Journal of Agricultural Technology 2011 Vol. 7(5): 1265-1273
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Studies on electrophysiology, olfactometric response and chemical analysis of groundnut extracts against groundnut bruchid (*Caryedon serratus*)

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Panday, G., Bhatt, P., Kanaujia, S., Kanaujia, K.R., Jyothi K.N., and Prasuna, A.L. (2011) Studies on electrophysiology, olfactometric response and chemical analysis of groundnut extracts against groundnut bruchid (*Caryedon serratus*). Journal of Agricultural Technology 7(5): 1265-1273.

Caryedon serratus (Olivier) (Bruchidae: Coleoptera) is one of the major pest of groundnut.It infests groundnut by making characteristic round holes on them which cause qualitative and quantitative losses.Considering limitations of chemical use, there is a need for alternate methods. Electrophysiology and olfactometric responses based studies were conducted to find out the food lure as an attractant. Extracts of pods and kernels in different solvents were tested. Methanol extract of shelled groundnut showed highest attractance to both male and female insects.Gas chromatograph-mass spectrometry analysis of methanol extract of pods revealed the presence of Glycerin, 9, 12-Octadecadienoic acid, n-Hexadecanoic acid and ribitol as major constituents which may be responsible for its attractance.

Keywords: *Caryedon serratus*, groundnut extract, methanol, GC-EAD, GC-MS

Running Title: High Attraction Elicitation in *Caryedon serratus* Against Shelled Groundnut Methanol Extract.

Introduction

Groundnut holds a 34% share of the total oil seed area (24 million hectare) and contributes nearly 40% of the total oil seed production (20 million tonnes) (Sahayaraj & Amalraj, 2006). During a study in Andhra Pradesh, India,

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losses caused by insects to groundnut stores were 20% (Dick, 1987). Among the insect pests attacking the groundnut in stores, the groundnut seed beetle, *Caryedon serratus* (Olivier) is the only insect species known to infest kernels and intact pods and is thus potentially the most important pest of kernels (Ramadevi & Rao, 2005). *C. serratus* is a generalist coleopteran bruchid developing at the expense of six *caesalpiniodeae* species and one *fabaceae*, *Arachis hypogaea* (Ali & Huignard, 1993). Studies on feeding potential of pod bruchid revealed the presence of 4 grub instars (Singh *et.al*., 2002). Attempts were made to find out suitable food medium to prolong the life span of adult bruchid *Caryedon serratus*, which showed that D-mannose significantly increases the life span of male and female from 19 days & 24 days in control to 63 & 70 days respectively **(**Mittal,1971**)**.Observation on the life cycle of pod borer calculated that the successful survival of the larvae and pupae was 75.71% and 68.57% respectively while the net reproductive rate was 3.80 females per generation in generation time of 59.91 days **(**Joshi & Ghorpade, 2001**)**. Studies on the biology of the beetle *C. serratus* on groundnut and other host showed the incubation period, grub development and pupal period were 9.58 days, 42.62 days and 27.16 days respectively**(**Halle *et.al.,*2002).

For the control of eggs, larvae and adults of *C. serratus* in stored groundnut vacuum fumigation was used with phosphine and methyl bromide(Rao *et . al.,* 1993**)**. Residual toxicity of some pesticides against seed beetle followed the order: Fipronil>Carbosulfan>Fenobucarb [each at 20, 40,60 and 80 ppm]**(** Tripathi *et. al*., 2000).Evaluation of the host resistance, solar heat and insecticidal essential oils for the management of *C. serratus* showed that the clove oil significantly suppressed the adult progeny (Lale *et. al.,* 2002).

Eucalyptus leaf oil and neem oil at 3% and 5% were as efficient as BHC in reducing egg laying (Atta & Ahmad, 2002). Neem oil (0.5 and 0.75%), neem leaves (2.5 and 5%w/w), custard apple leaves (2.5 and 5%w/w), neem seed coat(10%w/w) and neem cake(10%w/w) were also effective against the bruchid (Manjula, 2003**).**Efficacy of few botanicals against *C.serratus* was studied under Bhubaneswar (India) conditions and found that vegetable oil of neem, pongamia, coconut and mustard @ 4% ml per kg inhibits oviposition and growth of adult beetle **(**Tripathi *et. al.,* 2004). Females of *Caryedon serratus* release sex pheromone from the beginning of the scotophase which triggers a positive chemoanemotaxy in males and observed that about 70.37% of males began to react to the sex pheromone within the first 24 hours after emergence. At this age, only 1 female out of 31 was attractive. The existence of receptors on male antennae for this pheromone was demonstrated by electrophysiology (EAG) (Chaibou *et. al.,* 1993).In view of serious losses in storing the groundnut pods, due to bruchid infestation and considering limitations of chemical use, a

search of other measures of pest control is required i.e. why the present study is conducted.

Materials and methods

The present study was conducted in the Department of Chemistry, College of Basic Science and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar and Indian Institute of Chemical Technology(IICT), Hyderabad, India. Nucleus culture of *C. serratus* was obtained from IICT .The chemicals and solvents used in the present study were HPLC grade and were obtained from SD fine –chem limited, Mumbai. All the Solvents were distilled prior to use.Nucleus culture of insect was reared on groundnut in B.O.D incubator at 35±2^{0}C temperature and 70 per cent relative humidity at Department of Chemistry, Pantnagar**.** The insects were separated into male and female with the help of microscope on the basis of fifth sternite which is emarginated in female insects while non emarginated in male insects

Preparation of groundnut extract

Both shelled and unshelled groundnut (250g each) were extracted separately and successively using hexane, dichloromethane and methanol using Soxhlet apparatus of 500ml capacity.The solvents were concentrated first by using thin film rotary vacuum evaporator and then by N_2 vapours upto a volume of 10 ml. After extraction with solvent, the left over shelled and unshelled groundnut were extracted successively with triple distilled water (100ml) to obtain water extracts.

Olfactometer bioassays

The adult bruchids were subjected to a behavioural bioassay for the determination of food lure using a glass Y tube olfactometer (Analytical research Systems, Gainesville, FL, USA) .The Y tube consisted of a 14cm long stem, the release chamber and two 6.5cm long arms, each with a 1cm i.d.A screened glass plug at the base of the stem was used to introduce insects into the Y tube. At the upwind end of each arm was a glass tube (1cm i.d×10cm long) within which odour sources were placed with a wire screen that prevented insects from entering these chambers and contacting odour sources. Charcoal-filtered humified air from Syntech air delivery unit was metered through the two arms of the Y tube via Teflon tubing at 250ml/min.The bioassays were conducted uniformly between 16-18 hours at 28±2°C. Presence of food lure

among the *C.serratus* adults was investigated in the olfactometer by exposing both the sexes separately to the air current carrying the odour of the each concentrated groundnut extract(Vassilis *et.al*., 2008).

Groundnut extract in different solvents were applied one by one to the pieces of Whatman filter paper of the size 1×2 cm and placed in one of the Y tube chambers against the air stream and with a control (equal volume of HPLC grade solvent) in the other.A single bruchid (with 5min interval) was introduced into the Y tube at the entrance of the stem of the release chamber so that it can make choice between the test odour and the control. The behaviour of the bruchid was classified as one of the three categories, choosing between control(solvent) or the treatment or no choice (individuals that had not made a choice for either odour source within 5min of crossing the start line).In each experiment, 20 insects were used and all the experiments were conducted in triplicates. New filter paper with the extracts and control solvent were used for every 10 insects. Each crude extract was used one by one for the experiment and replicated 3 times using 20 males and 20 females insects of *C. serratus* separately.Olfactometer arms were flipped around (180º) to minimize positional effect after testing of 20 insects. All the insects in the Y tube were removed after each experiment and the olfactometer was thoroughly washed, rinsed with acetone and oven dried for the next experiment.

Procedure for bioscreening groundnut extract using gas chromatograph coupled with electroantennogram

The perception of olfactory stimuli in insects is mediated largely through their antennal receptors.Electroantennogram recording technique is a unique and versatile technique, which utilizes insect antenna as a finely tuned detector for rapid screening of various semiochemicals.The principle of EAG is to record voltage changes between the tip and base of an antenna during stimulation by a volatile while Gas chromatograph (GC) analysis is known for its utility for separation of compounds even at minute quantities.

Recordings were performed with a commercially available GC- EAD system (Syntech, Hilversum, The Netherlands), with a column split and an extra outlet which allows simultaneous flame ionization detection (FID) and Electroantennographic detection (EAD).A capillary column of 15m× 0.53 mm i.d.; SPBTM- 1701 was used with hydrogen as the carrier gas (4 ml/min). Injector temperature was 275°C, detector temperature 275°C, EAD-outlet 130°C and the split less injection (0.2 µl of concentrated crude extracts). The temperature programme started at 60°C oven temperature with a holding time of 2 min and at a rate of 7°C / 10°C /min to 250°C and held for 5 min. The column effluent was split into two equal (1:1) parts between a flame ionization

detector (FID) and electroantennograph detector(EAD). Electroantennographic system consisting of a dual electrode probe for antenna fixation, a CS-05 stimulus controller and an IDAC box for data acquisition. Antenna was excised along with the head and fixed between the two stainless steel electrodes (Head at one end and tip of the antenna on to the recording electrode)using electrically conductive gel (ECG gel).The most biologically active extract (shelled groundnut methanol extract) was evalvuated by this technique. First the antenna of bruchid was carefully excised along with the head (using micro scissors) and fixed between the two pores of an electrode holder with the help of conducting gel. The antenna was continuously bathed with a stream of charcoal –filtered humified air through the stainless steel flow tube of the stimulus applicator. The column of the EAD outlet was introduced into an 8 mm diameter glass tube with a constant air stream filtered through activated charcoal (Flow 0.5 ml/min). The mounted antenna was placed 0.5 cm from the end of the glass tube. The FID and EAD signals were both analyzed and monitored on a personal computer using GC-EAD software (Auto spike, IDAC 2/3 syntech, The Nether lands).

Chemical analysis

The GC-MS data of methanol extracts of shelled grounduts was obtained on GC-MS Quadrupole using HP-5MS non-polar capillary column (30m x 0.25mm, 0.25µm i.d.).Helium was used as carrier gas at a flow rate of 1.1 mL/min and the mode of ionization was EI (70 eV). The detector temperature and MS source temperature was 150^0C and 230^0C respectively. Temperature program applied was 1.5^0C/min up to 280^0C and finally isotherm for 30 min.

Results

Olfactrometric bioassay of both shelled and unshelled groundnut extracts (Hexane, DCM. Methanol and Water) against male and female *C.serratus* revealed that methanol extract of shelled groundnut differ significantly for both male (75.00± 13.23**) and female insects (55.00± 17.00**). i.e. methanol extract of shelled groundnut has greatest attractancy towards male and female insects (Fig. 1, 2) No significant difference was observed among the unshelled groundnut extracts against male and female insects (Fig. 3, 4).

In view of this, GC-EAD bio-screening of antenna of male and female *C .serratus* against pod methanol extract was done.GC-EAD analysis of pod methanol extract to conspecific males of *C. serratus* revealed the presence of 5 bioactive peaks at retention times 15 min (1.9 mv), 18 min (0.5 mv), 21 min

(0.7 mv), 23 min (0.6 mv) and 34 min (0.4 mv) respectively (Fig. 5). GC-EAD analysis of shelled groundnut methanol extract to conspecific females of *C. serratus* revealed the presence of 4 bioactive peaks at retention times 11 min (0.7 mv),15 min (0.9 mv), 26 min (0.7mv) and 34 min (1.3 mv) respectively (Fig. 6).The GC-MS analysis methanol extract of shelled groundnut revealed the presence of more than fifty five compounds out of which twelve compounds in methanol extract of shelled groundnut contributing 37.14% were identified. The major identified constituents were Glycerin (18.05%),9,12-Octadecanoic acid (5.74%), n-Hexadecanoic acid (9.55%), ribitol (4.4%) and 4H-Pyran-4-one,2,3,dihydro-3,5,-dihydroxy-6-methyl (3.86%) besides other minor constituents (Table 1).

Discussion

The olfactometric studies have clearly shown that shelled groundnut methanol extract exhibits attractance for male and female *Caryedon serratus*. This attractance was further confirmed by GC-EAD response of male and female antennae. Male antennae responded for 5 bio-active components in the methanol extract and female responded for 4 bio active components at different retention times. But response at 34 min. was found in male as well in female.

GC-MS analysis showed some major chemical components along with the minor components (Table1). The attractance of methanol extract for male and female *C. serratus* may be due to presence of these components collectively or separately.The chemical components present in methanol extract may be used as food lure for this insect. Presence of food lure among the *C.serratus* adults was investigated in the olfactometer by exposing both the sexes separately to the air current carrying the odour of the each concentrated groundnut extract as similar to the work of Vassilis *et al.* (2008). When a gas chromatograph combines with electroantennograph detector it would simultaneously separate volatile compounds present in the extract and screen the eluted compounds for their bioactivity against the insect antenna which is also reported by David, *et al.*(2009); Satoshi, *et al.* (2003); Jyothi, *et al.*(2008); and Kanaujia & Kaissling (1985). Further studies in this direction are in progress.

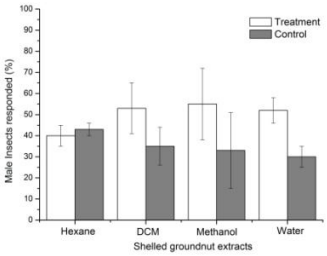

Fig. 1. Response of male *Caryedon serratus* insects (%) against extracts of Shelled Groundnut

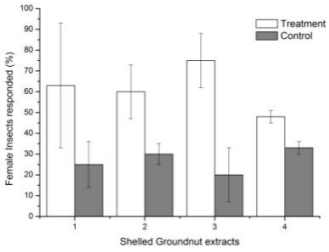

Fig. 2. Response of female *Caryedon serratus* insects (%) against extracts of Shelled Groundnut

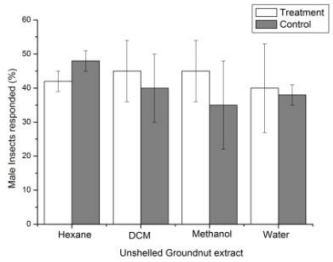

Fig. 3. Response of male *Caryedon serratus* insects (%) against extracts of Unshelled Groundnut

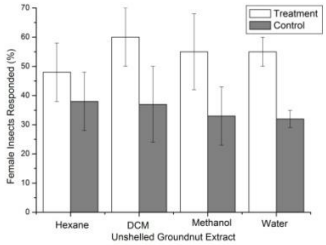

Fig. 4. Response of female *Caryedon serratus* insects (%) against extracts of Unshelled Groundnut

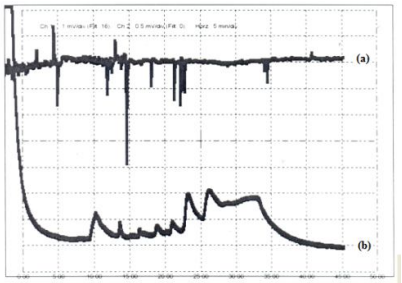

Fig. 5. GC-EAD profile of antenna of male *Caryedon serratus* response to Shelled groundnut methanol extract (a). Electrophysiolocal response (b). Gas chromatograph profile

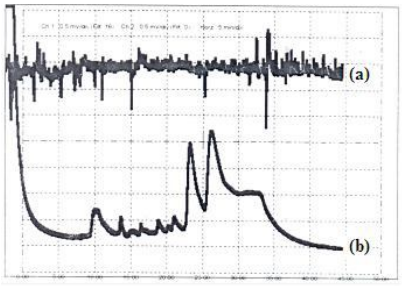

Fig. 6. GC-EAD profile of antenna of male *Caryedon serratus* response to Shelled groundnut methanol extract (a) Electrophysiolocal response, (b) Gas chromatograph profile

Table 1. Comparitive chemical composition of shelled groundnut methanol extract.

Name of compound	Shelled methanol extract (Area%)
Glycerin	18.05
9,12-Octadecadienoic acid	5.74
9-Octadecenoic acid	0.70
n-Hexadecanoic acid	9.55
8,11-Octadecadienoic acid,methyl ester	0.97
1,2-Benzenedicarboxylic acid, mono (2-ethyl hexyl) ester	0.48
Bicyclo [2,2,1] heptan-2- one,1,7,7,trimethyl(1R	0.37
4H-Pyran-4-one,2,3,dihydro-3,5,dihydroxy-6-methyl	3.86
1H-2-Benzopyran-1-one,3,4,dihydro-8-hydroxy-3-methyl	0.42
Ribitol	4.4
6H-Benzofuro[3,2-C][1] benopyran-3	0.62
Erucic acid	0.17

Acknowledgements

Thanks are due to authorities of G.B. Pant University of Agriculture & Technology, Pantnagar, India for providing necessary research facilities and IICT Hyderabad for providing technical support and at all stages of work.

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(Received 30 June 2011; accepted 5 August 2011)