

**MORPHOLOGICAL ABNORMALITIES INDUCED BY PLANT ESSENTIAL OILS ON  
THE DEVELOPMENT OF CALLOSBRUCHUS CHINENSIS****\*M. Madhavi**

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Article Received on 27/07/2017

Article Revised on 17/08/2017

Article Accepted on 07/09/2017

**ABSTRACT**

Vitex negundo and Allium sativum oil causes a sharp reduction in the morphogenesis and ovarian development of Callosobruchus chinensis. When freshly moulted IV and V instar larvae of Callosobruchus chinensis were treated with different concentrations of Vitex negundo and Allium sativum oil affected the mortality, moulting and metamorphosis variedly. These oils were found to suppress the population of pests at higher dosages, while lower dosages were found to induce several developmental defects. Thus, it is evident that the influence of oils on Callosobruchus chinensis resulted in, varied morphogenetic and ovarian deformities ruling out the possibility of further propagation of the stored pest.

**KEYWORDS:** Vitex negundo oil, Allium sativum oil, Callosobruchus chinensis, larvae, stored pest.**INTRODUCTION**

In the recent past, the preservation of cereals, pulses and other food commodities has relied heavily upon the insecticides to control the storage pests. But the increasing problems of resistance and residues of pesticides and contamination of biosphere have led the need for safer and eco friendly biodegradable pesticides. The present trend is towards the use of alternative environmental friendly and non-toxic control methods that pose no threat to the health of operator or consumer. It is demanding to develop the alternative methods that are economically feasible and ecologically safer to control the storage grain insects. The use of botanical pesticides is considered as one of the alternative substitute to hazardous chemicals. Among the botanicals, Vitex negundo is visualized as an eco-friendly pesticide having rich source of bioactive chemicals with a greater potential for use as successful pest control agent which can affect insects in several ways: they may disrupt major metabolic pathways and cause rapid death, act as attractants, deterrents, phago-stimulants, antifeedants or ovipositional deterrents, also retard or accelerate development or interfere with the life cycle of the insects.

Garlic is visualized as an eco-friendly pesticide. Even today, the medicinal use of garlic remains popular all over the world (Namaz 2008) through careful research, detailed information on the chemical compositions of garlic essential oil is now very clear: it is mainly dominated by sulphides (Kimbaris et al., 2009; Wu et al., 2004). Its strong insecticidal activity has likewise been

demonstrated (Dugravot et al., 2002; Park and Shin, 2005; Kimbaris et al., 2009). A number of studies showed that garlic essential oil and its two major components, methyl allyl disulfide and diallyl trisulfide, studied the effectiveness of garlic essential oil as grain protectants.

**MATERIALS AND METHODS**

A rich standard culture of this insect was maintained in the laboratory on normal dietary medium composed of coarsely ground green gram inside a glass container at 25°C temperature and 70% Relative humidity. Freshly harvested, insect free variety was used for experimental purpose. Allium sativum oil and Vitex negundo oil was used for experimentation. The Garlic oil and Vitex negundo oil is diluted with acetone to obtain the required doses 1, 2, 3, 4, 5% concentration for the evaluation. Freshly moulted IV and V instar larvae were treated on the abdominal region with 1µL/larva of Garlic oil and Vitex negundo oil dissolved in 2µl of acetone respectively each with the help of Hamilton micro syringe. 50 larvae were treated each time and the experiments were replicated 5 times. Controls were treated with 2µl of acetone. After treatments a suitable time gap of 5 minutes was given and they were transferred into diet. The treated larvae were observed daily to note the changes, and the resulting abnormal intermediates were collected from the diet media. The mortality rate of the larvae, moulting duration in the larvae and the reproductive changes in the female moths were recorded.

## RESULTS

### Influence of *Vitex negundo* oil and *Allium sativum* oil, on Morphogenesis and ovarian development.

Experiments were carried out to assess the possible changes in the morphology of the *Callosobruchus chinensis* with application of *Vitex negundo* oil and *Allium sativum* oil on the IV and V instar larvae of *Callosobruchus chinensis*. The effects induced by the bulb extract were evaluated in the resultant intermediate forms and in the abnormal larval, pupal and adult stages.

#### Effect of *Allium sativum* oil.

##### On the IV instar larva.

22% of the treated resultant IV larvae shrunk in size, became darker with anterior region melanization and finally terminated the life cycle. 24% of the treated IV instars larvae developed into larval pupal intermediates. In these intermediates, the retention of the head capsule was observed (Fig. 1, 2).

##### On the V instar

14% of the treated resultant V larvae developed brown patches and they became black and died. Malformation of pupa resulted in the death or delayed mortality due to the incomplete emergence of adults from the pupal cuticle and the larvae that completed the moult were not viable. 10% of the resultant adults were with abnormally large wings, malformed wings, shrivelled wings, short and stumpy wings. 7% of treated V instar larvae metamorphosed into malformed adults with short wings unable to expand. (Fig.3&4.)

### Influence of *Allium sativum* oil on the development of ovaries of *Callosobruchus chinensis*

The female reproductive system of *Callosobruchus chinensis* has two distinct ovaries, each with six ovarioles. each telotrophic ovariole is clearly demarcated into germarium and vitellarium, the terminal oocytes

were abnormally large and unable to ovulate due to dislodging of their linear position. The abnormal terminal large oocytes block the ovulation and deformities such as reduction in the size, number of ovarioles, varied in length of the ovarioles and loop formation of the ovarioles. In some, the ovaries were reduced to thin and filamentous ovarioles. The resultant abnormal adults appeared with ovarian abnormalities. The ovarioles developed with large sized oocytes and in some ovarioles with large oocytes unovulated. (Fig.5, 6).

### Effect of *Vitex negundo* oil on the IV instar larvae of *Callosobruchus chinensis*.

IV instar larvae on treatment has deformed mouth parts. 6% of the IV instar larvae slowly deteriorated and died after shrinkage. 18% of the IV instar treated larvae were unable to moult into the next instar and remained as "overaged" larvae for an extended period of time without moulting. These "over-aged" larvae were called as "Permanent larvae" in which stage they continue for several days and eventually died. 20% of the IV instar treated larvae developed as larval-pupal intermediate with exuvium attached (Fig.7).

### V instar larvae of *Callosobruchus chinensis*

Neem oil interfered with metamorphosis resulting in abnormal pupae. 16% of the treated V instar resultants could not free themselves from the pupal case and 9% of the V instar treated resultants metamorphosed into larval pupal intermediates with larval prolegs and pupal wing pads. 5% of the V instar treated resultants emerged as soft bodied bloated larva. 10% of the V instar treated larvae emerged as adults with large crumpled wings. 18% of the V instar treated larvae developed into adults with protruded ovipositor, the disturbance of the linear arrangement of the ovariole and ovaries with filamentous ovarioles (Fig.8).



Fig.1



Fig.2



Fig.3



Fig.5

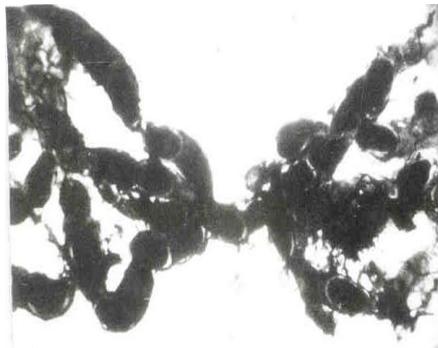


Fig.6

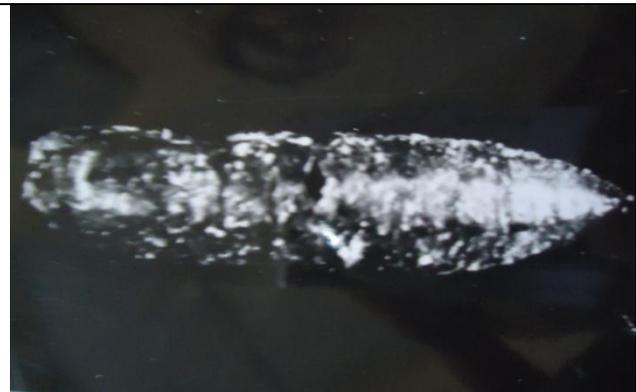


Fig.4



Fig.7



Fig.8

## DISCUSSION

The results of this study clearly demonstrate that the *Vitex negundo* oil and *Allium sativum* oil disrupted the development of the stored grain pest *Callosobruchus chinensis*. On application of oils to the IV and V instars, exhibited derangement of growth and development. Few of the IV instars died during moulting. The interference with pupal ecdysis, inhibition of egg maturation and induction of permanent larvae (Srivatsava.,1988). The results of the experiments carried out on the larvae of

*Callosobruchus chinensis*; suggest that the morphogenetic changes observed are brought about by ecdysteroid deficiency (Sieber and Rembold, 1983, Garcia and Rembold, 1984). The nonfeeding character of the resultant shrunken larvae of *Callosobruchus chinensis* is due to the displacement of mouthparts. It may also be due to the blockage of the gut. The action of *Vitex* oil on larvae suggests an effect on endocrine mechanism that regulates moulting and metamorphosis and on weight gain without being directly toxic to the

organism (Premaleela and Muraleedharan, 1995, Zhang, X. and Chin. 1987).

The larvae treated with *Vitex negundo* oil and *Allium sativum* oil in larval pupal intermediates, contracted pupae, malformed pupae and adults with varying degrees of wing deformities. They could not escape from the pupal case which resulted in potentially severe inhibition of flight. In certain cases the adult ecdysis was completed but the adult could not survive. These results are in conformity with Bollenbacher and Gilbert (1981). Deena (1997), Shyamala Pillay (2000), Renuka (2004), Sandhya (2009).

Ovarian ecdysteroid synthesis is probably under direct control of the brain hormone. These oils most probably reduced the total amount and concentration of edysteroids relative to untreated individuals by acting on the neuroendocrine system, which may also explain the decrease in the body weight and inhibition of oviposition. (Premaleela and Murleedharan, 1995, Zheng et al 2009) in red cotton bug. These findings support the pesticidal properties of oils in the present study against *Callosobruchus chinensis*.

Hence, the *Vitex negundo* oil and *Allium sativum* oil induces premature metamorphosis, which might be more appropriate for use as a pesticide against the stored grain pest *Callosobruchus chinensis* in which damage is mainly caused by the larval stages. It would widen new scopes in pest management as it will be cheaper, easily available and non-toxic to humans. Thus, it is evident that the oils influence the endocrine mechanism of *Callosobruchus chinensis*, which resulted in, varied morphogenetic and ovarian deformities ruling out the possibility of further propagation of the stored pest.

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