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Research Paper

Influence of phytochemical cleistanthin-c on morphogenetic development of Pulse pest *callosobruchus chinensis*.

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ABSTRACT: *Phytochemicals are natural plant products and most of them are used in pest control. Cleistanthin-C, a Phytochemical isolated from the leaf of heartwood Cleistanthus collinus exhibit the insect growth regulating activity in the last instar larvae and pupae of Callosobruchus chinensis. The larvae and pupae were treated with 1µg/ µl concentrations of Cleistanthin-C in solvent acetone. The important morphogenetic deformities such as formation of malformed adults and interference in ecdysis and the development of larval-pupal intermediates, pupal-adult intermediates were observed in treated resultants. The ovarian deformities were also observed in the treated resultant adult females. The resultant forms were unable to proceed for the further development and reproduction.*

Keywords:- *Phytochemical, Cleistanthin-C, Callosobruchus chinensis.*

I. INTRODUCTION

Higher plants are a rich source of novel natural substances that can be used for insect control (Arnason *et al.*, 1989). Insecticidal activity of many plants against several insect pests has been demonstrated (Jilani and Su, 1983; Carlini and Grossi-de-Sá, 2002). The deleterious effects of plant extracts or pure compounds on insects can be manifested in several manners including toxicity, mortality, antifeedant growth inhibitor, suppression of reproductive behaviour and reduction of fecundity and fertility.

Since the dawn of human history, the farmers tried to protect their harvest produce against arthropod pests. In many areas of the world, locally available plants are currently in wide use to protect stored products against damage caused by insect infestation (Hassanali and Lwande, 1989). Owusu (2001) suggested the natural and cheaper methods for the control of stored-product pests of cereals, with traditionally useful Ghanaian plant materials. More than 150 species of forest and roadside trees in India produce oilseeds, which have been mainly used for illumination, medicinal purposes and as insecticides from ancient times to early 20th century (Mariappan *et al*., 1988). The plant extracts, powders and essential oil from different bioactive plants were reported as repellent against different economically important stored product insects (Boeke *et al.,* 2004; Talukder *et al.,* 2004).

The beetles of the family Bruchidae are closely associated with the plant family Leguminosae , and many species are important primary insect pests of stored legumes . Grain Legumes are often the main source of protein for people in developing countries , but suffer heavy quantitave and qualitative losses from the attack of of *Callosobruchus* species during storage and harvest

Worldwide reports on the toxicity of different plant derivates showed that many plant products are toxic to stored-product insects (Tripathi *et al*., 2000; Islam and Talukder, 2005). *Callasobruchus chinensis* is considered as a major pest of stored grains. Hence the present study is an attempt made to evaluate the pesticidal activity of the extracts from the leaf of *Cleistanthus collinus* (Heart wood) against larvae and adults of *Callasobruchus chinensis*.

II. MATERIALS AND METHODS

2.1 The Pest and Culture:

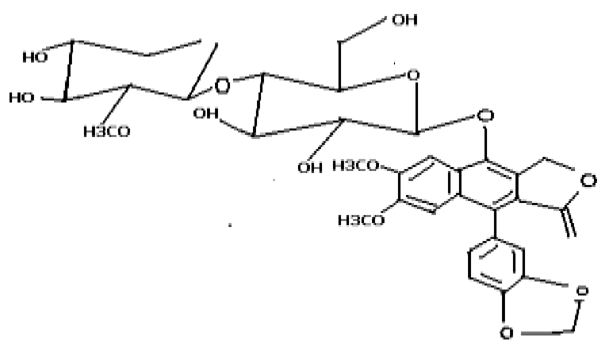
2.1. A.Callasobruchus chinensis: *Callosobruchus chinensis* life cycle takes 36 days at 25°±2C and 70% ±5 RH. The period of life cycle may vary according to the change of temperature, Relative humidity.

Females lay about 75-90 eggs. The eggs are doomed structures with oval, flat bases. Eggs hatch within 5-6 days. The life cycle of Callosobruchus *chinensis* consists of Five larval instars. Larval stages are the main feeding stages. During the pupal period(5 – 6 days) the tissues are broken down and changed for preparation of adult. Adults are short lived. Infestation commonly begins in the field, where eggs are laid on maturing pods. As the pods dry, the pest's ability to infest them decreases. Thus, dry seeds stored in their pods are quite resistant to attack, whereas the thrashed seeds are susceptible to attack throughout storage.

2.1.b.Insect Cultures : The pulse pest *Callasobruchus chinensis* were maintained under identical laboratory conditions of temperature 27 ± 1°C and 65 ± 5% RH and fed on red gram (*Cajanus cajan*).

2.2. Phytochemical Cleistanthin – C : *Cleistanthus collinus* Linn. (Family : Euphorbiaceae), is a small deciduous tree found in Deccan peninsula thrives well on dry rocky ground. Cleistanthin-C is extracted from the heartwood tree by the research students of the Department of Chemistry, (Natural Chemistry Lab), Osmania University, Hyderabad in the following manner :The heartwood powder was extracted with petroleum ether (8 litres X 3 times) in a soxhlet extraction. The pet ether, on concentration yielded a brown semi-solid , which was not worked out further. The defatted plant material was now extracted in soxhlet with CHCl3 , on concentration yielded a brown gum (20g). The column was then eluted ethyl acetate. Ethyl acetate fractions 91-150 on concentration yielded colorless solid which was identified as Cleistanthin- C.

Chemical structure of cleistanthin-c



Chemical Formula of Cleistanthin – C : $\mathbf{C_{34}H_{38}O_{16}}$

2.3. Treatment with Cleistanthin–C: Freshly ecdysed fifth instar larvae and pupae were treated topically on the abdominal region with 1 μg/ μl Cleistanthin - C dissolved in the carrier solvent acetone. Fifty larvae and fifty pupae were treated separately each time and the experiments were replicated five times. Controls were treated with an equivalent volume of carrier solvent acetone. After total absorption of the test compound the larvae were transferred into the diet and were observed daily to note the changes.

III. RESULTS

The optimum effects of the Cleistanthin-C were observed on the Vth instar larvae and the zero hour pupae. Treatment of 1 μg / μl of Cleistanthin-C induced varied morphological and physiological deformities.

3.1. Morphological Deformities in Treated Resultants:

The topical application of 1 μg / μl Cleistanthin-C on the larvae of *Callasobruchus chinensis* influenced the molting, metamorphosis and mortality.

3.1.a. Effect of cleistanthin–c on the v instar grubs: The topical application of 1 μg / μl of Cleistanthin-C on the grubs of Callasobruchus chinensis influenced the development, molting and metamorphosis. 34 % of the treated larvae died during moulting. The insects tried hard to cast their exuviae but they succeeded only partially. The exuviae began to detatch from the body of the insect, but could not be ruptured. In some cases the exuvium ruptured above the head and hence the insect could not extricate completely.

26% of the treated V instar grubs remained as over-aged larvae for an extended period of time they are also known as "permanent larvae". They live upto several days and eventually die. (Plate2 A and B). 30 % of the treated resultant V instar larvae metamorphosed into malformed pupae with malformed wing pads and abnormal constricted body in anterior and posterior regions. . (Plate2 C).10% of the treated resultant larvae developed into morphologically normal adults. But the fecundity of these adults was at reduced rate when compared with the normal control adults.

3.1. B. Effect of Cleistanthin–C on the Pupae: Fifty pupae were treated with 1 μg / μl of Cleistanthin - C. The resultants exhibited the following results: 30% of the treated pupae did not develop into adults. These were unable to extricate from the pupal case. They died with their exuviae still attached to the body.30% of the treated pupae transformed into "pupal-adult intermediates". These were characterized by incomplete shedding of the pupal case and incomplete liberation of the mouth parts and appendages. They move for limited extent and these forms lived for several days and died. . (Plate2 D).

20% of the treated pupae metamorphosed into abnormal adults. These were characterized by incomplete development of wings and legs, wings partially attached with the abdomen and legs and mouth parts were rigid. Some of them showed deformed appendages with malformed wings. Some of the abnormal adults had wings entangled with the exuvium . (Plate2 Eand F). 8% of the adult mortality was seen during adult emergence. 12% of the treated pupae metamorphosed into normal adults. The fecundity of these adults was reduced when compared with the control adults.

3.2. Ovarian Deformities In Treated Resultants:

The development of the ovary in insects depends upon the deposition of yolk i.e., vitellogenesis. Vitellogenin is a name given to a unique group of proteins that are synthesized extraovarially, specifically by the fat body,released and transported through the haemolymph and deposited in the oocytes as vitellins, the major yolk proteins(Hagedorn 1974).The vitellogenins have proved to be exceptionally useful for studying the control of reproduction in insects Vitellogenesis in Callasobruchus chinensis was influenced by Cleistanthin–C In control C. chinensis there are two distinct ovaries, each with six ovarioles. Each telotrophic ovariole is clearly demarcated into germarium and vitellarium. (Plate -1D).

The ovaries, of treated resultant adults which could not extricate from the pupal case, were small and reduced in number of ovarioles. The ovarioles remained underdeveloped and one or two chorionated oocytes blocks the common oviduct and some of the terminal oocytes were abnormally large and blocks the oviduct.. (Plate -3 A and B). In abnormal adults, the ovaries were in advanced stages of degeneration. The yolk was reabsorbed and the oocytes lost their shape and these were varied in sizes. In some ovarioles the dislodgement of linear arrangement of oocytes were observed. . Small and deformed ovarioles and a large chorionated oocyte blocking the common oviduct in un-extricated adult. (Plate -3 C and D).

IV. DISCUSSION

The Cleistanthin-C influences the development and morphogenesis like permanent larvae, pupal-adult intermediates and malformed adults. deformities in ovaries appear like large tropical oocytes, chorionated oocytes and malformed oocytes and ovarioles. Chorionated oocytes blocks the common oviducts which influence the capacity of egg laying in the insects thereby reducing their populations.

Treatment of *Callasobruchus chinensis* with the Cleistanthin – C influenced larval growth and moulting. The treated V instar larvae of C.*chinensis* were unable to moult into pupae and continued as the "permanent larvae". Some of the treated pupae of the *Callasobruchus chinensis* with Cleistanthin – C developed as the "pupal-adult intermediates". Appendages mouth parts and wing pads were not well marked in these forms. This abnormalities might be developed due to the interference of the Cleistanthin – C in molting.

The ovaries of the treated resultant *Callasobruchus chinensis* pupal-adult intermediates and abnormal adults showed oocytes degeneration and resorption and one or two bulky deposited chorionated eggs blocks the oviducts. Similar results were observed with different phytochemicals, in Locusta migratoria Azadirachtin inhibited both oogenesis and ovarian ecdysteroid synthesis hence preventing oviposition (Rembold and Seiber, 1981). The reduced amount of proteins in the ovaries of treated females of *Callasobruchus chinensis* clearly indicates that the Cleistanthin – C caused a lower uptake of proteins by the ovary. Hence the treated resultant adults exhibited inhibition of vitellogenesis and reduced fecundity

V. CONCLUSION

Thus the present study clearly indicates that Cleistanthin-C acts as insect growth regulator and it influences the morphogenetic developments and cause to form the morphological deformities like permanent larvae, pupal-adult intermediates and mal-formed adults. Ovaries also exhibited the deformities like tropical oocytes, chorionated oocytes , malformed oocytes and ovarioles. The protein levels also variable with that of control insects. The decreased protein levels indicate insufficient protein availability at the time of morphogenesis. These plant extract inhibited the growth and development of the pulse pest *C. chinensis* and suggesting its use as a insect growth regulator in controlling pests like *C. chinensis*.

Table –1.Larval Treatment:

Dosage of Cleistanthin - C	Mortality(%)	permanent larvae(%)	Malformed pupae (LPI & PAI) (%)	Morphologocally normal adults (%)
1 µg / µl /larva	34	26	30	10

LPI: Larval pupal intermediate , PAI: Pupal adult intermediate

Table-2. Pupal Treatment:

Dosage of Cleistanthin - C	Mortality(%)	Pupal adult intermediates (%)	Abnormal adults(%)	Morphologocally normal adults (%)
1 µg / µl /pupa	38	30	20	12

Plate - 1

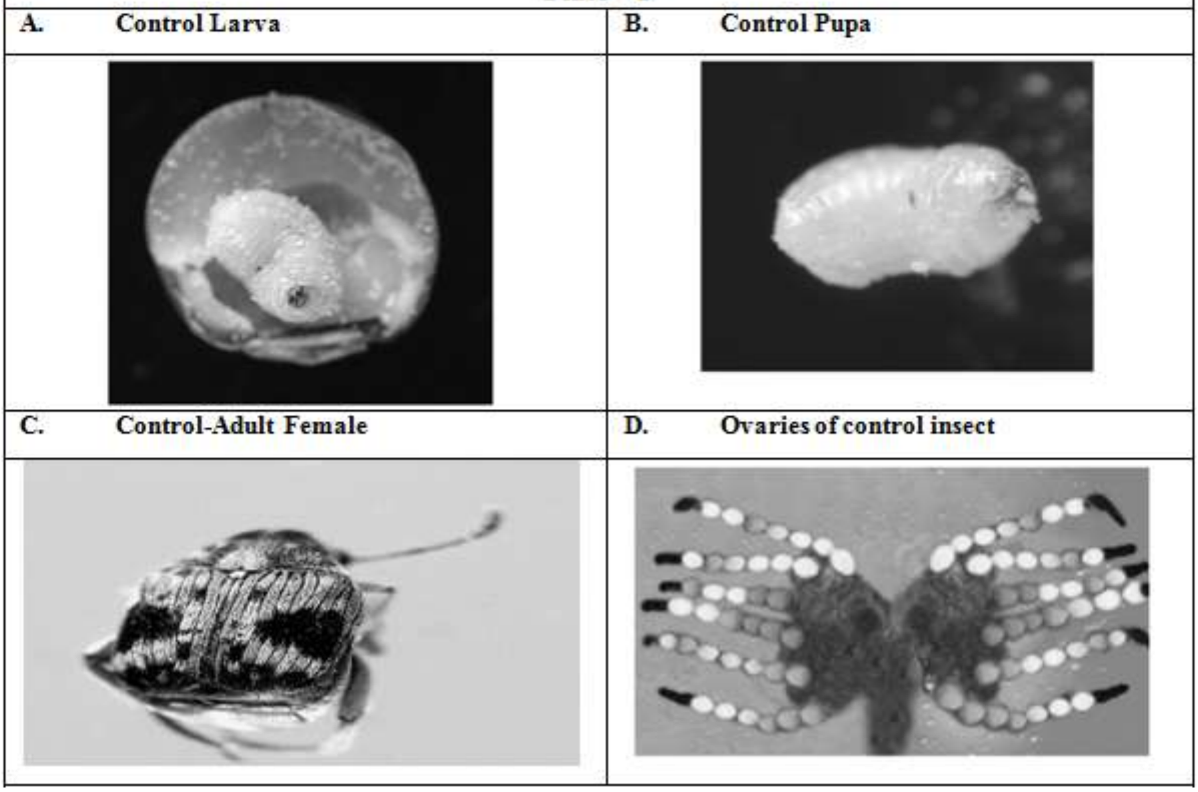


Plate – 2 Treated resultants

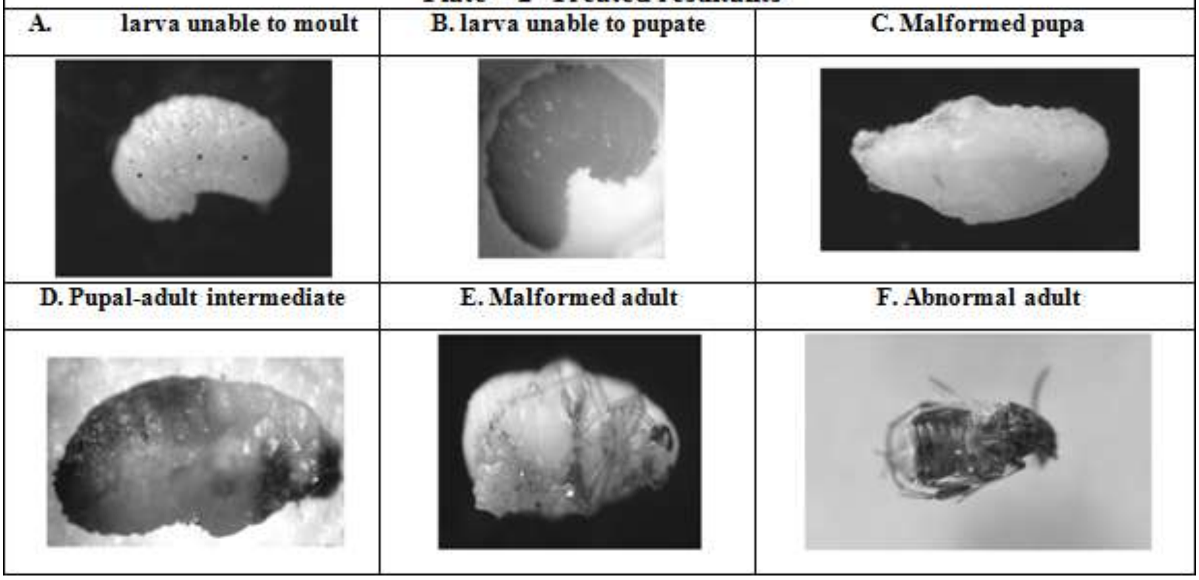
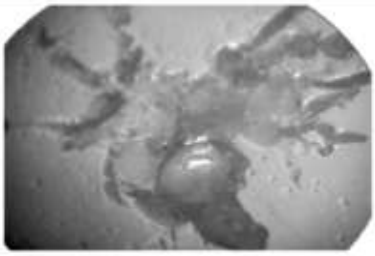


Plate –3 Ovaries of Treated resultants	
A. Terminal oocytes abnormally large	**B. Terminal oocytes abnormally large**
	
C. Dislodgement of linear arrangement of oocytes.	**D Chorionated oocyte block the common oviduct**
	

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