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Ovipositional deterrence by egg extracts of *Caryedon serratus* (Olivier)

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ABSTRACT

The grub of *Caryedon serratus* (Olivier) (Bruchidae: Coleoptera) infests the groundnut kernels by making a characteristic round hole on them which causes qualitative and quantitative loss to the kernels, so ovipositional behavior of *C. serratus* was studied by choice and no-choice experiments to check the presence of ovipositional deterrent activity of egg wash extracts using three different solvents. Dichloromethane (DCM) extract showed deterrent activity on oviposition of *C. serratus* (20 eggs/mL) in both choice and no-choice experiments. Only 23 to 57 percent of eggs hatched. The egg extracts and groundnut extracts did not interfere with the further development of larvae to adults. GC-MS analysis revealed the presence of more than 55 compounds in dichloro methane egg extract out of which eleven compounds contributing 23.13% of the total egg extract were identified.

Keywords: *Caryedon serratus*, ovipositional deterrent activity, dichloromethane egg extract, GC/MS

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a premier oil seed crop of India. It holds 34% share of the total oil seed area (24 million hactare) and contributes nearly 40% of the total oil seed production (20 million tons) (Sahayaraj and Amalraj,2006). Groundnut seed contains 44-56% oil and 22-30% protein on a dry seed basis and is a rich source of minerals (P, Ca, Mg, K) and vitamins (E, K, B group)(Savage and Keenan,1994). During storage, groundnut pods/seeds are susceptible to the attack of many insect pests including groundnut seed beetle (Bruchid), *Caryedon serratus* (Olivier). The grubs of *C. serratus* mostly cause damage to the kernels (Devi and Rao, 2005; Singh *et al*., 2002). For protection of groundnuts from the infestation of *C. serratus*, various insecticides and fumigants have been evaluated(Tripathi *et al.*, 2000 and Rao *et al*, 1993). These insecticides and fumigants have adverse health hazards and pose a high risk to the environment. To minimize use of insecticides, some plant products and aromatic oils were evaluated against *C. serratus* (Kumari *et al.*, 1998; Manjula, 2003). Pheromone technology is one of the most effective and ecofriendly tool for insect control. Pheromones are species specific and required in minute quantities (Kanaujia and Kaissling, 1985; Malik *et al.*, 2003). Ovipositional deterrent activity was observed in egg extract of *Callosobruchus maculatus* (Messina *et al.*, 1987). In view of this, present study has been undertaken to assess the ovipositional deterrent activity in the egg extracts of *C. serratus* and GC/MS analysis of the egg extracts.

MATERIALS AND METHODS

Source and maintenance of culture

Nucleus culture of *Caryedon serratus* (Olivier) was obtained from Indian Institute of Chemical Technology (IICT) Hyderabad and maintained on groundnuts in the incubator at 35 ± 2^0 C temperatures and 70% relative humidity in the laboratory of Department of Chemistry.

Sample preparation

For collection of eggs of *C. serratus*, 10 groundnut kernels were taken in a plastic container and five pairs of freshly emerged adults (less than 24 hours old) were released in it. The insects were left for mating and egg laying. The experiment was replicated five times. After 24 hours, the kernels bearing eggs were replaced by fresh kernels and dead insects (if any) were replaced by fresh insects. Freshly laid eggs of *C. serratus* along with negligible portion of groundnut kernels adhering to the egg was scrapped with the help of a surgical blade and collected in a sample bottle (5mL). The eggs were treated successively with 2 ml of the solvents of increasing polarity (1 hour in hexane followed DCM and 24 hours in methanol), to prepare egg extracts. Similarly groundnut kernel extracts (hexane groundnut extract, dichloromethane groundnut extract and methanol groundnut extract) were also prepared as control for respective egg extracts by successive elution of small portion of groundnuts kernels in hexane, dichloromethane and methanol.

Behavioural bioassay

Evaluation of ovipositional deterrent activity of egg wash extracts against *C. serratus* adults was done by choice experiment and no-choice experiment. Different concentrations of the egg extracts and groundnut kernel extracts (control) expressed as 50 eggs/mL, 20 eggs/mL and 10 eggs/mL were used. In the similar manner, test solutions of concentration similar to dilutions as 50 eggs/mL, 20 eggs/mL and 10 eggs/mL of each of hexane, dichloromethane and methanol groundnut extracts were prepared.

No-choice experiment

No-choice experiment was conducted at room temperature in which 22 treatments were carried out involving groundnuts treated with different concentrations of the egg extracts (T_5 - $T_{13)}$ and groundnut kernel extracts (T_{14}-T_{22}), groundnuts treated with pure solvents hexane (T_1), DCM (T_2) and methanol (T_3), used as solvent control and the untreated groundnut kernels (T_4) as control. All the treatments were replicated thrice and performed in separate plastic containers taking 10 groundnut kernels for each treatment. The kernels were dipped in different concentra tions of egg extracts, groundnut kernel extracts and in pure solvent control (the kernels were taken out just after dipping in the respective extracts/solvents) and dried at room temperature to evaporate the solvent. The treated and untreated groundnut kernel were placed in the respective labeled containers. Five pairs of freshly emerged adults were released into the containers and left for mating and egg laying. The number of eggs laid on groundnut kernels of each box and number of dead insects were counted at a regular interval of 24 hours and the groundnut kernels with eggs were replaced by freshly treated groundnut kernels. The experiment was carried out till all the insects were dead (maximum longevity of male and female *C.serratus* was 12 to 13 days). The egg bearing groundnut kernels of all the treatments were counted daily and kept separately for further observations on development of eggs to adults. Based on the observations, the egg hatching, pupal develop ment, adult emergence and eggs converting to adults were calculated.

Choice experiment

Based on the results of previous experiments, choice experiment was carried out at room temperature using concentration of 20 eggs/mL of egg extracts and groundnut extracts. For egg extracts, four equidistant circles were marked in one glass petri-dish (diameter 180 mm) each for hexane (T_6), DCM (T_9), methanol (T_{12}), treated and untreated groundnuts (T_4). Similarly for groundnut extracts, in another glass petri-dish four equidistant circles were marked each for hexane (T_{15}), DCM (T_{18}), methanol (T_{21}), treated and untreated groundnuts (T_4). Ten groundnut kernels were used for each treatment and five pairs of *C. serratus* were released in each petri- dish. Other methodology was similar to that of no-choice experiment.

Chemical analysis

The GC-MS data of hexane and DCM egg extracts 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 were 150^0C and 230^0C respectively. Temperature program applied was 1.5^0C/min upto 280^0C and finally isotherm for 30 min.

RESULTS AND DISCUSSION

Fecundity of *C. serratus*

Investigations on ovipositional preference of *C. serratus* in no-choice experiment revealed that maximum and minimum number of eggs was laid on groundnut kernels treated with DCM and hexane treated groundnut kernels respectively (T_C in Fig. 2). Higher number of eggs were laid on first day in all treatments which slowly declined each day and ceased after seventh day (Fig. 1). Egg laying behavior on groundnut extract treated kernel showed that methanol extract was most preferred in all three concentrations (T_{g10},T_{g20} and T_{g50} in Fig. 2) Out of three concentrations of egg extracts of hexane, DCM and methanol, highest egg laying was observed on groundnuts treated with concentration of 50 eggs/ml in all the three egg extracts (T_{50} in Fig.2) and DCM , egg extract of concentration 20 eggs/mL was least preferred for egg laying (T_{20} in Fig.2) even less than untreated groundnuts and groundnuts treated with DCM.

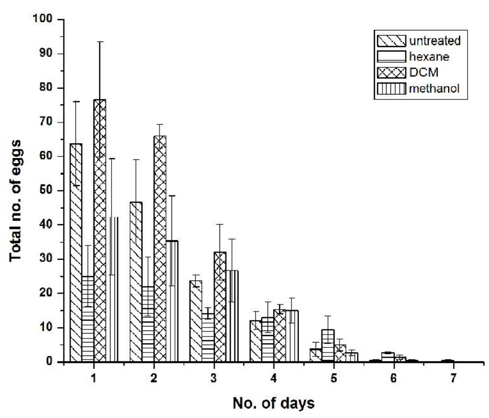

Figure 1. Eggs laid by *C. serratus* on groundnuts treated with different solvents (control) and untreated groundnuts (no-choice experiment)

Table 1. Larval hatching, pupal formation, adult emergence and total emergence (%) of *C. serratus* in relation to various treatments.

Treatments	Concentrations (Eggs/mL)	Larval hatching	Pupal formation from larvae	Adult emergence from pupae	Egg conversion to adults
T_1-Pure hexane	-	48.83	100	88.09	43.02
T_2-Pure DCM	-	33.16	100	98.46	32.65
T_3-Pure methanol	-	46.72	100	96.49	45.08
T_4-Untreated	-	23.33	91.42	100	21.33
T_5-Hexane egg extract	50	40.36	100	88.05	35.54
T_6-Hexane egg extract	20	44.02	100	89.83	39.55
T_7-Hexane egg extract	10	44.72	100	94.44	42.23
T_8-DCM egg extract	50	39.37	100	94.73	37.30
T_9-DCM egg extract	20	43.24	100	87.50	37.83
T_{10}-DCM egg extract	10	46.42	100	92.30	42.85
T_{11}-Methanol egg extract	50	35.16	100	98.43	34.61
T_{12}-Methanol egg extract	20	48.29	100	98.59	47.61
T_{13}-Methanol egg extract	10	49.23	100	98.43	48.46
T_{14}Hexane groundnut extract	50	46.77	100	98.27	45.96
T_{15}-Hexane groundnut extract	20	38.05	100	98.50	37.93
T_{16}-Hexane groundnut extract	10	53.65	98.48	96.92	51.21
T_{17}-DCM groundnut extract	50	41.33	98.48	100	40. 66
T_{18}-DCM groundnut extract	20	57.14	100	100	57.14
T_{19}-DCM groundnut extract	10	44.26	100	100	44.26
T_{20}-Methanol groundnut extract	50	49.70	100	97.61	48.52
T_{21}-Methanol groundnut extract	20	40.21	100	98.64	39.67
T_{22}-Methanol groundnut extract	10	46.90	98.48	98.88	45.87

Abbreviations

T_C = Treatment of groundnut kernels with different controls, T_{g10} = Treatment of groundnut kernels with groundnut kernels extract of conc. equivalent to 10 eggs/mL, T_{g20} = Treatment of groundnut kernels with groundnut kernels extract of conc. equivalent to 20 eggs/mL, T_{g50} = Treatment of groundnut kernels with groundnut kernels extract of conc. Equivalent to 50 eggs/mL, T_D=Treatment of groundnut kernels with hexane conc., T_H = Treatment of groundnut kernels with hexane conc., T_M = Treatment of groundnut kernels with hexane conc., T_U =untreated groundnut kernels, T_{10} = Treatment of groundnut kernels with egg extract of conc. 10 eggs/mL, T_{20}= Treatment of groundnut kernels with egg extract of conc. 20 eggs/mL, T_{50} = Treatment of groundnut kernels with egg extract of conc. 50 eggs/mL, GC/MS = Gas chromatography mass spectrometry, EI = Electronic ionization and eV = Electron Volt

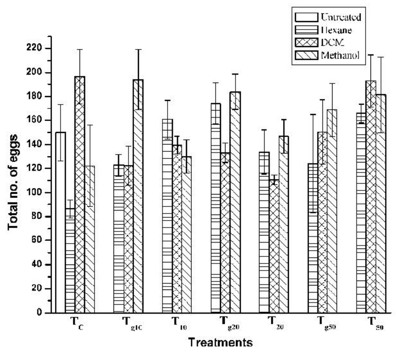

Figure 2. Comparative data of total number of eggs laid by *C. serratus* on untreated groundnut kernels and kernels treated with different concentrations of groundnut extracts and egg extracts (no-choice experiment)

On the basis of results of no-choice experiment, free choice chamber test was performed with concentration of 20 eggs/mL. It was observed that in control experiment, groundnuts treated with hexane groundnut extract DCM groundnut extract and methanol groundnut extract showed similar preference as untreated ground kernels by *C. serratus* for egg laying (Fig. 3). In case of egg extracts, highest preference for egg laying was given to groundnut kernels treated with methanol egg extract and minimum number of eggs were observed on groundnuts treated with DCM egg extract (Fig. 3). These observations were similar to that of choice experiment. This showed some ovipositional deterrent activity in DCM egg extract which repelled the insect for ovipositioning on groundnuts treated with this extract. Similar oviposition deterrent activity has also been observed in etheral egg extract of *Callosobruchus maculates* (Messina *et al.*,1987)

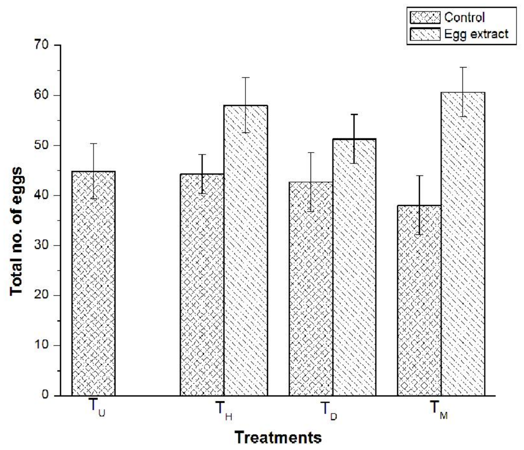

Figure 3. Eggs laid by *C. serratus* on groundnuts treated with different groundnut extracts and egg extracts (choice experiment)

GC-MS analysis

Results of behavioral bioassay showed some ovipositional deterrent activity in DCM egg extract of *C.serratus* .The GC-MS analysis of DCM egg extract of *C. serratus* revealed the presence of more than 55 compounds in the extract, out of which eleven compounds contributing 23.13% of the total egg extract were identified. phthalic acid, nonyl 4-octyl ester (6.55%), phthalic acid, bis (7-methyloctyl) ester (6.26%), 1,2-benzenedicarboxylic acid, dinonyl ester (5.50%), 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester (1.21%) and oleic acid (1.16%) were the major constituents besides other minor constituents .It has been reported that esters of pthalic acid (phthalates) are known to possess insect repellent activity (Mustafa *et al.,* 2007) . It has also been reported that oleic acid posses ovipositional deterrent activity in the egg extracts of adzuki bean borer (*O.furnacalis*) and *Helicoverpa armigera* (,Guoqing and yukio,2005, Mulan et al.,2008). Therefore oviposition deterrent activity of DCM egg extract of *C. serratus* may be attributed to the presence of phthalates and oleic acid along with other components.

Biology

The eggs laid by female *C. serratus* on groundnuts in no-choice experiment were monitored daily for hatching, larval development, pupal formation and adult emergence. The results are given in table 1. It was evident from the results that only 23 to 57% of eggs hatched and developed into larvae. From the larvae to pupae and from pupae to adult development was almost 100%. The total percentage of eggs developing into adults was found to be less than 50% in all the treatments. There was no effect of groundnut extract or egg extract on the development of eggs to adults.

The results have clearly shown that DCM egg extract (20 eggs/mL conc.) showed oviposition deterrent activity although maximum number of eggs were obtained on groundnuts treated with pure DCM (control). Hence, it may be concluded that there is some ovipositional deterrent activity in DCM egg extract. Ovipositional deterrent activity of DCM egg extract may be due to some chemical components (such as phthalates and oleic acid) present in this extract and the chemical components responsible for it may be used for control of this insect along with other control measures. Although ovipositional deterrent activity was shown by DCM egg extract (20 eggs/mL) but no effect of egg extracts and groundnut extracts was observed on further development of larvae to adults. The nature of active components in the egg extract, which could possibly be responsible for its deterrent activity, will be subject of further investigations.

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