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Insect attractant and oviposition enhancing activity of hexadecanoic acid ester derivatives for monitoring and trapping *Caryedon serratus*

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ABSTRACT

This paper describes behavioural changes elicited in *Caryedon serratus* by some synthesized hexadecanoic acid ester derivatives, which can be effectively used for monitoring pest populations thereby reducing the risk of pests becoming established in stores. Six hexadecanoic acid ester derivatives were synthesized. Choice and no-choice experiments with different concentrations (0.1, 0.5 and 1.0 mg l^{-1}) of derivatives against *C. serratus* revealed significant ($p < 0.001$) oviposition activity for five Hexadecanoic acid ester derivatives. In contrast, 1.0 mg l^{-1} benzoyl hexadecanoate reduced oviposition (4.7 ± 0.6) significantly ($p < 0.001$) as compared with control. In Y-tube olfactometer bioassays performed under laboratory conditions, synthesized derivatives elicited significant ($p < 0.001$) attractiveness (70–98%) in female *C. serratus*. Additionally, trap experiments performed in mini-stores also support highly significant ($p < 0.001$) attractiveness of 3-methylphenyl hexadecanoate (0.1 mg per 10 ml) and 4-bromophenyl hexadecanoate (1.0 mg per 10 ml). Conversely, benzoyl hexadecanoate significantly attracted male *C. serratus*. This study established the dose specific application of hexadecanoic ester derivatives in traps for effective management of this groundnut pest. The specificity of synthesized ester derivatives in attracting both sexes differently also indicates their probable resemblance to pheromone components of *C. serratus*.

1. Introduction

Caryedon serratus (Olivier) (Coleoptera: Bruchidae), the groundnut bruchid is a key pest of groundnuts (*Arachis hypogaea* L.) which is known to cause damage to groundnut pods/seeds and kernels. Studies have shown that maximum losses due to the attack of *C. serratus* occur at the time of storage (Mishra and Dash, 2009). Infestation rate and population levels increase rapidly in stores leading to heavy weight losses in stored groundnuts (Sembene, 2006). It is therefore necessary to control the pest *C. serratus* of which the destructive stages have a hidden and protected life style within stored groundnuts. In response to this, farmers and traders have utilized insecticides and fumigants for the protection of groundnuts from infestation. However, major problems with pesticide application include adverse health hazards, non-biodegradability and high risk to the environment (Tripathi et al., 2000; Rao et al., 1993).

Insect contamination in food commodities is an important quality control problem for food industries. The extent of post harvest losses has made groundnuts less competitive compared to palm oil, cotton seed oil and others. To regain its competitiveness, groundnut yield and production need to be increased significantly. Technologies to enhance its production through proper pest management must be promoted (Waliyar et al., 2007). The focus over the last few years has been on the pheromone technology which is more likely to succeed for its effectiveness and ecofriendly way to control insects. Moreover, pheromones are species-specific, required in minute quantities and best suitable for the management of pests with internal feeding life styles (Kanaujia and Kaissling, 1985; Malik et al., 2003).

This work is focused on management of *C. serratus* in groundnut stores to reduce serious losses incurred in groundnut pods, due to bruchid infestation. Studies have established that alkanes, monoterpenes, sesquiterpenes, phenyl propanoids, and fatty acids could serve as olfactory cues to the insect for finding its host (Harborne, 2003; Schoonhoven et al., 2005; Fernandez and Hilker, 2007; Matsuki et al., 2011). This guided the authors to synthesize

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hexadecanoic acid ester derivatives and study in detail the behavioural response of *C. serratus* towards them. In this study, a series of experiments was designed to examine the effect of different concentrations of synthesized hexadecanoic acid ester derivatives via choice and no-choice testing at two scales and Y-tube olfactometer bioassays. Also, experiments were designed in mini stores to determine the potential role of sticky traps impregnated with an appropriate concentration of these esters in monitoring and trapping *C. serratus* population in stored groundnuts.

2. Materials and methods

2.1. Synthesis of hexadecanoic acid, ester derivatives

We used methods described by Kazuhito et al. (2004), for the synthesis of hexadecanoic acid ester derivatives (Tables 1 and 2). The chemicals and solvents used in the present study were obtained from Sigma–Aldrich, U.S.A. and E. Merck Ltd. Mumbai India and were HPLC grade. All the solvents were distilled prior to use. In a two-neck flask, reactant 1 was poured into reactant 2 and reactant 3 was added drop-wise to it. The solution was cooled in an ice bath, magnetically stirred for 2 h and then transferred to a separating funnel and washed successively with 1M HCl, 50 g l^{-1} bicarbonate solution, 100 g l^{-1} brine solution and water. The organic layer was dried over anhydrous $MgSO_4$ and the solvent was evaporated under reduced pressure to give the product. The synthesized derivatives were all characterized by spectroscopic means using ^{1}H NMR and IR.

Different concentrations (0.1, 0.5 and 1.0 mg l^{-1}) of each of the six synthesized derivatives were made in hexane for bioassays.

2.2. Test insects

The groundnut beetle, *C. serratus* was used for the experiment. Starter cultures of *C. serratus* (Olivier) (Coleoptera: Bruchidae) were brought from the Indian Institute of Chemical Technology (IICT), Hyderabad. The insect colonies were raised on both shelled and unshelled groundnuts in plastic containers at 35 ± 2 °C and 70% relative humidity (r.h.) (Jyothi et al., 2014). Newly-emerged adults (0–24 h) old were collected and used for the experiments as early stages determine the number of adult beetles that will eventually reproduce in the F_2 generation (Rotimi and Evbuomwan, 2012). Segregation of sexes by microscope (Olympus SZ40) was done by fifth sternite which was found to be emarginated in female while non-emarginated in male insects (Delobel et al., 2003).

2.3. Evaluation of oviposition enhancing activity

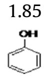
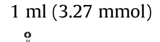
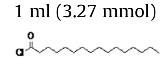
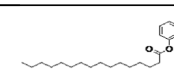
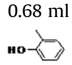
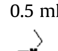
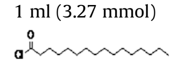
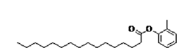
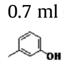
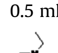
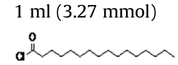
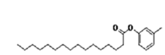
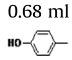
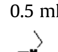
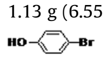
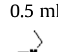
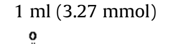
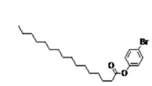
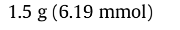
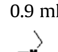
Groundnuts used for the experiments were grown in the field of G.B. Pant University of Agriculture and Technology, Pantnagar, India, by organic farming and were free from pesticides. Oviposition activity of *C. serratus* was assessed by conducting no-choice experiments with groundnuts dipped in 0.1, 0.5 and 1.0 mg l^{-1} solutions of derivatives (in hexane) and using hexane as control. For each treatment, ten fresh unshelled groundnuts were dipped in solutions for 2 min and taken out to evaporate the solvent at room temperature. Groundnuts were then placed in glass Petri dishes (diameter 90 mm) and ten pairs of freshly-emerged *C. serratus* (0–1 day old) were released in the centre of each Petri dish and covered. The number of eggs was scored at regular intervals of 24 h for 10 days. The groundnuts which contained eggs were removed and replaced with freshly-treated groundnuts. This experiment was repeated for each synthesized derivative.

Choice tests utilized the two most effective concentrations (0.1 mg l^{-1} 3-methylphenyl hexadecanoate and 1.0 mg l^{-1} 4-bromophenyl hexadecanoate), which enhanced oviposition in preliminary no-choice experiments. The unshelled groundnuts were dipped in these concentrations and the control and dried at room temperature for 2 min. Ten pairs (10 males and 10 females) of freshly-emerged (0–1 day old) adult *C. serratus* were then confined in the centre of the Petri dish (180 mm diameter) and left for mating and egg laying after the groundnuts were placed in three equidistance circles marked on the Petri dish. The number of eggs in each kernel was counted at the regular intervals of 24 h for 10 days. The groundnuts which contained eggs were removed by sterile forceps and replaced with freshly-treated groundnuts. The procedure was repeated with 4-methylphenyl hexadecanoate (0.1 mg l^{-1}) and phenyl hexadecanoate (0.5 mg l^{-1}) placed along with a hexane control. Each experiment was conducted at 35±2 °C, using 20 bruchids in each of three replicates for each trial.

2.4. Olfactometer experiments

Y-tube olfactometer (Analytical Research Systems, Gainesville,

Table 1
Chemical structures of synthesized and tested hexadecanoic acid ester derivatives.

	Reactant 1	Reactant 2	Reactant 3	Product
1.	1.85 g (19.6 mmol) 	1 ml (3.27 mmol) 	1 ml (3.27 mmol) 	
2.	0.68 ml (6.55 mmol) 	0.5 ml (3.59 mmol) 	1 ml (3.27 mmol) 	
3.	0.7 ml (10.55 mmol) 	0.5 ml (3.59 mmol) 	1 ml (3.27 mmol) 	
4.	0.68 ml (6.55 mmol) 	0.5 ml (3.59 mmol) 	1 ml (3.27 mmol) 	
5.	1.13 g (6.55 mmol) 	0.5 ml (3.59 mmol) 	1 ml (3.27 mmol) 	
6.	1.5 g (6.19 mmol) 	0.9 ml (6.46 mmol) 	1.44 ml (12.37 mmol)