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Insect Growth Regulating Activity of Phytochemical (+)-O-Methylarmepavine, Against the Stored Pulse Pest Callosobruchus Chinensis

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Abstract*: Topical application of (+)-O-Methylarmepavine isolated from the leaves of Annona squamosa caused inhibition of growth and development of fifth instar larvae of red gram beetle Callosobruchus chinensis. There was production of larval-pupal intermediates and pupal-adult intermediates and adults with various ovarian abnormalities.*
Key words: *Phytochemical, (+)-O-Methylarmepavine, Stored grain pest, Insect Growth regulator*.

I. Introduction

Increased concern by consumers over insecticide residues in food products, the occurrence of insecticide resistant insect strains (Subramanyam and Hagstrum, 1995) and the precautions necessary to work with traditional chemical insecticides call for new approaches to control stored product insect pests. Management of stored product pests using materials of plant origin, is now-a days the subject which received much research attention because of their little environmental hazards and low mammalian toxicity (Isman1994). Plants are a rich source of novel natural substances that can be used to develop environmental safe methods for insect control (Jbilou et al., 2006) The responses of insects to various juvenile hormone analogues derived from plants have been studied previously by different workers (Raja et al., 1987, Cespeded et al., 2000, Vardhini et al., 2001, Jeyabalan et al., 2003, Homson et al., 2007). Hence in this communication we report Insect growth regulating effects of (+)-O-Methylarmepavine isolated from the leaves of *Annona squamosa* on the pulse pest.

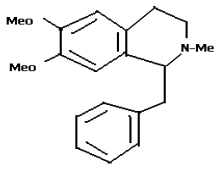
II. Materials And Methods

Pest: The red gram 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*). Freshly ecdysed fifth instar larvae were treated topically on the abdominal region with varying concentration 1 µg/ µl / larva, 2 µg / µl / larva and 4 µg / µl / larva of (+)-O-Methylarmepavine dissolved in the carrier solvent acetone. Fourty larvae were treated 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.

Plant: *Annona squamosa* L., a small evergreen tree, occurs wild and is also cultivated throughout India for its fruits. The reason for selecting the leaf extract of *Annona squamosa* is that the Foliar extracts of *Annona squamosa* (Family: *Annonaceae)* were screened for antimicrobial and insecticidal activity against the common microbial infestants of pulses and the stored grain pest pulse beetle, Callosobruchus chinensis (Coleoptera: Bruchidae). Flavonoids isolated from aqueous extracts of *A. squamosa* showed anti smicrobial activity against all the common microbial contaminants of pulses and 80% insecticidal activity against C chinensis at a concentration of 0.07 mg ml-1. Various physico-chemical tests, chromatographic and spectroscopic studies with partially purified aqueous extract indicated the presence of flavonol type flavonoids. This may provide a useful beginning for the development of botanical pesticides for post-harvest safeguard of pulses.

Isolation of (+)-O-Methylarmepavine: This Phytochemical was isolated, purified, analyzed and provided by V. Danaiah, Natural Chemistry *Lab, Department of Chemistry, Osmania University, Hyderabad. The dried leaf* powder (15 Kg) was extracted with methanol at room temperature. The methanol extract on concentration under reduced pressure gave dark green viscous mass (8.0g), which was extracted with 5% HCL, and the acidic solution defatted with pet ether, basified with 5% NaOH and extracted with Et2O . The Et2O extract was washed with water, dried and evaporated to give alkaloidal mixture. It was chromatographed over neutral alumina by eluting with CHCl3 to give a viscous liquid identified as a (+)-O-methylarmapavine and this is used for the entire experiment.

Structure of (+)-O-Methylarmepavine



Chemical formula of (+)-O-Methylarmepavine C20H25NO3

Analysis of growth and development of pest: *Callasobruchus chinensis* life cycle takes 36 days at the 25°C and 70% RH. Usually, six to seven generations occur in a year. The adult beetles, which do not feed on stored produce, are very short lived (usually not more than 12 days under optimum conditions) and during this time the females lay many eggs (85-100). Egg hatches and larva enters into the seed.The developing larvae feeds entirely within a single seed, excavating a chamber within the cotyledons as it grows. The hatching larvae bites through the base of the egg, directly through the testa and into the cotyledons. The larvae is scarabaeiform and the 5 instars (moulting 4 times) develops in about 22-24 days at 25°C and 70% RH. The whole time of the larvae are spent within a single seed. Larval stages are the main feeding stages. Pupation takes place inside the seed in a chamber covered by a thin window of testa material and requires about 5 – 6 days. Pupa metamorphoses into adult. The IGR activity of the (+)-O-Methylarmepavine studied on *Callosobruchus chinensis* which induce the biochemical changes (Table-2) in the protein content of the ovaries of insects finally leading to the ovarian deformities and abnormal offspring.

III. Results

Application of the compound (+)-O-Methylarmepavine at the concentrations of 1µg ,2µg ,4µg/ µl / larva caused to develop into larvl pupal intermediates ,pupal adult intermediates and morphogenetic aberrations which were expressed in the metamorphosed forms. Treatment with 4 µg / µl / larva resulted in prolongation of larval period from 5 days to 11 days when compared to that of the controls. 90% of the treated larvae resulted in larval pupal intermediates(Plate -2 ,Fig A,B) and pupal adult intermediates(Plate -2 ,Fig C,D). having more larval characters. Such forms were inactive and could not pupate normally.10% of the treated larvae developed in to malformed adults which could not perform and finally die. Similar results were reported by Vardini *et al* (2001) with Sujoil.

The treatment with 2 µg / µl / larva and 1 µg / µl / larva of (+)-O-Methylarmepavine, some of the treated larvae pupated normally but ovaries of these resultant adult were drastically affected. However The ovarioles of the affected ovaries showed variation in the number and the size of the oocytes. (Plate-3, Fig – B,C,D.)There were a large number of these resultant adults where in the ovarioles had matured oocytes but remained unovulated.

IV. Discussion:

Non oviposition of the matured oocytes may be due to the imbalance in the hormonal milieu caused by the application of (+)-O-Methylarmepavine. Oocyte development was inhibited by botanicals were observed in *Helicoverpa armigera* (Patel and Patel 1997). Various botanicals were tested and the growth of the larvae was hampered in *Corcyra cephalonica* (Dwivedi *et al.,* 2006) .In the present study the effects of administering (+)-O- Methylarmepavine, leaf extract of *Annona squamosa* topically have detrimental effects on the development of larvae and pupae of *Callasobruchus chinensis* which led to eclosion of abnormal adults. The treated insects could not moult normally due to which flight and reproduction were hindered. The prolongation of larval periods were observed.

V. Conclusion:

Thus the present study clearly indicates that (+)-O- Methylarmepavine acts as insect growth regulator and it influences the metamorphosis in the treated resultant larvae and pupae metamorphosed into larval-pupal intermediates, pupal-adult intermediates and mal-formed adults. Ovaries also exhibited the deformities like large oocytes, chorionated oocytes which blocked the ovarioles and reduced the fecundity. The protein levels also were variable with that of control insects. The decreased protein levels indicates the effect of (+)-O-Methylarmepavine on protein synthesis and uptake during larval pupal transformation and vitellogenesis. (+)-O-Methylarmepavine inhibited the growth and development of the stored grain pest *C.chinensis* and suggesting its use as a cheap, safe and eco-friendly pesticide.

Table-1: Effect of (+)-O-Methylarmepavine at different concentrations on the larvae of *Callasobruchus chinensis*

S.No.	Dosage µg/µl/insect	No.of insects treated	LPI and PAI	Abnormal Adults	Normal adults
1	Control acetone	40	0	0	40
2	1	40	20	14	6
3	2	40	30	9	1
4	4	40	36	4	0

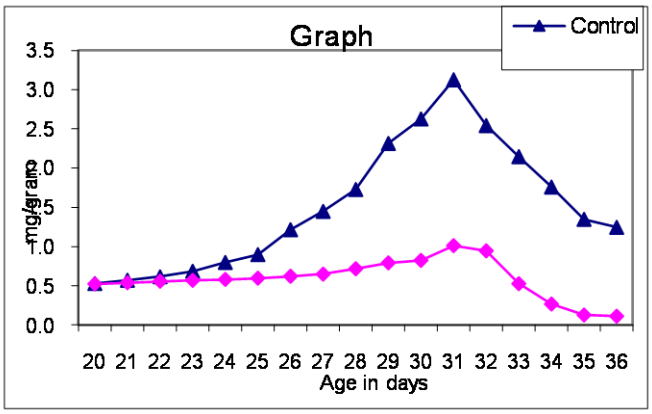
LPI:larval pupal intermediates , PAI:pupal adult intermediates

Table - 2

Protein content in the Ovaries of *Callosobruchus chinenseis* in control and treated ((+)-O-methylarmepavine) resultant insects.

Stage	Age in days	Control	Treated with (+)-O-methylarmepavine
V Instar	20 days	0.527±0.0008	0.524±0.0004 ^{NS}
	21 days	0.569±0.0006	0.538±0.0006
	22 days	0.613±0.0008	0.558±0.0008
	23 days	0.683±0.0006	0.569±0.0005
	24 days	0.796±0.0006	0.582±0.0008
Pupal period	25 days	0.896±0.0006	0.598±0.0004
	26 days	1.214±0.0004	0.622±0.0006
	27 days	1.448±0.0008	0.649±0.0004
	28 days	1.727±0.0008	0.717±0.0011
	29 days	2.315±0.0004	0.793±0.0006
	30 days	2.628±0.0008	0.825±0.0008
Adult period	31 days	3.128±0.0009	1.014±0.0008
	32 days	2.543±0.0008	0.947±0.0006
	33 days	2.147±0.0005	0.526±0.0009
	34 days	1.759±0.0008	0.266±0.0009
	35 days	1.347±0.0006	0.126±0.0008
	36 days	1.245±0.0008	0.110±0.0006

The values are expressed in mg of protein/gm.
Each value is the mean ± Standard error of Six individual observations.
The difference between control and treated is statistically significant (P>5%).
^{NS} denotes not significant (P<5%).



Graph: Quantitative changes in the protein content of the Ovaries of *Callosobruchus chinenseis* in control and with (+)-O-METHYLARMEPAVINE treated resultant insects during the different stages of lifecycle. Values expressed as protein content in mg/gram wet weight of tissue.

PLATE – 1

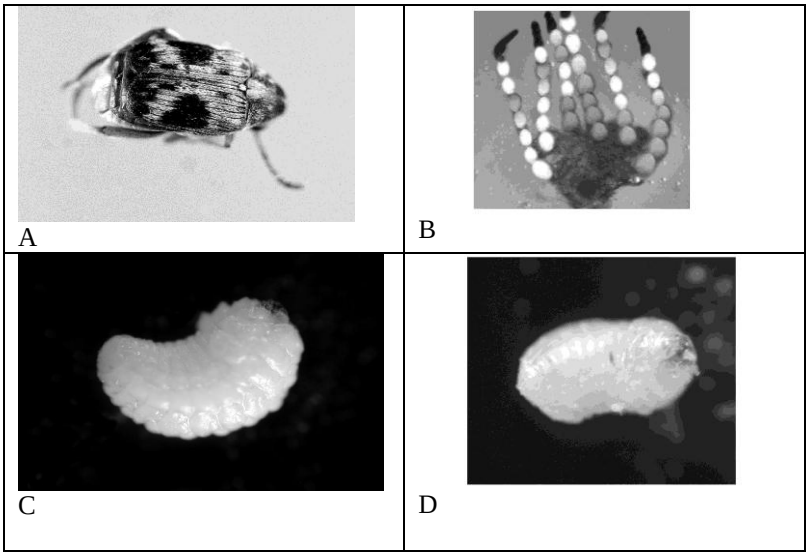


Fig : 1 A. Control Adult female
Fig : 1 B. Ovary of . Control Adult female
Fig : 1 C. 5^{th} instar larva (Control)
Fig : 1 D. Control pupa

PLATE – 2
TREATED RESULTANTS WITH DEFORMITIES

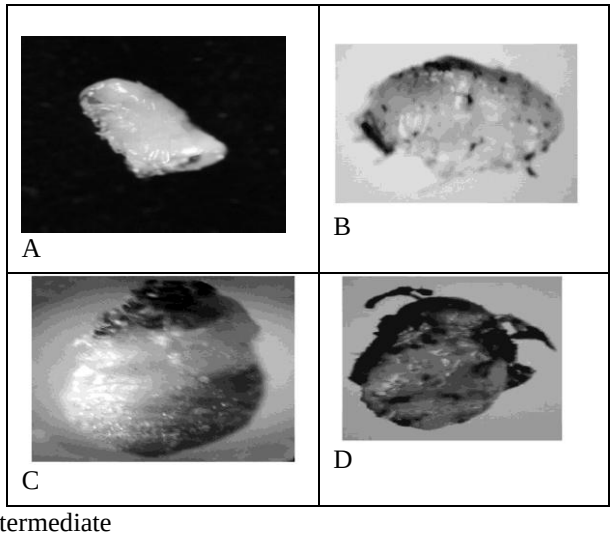


Fig : 2 A,B Larval-Pupal intermediate
Fig : 2 C and D . Pupa-Adult intermediates

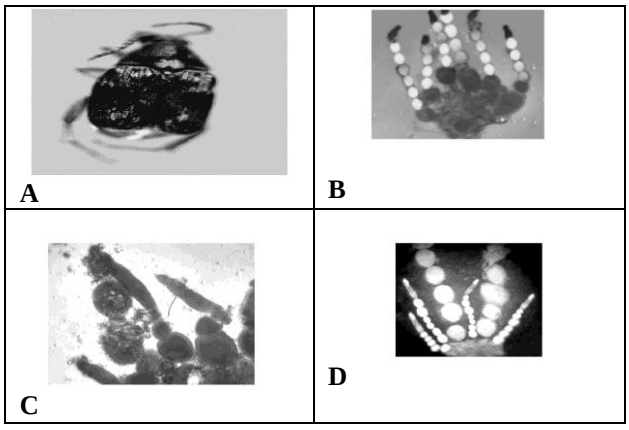


PLATE – 3
DEFORMITIES OBSERVED IN TREATED RESULTANTS

Fig : 3 A. Abnormal Adult with deformed wings and appendages
Fig : 3 B,C and D Ovarian deformities in treated resultant adult females.

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