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Reproductive Study of *Callosobruchus Chinensis* L (Coleoptera: Chrysomelidae: Bruchinae) on Treating with Medicinal Plants

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Abstract: ***Medicinally important plants Centella asiatica and Boerhavia diffusa were tested against the pulse beetle. Certain insecticidal 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 in controlling the pest.***

Keywords: *Callosobruchus chinensis, Centella asiatica, Boerhavia diffusa* ,insecticidal compounds, ovary, fecundity

1. Introduction

Many factors affect the number of eggs laid by the female insects. 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).

Petrolium ether extrat 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, *Centella asiatica* and *Boerrhavia diffusa* on the fecundity of adult females of *Callosobruchus chinensis.*

2. Materials and methods

Test insects
Experiments were conducted in the Entomology Research Laboratory, Department of Zoology, University College Thiruvananthapuram. The pulse beetle, *Callosobruchus 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
20 gm of powdered leaves of the plants were weighed and dissolved in 100 ml distilled water and kept for 24 hours. After 24 hours the mixture was heated at low heat for 2-3hours 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 What man 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 What man filter paper 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 control was also 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 *Centella asiatica* by histological studies. Cross sections of the ovary of adult female *Callosobruchus 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.

3. Results

Effect of plant extracts on the number of eggs laid
The number of eggs laid in control sets were comparatively more compared to treated sets. Number 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 1,2,3 and 4

4. 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).

Table 1: Number of eggs laid in control and treated insects

Extracts	Dose (%)	plants			
		Centella asiatica		*Boerhavia diffusa*	
		control	test	control	test
Aqueous	0.5	32±0.03	17±0.02	30±0.02	19±0.03
	1.5	32±0.03	17±0.03	28±0.03	19±0.02
	2.5	30±0.02	15±0.03	29±0.03	18±0.01
	3.5	30±0.02	15±0.03	28±0.03	17±0.02
Ethanol	0.5	26±0.03	14±0.02	26±0.01	15±0.03
	1.5	26±0.03	13±0.03	25±0.03	15±0.00
	2.5	25±0.02	13±0.03	26±0.01	16±0.01
	3.5	25±0.01	13±0.02	25±0.03	16±0.03
acetone	0.5	25±0.03	14±0.01	26±0.00	15±0.01
	1.5	25±0.02	14±0.03	25±0.01	15±0.00
	2.5	24±0.00	13±0.00	24±0.02	16±0.00
	3.5	26±0.03	14±0.03	25±0.03	16±0.03

Values are mean ±SE; all values are significant at $p \leq 0.05$ level of significance

5. Discussion

In this study the number of eggs laid decreased on treatment with extracts.However in this study no distinct morphological change in ovarian structure was noticed,though the number of eggs 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 be also based on the extract action on the insect nutrition.When incapable of feeding,the number of eggs would be reduced as a response to the stress provoked by the extract. .(Dimetry et al,1993).

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.

In this study all the plant extracts caused fecundity reduction possibly due to significant oviposition deterrent properties of the plant extracts against *Callosobruchus chinensis*.

C.S. of ovariole

C.S of ovariole stained with eosin and haematoxylin

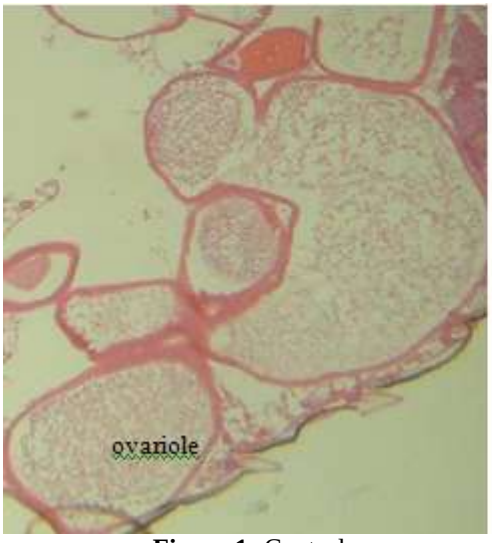

Figure 1: Control

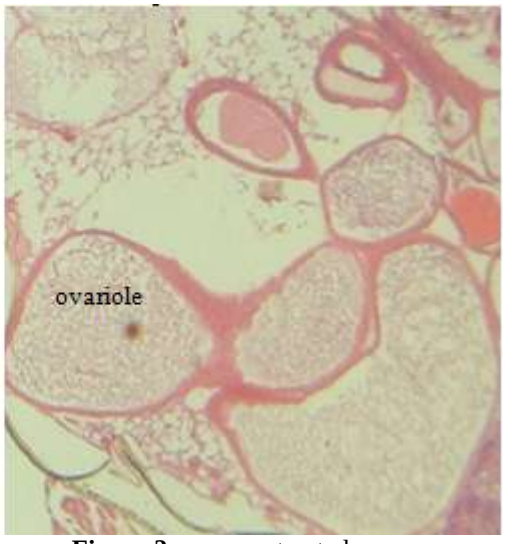

Figure 2: aqueous treated

Figure 3: Ethanol treated

Figure 4: Acetone treated

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