Effect of plant leaf extracts on fecundity in *Callosobruchus chinensis* L (Coleoptera: Chrysomelidae: Bruchinae)

Bindhu V.R., Ganga, S. and Susha Dayanandan

ABSTRACT

Medicinally important plants such as *Centella asiatica, Boerhavia diffusa ,Bacopa moneri* and *Trichosanthes cucumarina* were tested against the pulse beetle, *Callosobruchus chinensis.* Certain compounds in the plants extracts were found to be effective in reducing the egg laying capacity of the beetle. Thus the plants are proved to be effective as insecticides in controlling the pest.

Keywords: *Callosobruchus chinensis, Centella asiatica, Boerhavia diffusa, Bacopa moneri Trichosanthes cucumarina*, insecticidal compounds.

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INTRODUCTION

Insects are a highly specialized group of invertebrates belonging to the largest of animal phyla, the Arthropoda. Stored products are attacked by many species of beetle pests and insect contamination is an important problem for food industries. Stored products of agricultural and animal origin are attacked by more than 600 species of beetle pests, 70 species of moths, and about 355 species of mites causing quantitative and qualitative losses and insect contamination in food commodities is an important quality control problem of concern for food industries (Nakakita, 1998).

The pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) is one of the most important insect pests of stored legumes. It comes under the order of coleoptera and family of bruchidae. Female beetle lays eggs on the seeds. Hatching larvae bore inside and spend their life within the seed. Thus the pulses become completely hollow and unsuitable for human use. Many factors affect the number of eggs laid by the female insects (use the word fecundity). Number of host seeds available to each female (Credland and Dick 1987), characteristics of host seeds such as roughness of seed coat (Nwanze and Horber, 1976), seed size and shape (Nwanze *et al.,* 1975), temperature, humidity (Giga and Smith, 1983) and density of adult beetles (Bellows, 1982), all contribute to the variation in oviposition rate.

Many plant compounds, the majority of which are alkaloids and terpenoids, have now been known to affect insects' behaviour, growth and development, reproduction, and survival. (Warthen *et al.,* 1990) Various plant powders and their extracts have been reported to possess insecticidal, oviposition deterrent and ovicidal activity against bruchids and some other insects (Nyamador, *et al.,* 2010).

Petroleum ether extract of neem (Ranjana Saxena and Beenam Saxena 2000), dichloromethane and methanol extract of *Acorus calamus* and *Cassia siamia* (Jayakumar *et al.,* 2005), powdered leaves and extracts of *Vitex negundo* (Rahman and Talukder, 2006) and powder of *Terminalia chebula* and *Cassia auriculata* (Govindan and Jeyarajan Nelson, 2008) were reported to have significant oviposition deterrent and other biological activity against *C. maculatus.*

The present study focuses on the effect of aqueous, ethanol and acetone leaf extracts of

plants, *Boerrhavia diffusa, Bacopa monnieri, Centella asiatica* and *Trichosanthes cucumerina* on the fecundity of adult females of *Callosobruchus chinensis.*

MATERIALS AND METHODS

Test insects

Experiments were conducted in the Entomology Research Laboratory, Department of Zoology, University College, Thiruvananthapuram. The pulse beetle, *C. chinensis* L. adults were obtained from naturally infested green gram seeds from local markets. The adult male and female beetles were reared on clean and un-infested green gram (*Vigna radiata* L).The seeds were made pesticide free by washing with clean water. Newly emerged adults were used for the study.

Preparation of aqueous extract

A 20 gm of powdered leaves of the plants were weighed and dissolved in 100 mL distilled water and kept for 24 hrs. After 24 hrs the mixture was heated at low heat for 2-3 hrs continuously in a hot plate .While boiling the solution was mixed thoroughly with a glass rod at regular intervals to prevent overflow. After boiling the mixture was filtered through Whatman No. 1 filter paper. The supernatant was collected and centrifuged for 10 minutes at 2000 rpm. The concentration of prepared extract was considered as 100%. Finally it was stored in air tight glass containers under refrigeration.

Preparation of ethanol and acetone extracts

Ethanol and Acetone extracts were prepared using the soxhlet apparatus.

Treatment with aqueous, ethanol and acetone extracts.

Treatment was done by residual film method. No.1 Whatman filter papers were cut in round shape and placed in the plastic containers. Aqueous, ethanol and acetone extracts of the four plants were applied separately in different doses (i.e 0.5%, 1.5%, 2.5% and 3.5%) respectively to these filter papers using a micropipette and allowed to dry so that the solvent may evaporate completely. Then the feed (20 gm) was weighed out and placed in the containers and five pairs of newly emerged adult male and female beetles were then transferred to the containers.

For each treatment suitable control was set up without applying plant extract. Six replicates were kept for each treatment and its control. After five days, eggs laid by female beetles on the seeds were counted and recorded.

The structure of ovary of adult females was also studied after treating with aqueous, ethanol and acetone extracts of the plant *C. asiatica* by histological studies. Cross sections of the ovary of adult female *C. chinensis* were taken using an ultra microtome. Sections of 2.0 µm thickness were stained with haematoxylin and eosin using standard staining protocol. The histological analyses were performed on images of the ovaries, which were obtained under a photonic microscope (Leica) coupled to the image capture system (Samsung SHC 410 NAD digital color camera), using objective lenses of 10, 20, 40 and 100X.

Statistical analysis of data

The data obtained were recorded as mean± standard deviation. For testing the significance

of the data obtained, statistical analysis were carried out using ANOVA ($p \leq 0.05$) using SPSS software (Daniel 2006).

RESULTS

Effect of plant extracts on the number of eggs laid

The number of eggs laid in control sets was comparatively more than that in treated sets.

Numbers of eggs laid were significantly less in the case of ethanol and acetone extracts treated sets. Number of eggs laid in control and treated sets are shown in the Table (1).

Effect of plant extracts on ovaries of adult *Callosobruchus chinensis*

Female reproductive system of adult has a pair of ovaries. Each ovary is made up of four ovarioles. Ovariole is formed of germarium

and vitellarium. The germarium is spherical in shape and surrounded by an epithelial layer of very flat cells. It also contains many nurse cells and one oocyte. Vitellarium is also spherical in shape. Its nucleus is centrally located. Vitellogenesis and oocyte maturation occurs in the vitellarium.

The structure of ovaries in control insects and aqueous, ethanol and acetone extracts treated insects in sections were not found to be significantly different. No noticeable morphological difference is seen in the structure of ovaries of control and treated insects Figure 1.

Table 1. Number of eggs laid in control and treated insects result response to the stress provoked by the extract (Dimetry *et al.*,1993)

Extracts	Dose (%)	Plants							
		Centella asiatica		*Boerhavia diffusa*		*Bacopa moneri*		*Trichosanthes cucumarina*	
		control	Test	Control	Test	Control	Test	Control	Test
Aqueous	0.5	32±0.03	17±0.02	30±0.02	19±0.03	30±0.03	21±0.00	30±0.03	21±0.03
	1.5	32±0.03	17±0.03	28±0.03	19±0.02	30±0.02	20±0.03	29±0.03	22±0.01
	2.5	30±0.02	15±0.03	29±0.03	18±0.01	29±0.01	19±0.03	28±0.02	22±0.02
	3.5	30±0.02	15±0.03	28±0.03	17±0.02	27±0.03	19±0.01	28±0.00	23±0.01
Ethanol	0.5	26±0.03	14±0.02	26±0.01	15±0.03	25±0.01	16±0.01	27±0.03	18±0.03
	1.5	26±0.03	13±0.03	25±0.03	15±0.00	25±0.03	17±0.03	25±0.03	18±0.00
	2.5	25±0.02	13±0.03	26±0.01	16±0.01	25±0.03	17±0.03	25±0.03	19±0.03
	3.5	25±0.01	13±0.02	25±0.03	16±0.03	26±0.03	17±0.00	25±0.03	19±0.01
acetone	0.5	25±0.03	14±0.01	26±0.00	15±0.01	24±0.02	16±0.03	25±0.02	19±0.03
	1.5	25±0.02	14±0.03	25±0.01	15±0.00	25±0.01	17±0.02	24±0.01	19±0.02
	2.5	24±0.00	13±0.00	24±0.02	16±0.00	24±0.01	16±0.04	25±0.04	18±0.01
	3.5	26±0.03	14±0.03	25±0.03	16±0.03	25±0.00	16±0.03	24±0.03	19±0.00

DISCUSSION

In this study the number of eggs laid decreased on treatment with the plant extracts. However, no distinct morphological change in ovarian structure was noticed, though the number of laid decreased on treatment with extracts. Pandey and Khan (1998) reported that on treatment with *Lentis esculentes* the decline in oviposition was attributed to the interference of plant extract with vitellogenesis and also to the damage caused to the egg chambers in the ovaries of *C. chinensis*. Oviposition reduction may also be based on the extract action on the insect nutrition. When incapable of feeding, the number of eggs would be reduced as Dwivedi and Maheshwari (1997) screened extracts of ten plants prepared in two different solvents and reported that *Croton* bonplandianum (acetone), *Verbisinia enceliodes* (petroleum ether) and *Cassia occidentalis* (in both solvents) exhibited better oviposition deterrent properties against *C. chinensis*. It is determined that the extract of *T. vulgare* inhibited the development of *Dermanyssus gallinae* (Mesostigmata: Dermanyssidae). In addition, the same plant extract showed that it is effective on *T. urticae* (Chiasson *et al.,* 2001). The extract of garlic leaves caused high mortality and reduced reproductive capacity on *T. urticae.* (Attia *et al.,* 2011). It was determined that the extracts of yew showed a high mortality, decrease in female fecundity and shortened longevity (Furmanowa *et al.,* 2001) Pervin

Erdogen *et al.* (2012) concluded that the extract of *V. album* and *T. parthenium* caused high rate mortality and reduced fecundity for *T. urticae.* Olaifa and Erhun (1998) found that higher concentration of the powder of *Piper guineense* significantly reduced the oviposition. Mathur *et.al.* (1985) observed a reduction in oviposition of *C. chinensis* by using neem kernel powder. He also reported similar effect of black pepper on C. chinensis. High anti-ovipositional activity of neem oil has also been shown in *C. maculatus* by Naik and Dumbre (1984). Ivbijaro (1990) reported reduced oviposition after neem seed treatment at 2.0 and 3.0mL/kg concentration on cow pea seeds. The significant reduction in oviposition of *C. maculatus* by Eucalyptus leaf extract has also been reported by Gehlot and Singhvi (2006). Tripathi *et al.* (2002) who observed reduced oviposition of *Tribolium castaneum* by Curcuma longa. Mulatu and Gebremedhin (2000) found significant effect on the egg viability *C. chinensis* when they were released on seed treated with plant extracts at different doses. Pandey *et al.* (1986) reported that a petroleum ether extract of neem leaves and twigs mixed with green gram seeds inhibited the oviposition of *C. chinensis* (Figs. 1, 2). In this study all tested plant extracts caused fecundity reduction possibly due to significant oviposition deterrent properties of the plant extracts against *Callosobruchus chinensis.*

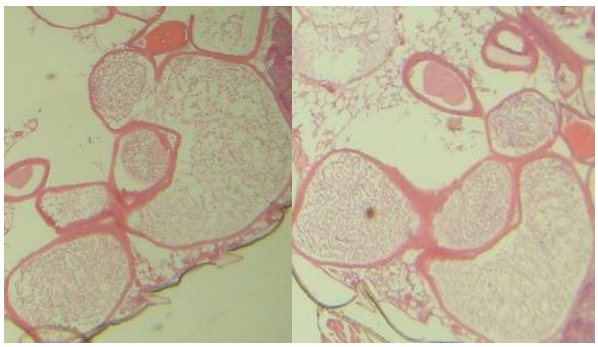

Fig. 1. Controle (left) and aqouas (right) extraxct trated *Callosobruchus chinensis*

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