 200 ml of hexane (HPLC grade). The flask was kept in the microwave oven at full power (800 W) for one min. The bottle was removed and cooled at room temperature. The clear supernatant liquid was transferred to a flask and reduced to a volume of 1 ml (10µl/g equivalent) using rotary evaporator [6]. This sample was used for further studies.

(ii) *Simultaneous solvent extraction*

Solvent extraction is basically a process of diffusion of a solvent into oil-bearing cells of the raw material, resulting in a solution of the oil in solvent. The host plant extract was prepared from 100 g of banana pseudostem pieces soaked in hexane (HPLC grade) and kept on a shaker overnight to facilitate extraction. The clear supernatant was carefully transferred into a flask and reduced to a volume of 1 ml (10 µl/g equivalent). The sample was used for bioassay studies [7].

(iii) *Air-entrainment technique*

The volatiles were collected from banana pseudostem using the air - entrainment technique [8]. The volatile collection system consists of a glass chamber (25.7 cm long and 7.6 cm ID) constructed of pyrex glass with a an inlet and a ground-glass joint outlet. A glass cartridge containing 50 mg of 'Porapak Q' adsorbent was connected to the collection chamber at one end. Another glass cartridge containing 50 mg activated charcoal was connected to the other end. This unit was connected to an aquarium pump by a Teflon tube. Air was pushed through the charcoal cartridge which purified the air passing inside the chamber containing sample. The air-flow was adjusted to 600 ml/min by a Flow meter (Syntech, Hilversum, The Netherlands). The unit was run for 72 hrs. Volatiles collected on the 'Porapak Q' were eluted using 100 µl of high purity methylene chloride.

Electroantennogram recording

EAG recording technique was employed for testing the olfactory sensitivity of male and female banana pseudostem weevil against extracts prepared by the above methods. The EAG (Syntech,

Hilversum, The Netherlands) apparatus was used for this study. Male and female antennae were excised carefully and fixed between two stainless steel electrodes with two droplets of 'Spectra gel' (electrically conductive), applied to the electrodes [6]. The stimuli obtained from i, ii and iii (experimental section) and control (solvent hexane) were used in the EAG. The stimulus cartridges were loaded with filter-paper strips previously impregnated with 10 µl of each stimulus extract after allowing the solvent to evaporate. The experiment was repeated for five times with male and female weevils for each test stimulus. Control stimulations using filter paper impregnated with 10 µl of hexane solvent were puffed on to the antenna before and after each stimulus presentation. Two puffs of each treatment and control spaced 10 s apart were administered to yield depolarization amplitudes for each replicate weevil.

Statistical analysis

Two way ANOVA was performed with six replication keeping gender of the weevils and hexane extracts of different source as factors. LSD was performed for separation of means, where statistical differences were detected.

RESULTS AND DISCUSSION

EAG results obtained from stimulating male and female antennae with control (hexane) and extraction of (i), (ii) and (iii) (experimental sections) are presented in Table 1. Microwave oven assisted extract elicited significant EAG response in male and female banana pseudostem weevils in comparison with control indicating the presence of certain antennae active constituents in this extract. The results obtained from microwave assisted extract were compared with the results of other stimuli (ii), (iii) and found similar in female. However, the solvent extract (ii) elicited higher EAG response in male than that of (i) and (iii). Analysis of results with LSD test for multiple mean comparisons revealed significant differences in EAGs in male and female to the treatments -columns: $F = 7.99$, $P < 0.05$; df = 7, 47) for male and female. Solvent extraction produced larger EAGs (4.244 ± 0.276) than any other stimulus sources. All the odourous stimuli elicited higher EAGs in male compared to female indicating that male perceive kairomones and may involve in the process of host plant selection. The results are helpful in identification of the kairomones for the control of this pest.

Table 1. Electroantennogram response of male and female banana pseudostem weevil to odourous stimuli

Treatments	EAG response in mV ± SE*†	
	Male	Female
Control (hexane)	0.763 ± 0.095^{e}	0.655 ± 0.170^{e}
(i) Micro oven extract	2.767 ± 0.281^{b}	1.478 ± 0.150^{c}
(ii) Solvent extraction	4.244 ± 0.276^{a}	1.422 ± 0.069^{c}
(iii) Air-entrainment extract	2.770 ± 0.079^{b}	1.262 ± 0.167^{cd}

** EAG values between the columns except for control are significantly different*
† Values within columns followed by the different letters in the superscript are significantly different.
Mean comparison by LSD df 7, 47 for males df 7, 47 for females
a = best, b = better, bc = fair and c = poor
LSD = Least significant deviation

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CONCLUSION

Microwave assisted extract elicited higher EAGs in female compare to male. This method is simple and needs short time compared to other two methods which are time consuming. Further, microwave assisted extract can be used as kairomones for attracting weevils particularly in the fields where, female population is outnumbered the male as in the case of *O. longicollis*.

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