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Anti Gonadotropic Effects Of a Phyto Chemical Cleistanthin-C during the Morphogenetic Development of the Pulse Pest Callosobruchus Chinensis

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Abstract: *The Pulse beetle Callosobruchuschinensis(Linn.) is a major pest of economically important leguminous grains, such as cowpeas, lentils, green gram and black gram. The larvae bore into the pulse grain which become unsuitable for the human consumption. Proteins are the characteristic components of the tissues which play a major role in morphogenetic events. The quantitative estimation of the proteins in the fatbody, haemolymph and ovary of larvae, pupae and the adult was carried out to show their interrelationship. Anti gonadotropic action of Cleistanthin-C on Callosobruchus chinensis was studied in the fatbody, haemolymph and the ovarian proteins during the morphogenetic development.*

Keywords: *Callosobruchus chinensis, Cleistanthin-C, fatbody, haemolymph and ovary*.

I. Introduction

Proteins are present in all vital cells acting as nucleoproteins enzymes and hormones, which control many chemical processes necessary for metabolism. The growth period develops along with the storage of proteins as these proteins are necessary for the development of imaginal organs during the process of metamorphosis. (Schmidt, 1967). Proteins associated with various reactions such as the hormonal regulation and they integrated in the cell as a structural element at the same time as the carbohydrates and the lipids (Cohen ,2010 and Sugumaran,2010)

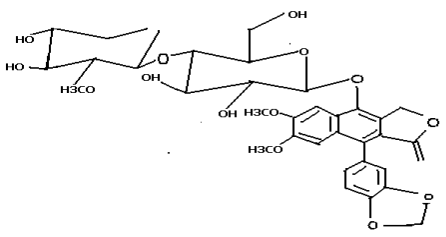
Insect haemolymph is in direct contact with the body tissues and any disturbances in the insect body are reflected by changes in the protein level and protein pattern of the haemolymph. It is influenced by the complex relations of the metabolism, synthesis and uptake of protein by the fat body tissue. (Miller S.G and Silhacek, D.L. b. **(1982)**; Raja etal., 1986).Insect fat body is a site of intermediary metabolism and biosynthesis. It is a store house for nutrients, a regulator of some haemolymph constituents, a site for detoxification and some times a dump for waste products. The insect fat body has been described as the principal site of insect metabolism by Kilby, 1963 ; Wigglesworth, 1972 and Raja et al., 1986. Prominent biological processes underlying the morphogenesis and development in the insect life cycle is the protein synthesis and these proteins may either be the structural proteins or the functional proteins. (Levenbook, 1985 ;Scheller, 1987).

Hence an attempt was made to study the effect of Cleistanthin – C on the protein content in the fat body, haemolymph and ovaries during the morphogenetic development of Callosobruchus chinensis.

II. Material And Methods

The pest Callosobruchus chinensis is reared on red gram diet at the temperature of 27 ± 1°C and RH 65 ± 5 for experimental purpose . The test compound Cleistanthin-C Procured from the Natural chemistry lab ,Department of chemistry ,Osmania University. Cleistanthin-C was extracted from the Cleistanthus collinus (heartwood tree) by the research students of the Department of Chemistry, Osmania University.

Chemical structure of Cleistanthin-C



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

The freshly moulted 3rd , 4th and 5th instar larvae and 0 hour pupae were topically treated on the abdominal region with 2µg /µl of Cleistanthin-C of acetone / larva. Controls were treated with only acetone. 40 larvae were treated each time and the experiment was replicated five times .

Haemolymph was collected using the rapid centrifugation method of Nation &Thomas (1965) Phenyl thio urea was added to the haemolymph to inhibit the tyrosinase activity. Haemolymph was centrifuged at 2500 rpm to remove haemocytes. The fatbody and ovaries of control and treated resultants were dissected in freshly prepared Ringer's solution. The proteins were extracted from these tissues and the protein was estimated by Lowry et al., (1951) method.

III. Results

Estimation of protein content in the fat body, haemolymph and ovaries of the cleistanthin – c treated resultant larva, pupa and adult

1.Estimation of fat body protein

The protein content in the fat body of different stages of life cycle of Callsobruchus chinensis treated with Cleistanthin-C exhibits a remarkable difference when compared with the controls.

1.1.Larval stages: The protein content in the fat body on the 1st day of III instar (10 days old) larvae was 0.517 ±0.0008 mg/gm and on the last day of the III instar (14 days old) larvae the protein content was 0.567 ± 0.0007 mg/gm. When the protein values of the treated resultant larvae were compared with that of the control larvae the early days (10th day) of the IIIrd instar was not significant. { Table 1 (a) }

On the first day (15th day of the lifecycle)of the IV instar larvae the protein content in the fat body was 0.599 ± 0.0008 mg / gm and on the last day of the IV instar (19th day of the lifecycle) larvae the observed value of the protein content in the fat body was 0.797 ± 0.0008 mg/gm. It is evident from the values noticed that all the recorded values of the IV instar larvae were significant over the control larvae. { Table 1 (a) }

On the first day (20th day of the lifecycle)of the Vth instar larvae the protein content in the fat body was 0.820 ± 0.0004 mg / gm and on the last day of the Vth instar (24th day of the lifecycle) larvae the observed value of the protein in the fat body was 0.987 ± 0.0005 mg/gm. It is evident from the values noticed that all the recorded values of the Vth instar larvae were statistically significant over the control larvae. { Table 1(a) }

1.2.pupal stage : On the first day (25th day of the lifecycle)of the pupal period, the protein content in the fat body was 1.016 ± 0.0008 mg / gm and on the last day of the pupal period (30th day of the lifecycle) the observed value of the protein in the fat body was 0.713 ± 0.0007 mg/gm. { Table 1(b) }

1.3.adult stage: On the starting day of the adult satge(31st day of the life cycle) the recorded value of the protein in fat body was 0.524 ± 0.0008 mg/gm and on the 6th day of the adult period (36th day of the life cycle) the protein content was 0.256 ± 0.0009 mg/gm of the fat body. { Table 1(b) }

2. Estimation of protein content in the haemolymph

The protein content in the haemolymph of different stages of life cycle of Callsobruchus chinensistreated with Cleistanthin-C exhibits a prominent variation when compared with the controls.

2.1.Larval stages : The protein content on the 1st day of IIIrd instar (10 days old) larvae was 0.683 ± 0.0006 mg/ml and on the last day of the IIIrd instar (14 days old) larvae the protein content was 0.696 ± 0.0006 mg/ml. When the protein values of the treated resultant larvae are compared with that of the control larvae (10th day) of the IIIrd instar was not significant on the last day of the IIIrd instar larvae the protein content significantly decreased. { Table 2(a) }.

On the first day (15th day of the lifecycle)of the IV th instar larvae the protein content in the haemolymph was 0.714 ± 0.0006 mg / ml and on the last day of the IVth instar (19th day of the lifecycle) larvae the observed value of the protein in the haemolymph was 0.777 ± 0.0006 mg/ml. It is evident from the values noticed that all the recorded values of the IVth instar larvae were significant over the control larvae { Table 2(a) }.On the first day (20th day of the lifecycle)of the Vth instar larvae the protein content in the haemolymph was 0.795 ± 0.0010 mg / ml and on the last day of the Vth instar (24th day of the lifecycle) larvae the observed value of the protein in the haemolymph was 0.955 ± 0.0009 mg/ml. It is evident from the values noticed that all the recorded values of the Vth instar larvae were statistically significant over the control larvae. { Table 2(a) }

2.2.Pupal stage : On the first day (25th day of the lifecycle)of the pupal period, the protein content in the haemolymph was 0.987 ± 0.0008 mg / ml and on the last day of the pupal period (30th day of the lifecycle) the observed value of the protein in the haemolymph was 0.713 ± 0.0007 mg/ml. {Table 2(b) }

2.3.Adult stage : On the 1st day of the adult stage (31st day of the life cycle) the recorded value of the protein in the haemolymph was 0.516 ± 0.0006 mg/ml and on the 6th day of the adult period (36th day of the life cycle) the protein content was 0.154 ± 0.0008 mg/ml of the haemolymph. { Table 2(b) }

3.Estimation of protein content in the ovaries:

The protein content in the fat body of different stages of life cycle of Callsobruchuschinensistreated with Cleistanthin-C exhibits a prominent variation when compared with the controls.

3.1.Larval period : The ovaries on the first day of the Vthinstar (20 day old larvae) recorded a protein content of 0.524 ± 0.0008 mg/gm weight of the tissue. It further increased to 0.565 ± 0.0008 mg/gm weight of the tissue on the third day. The last day of the Vthinstar (24 day old larvae) recorded a value of 0.579 ± 0.0003 mg/gm weight of the tissue. (Table 3). As observed from the control insects the values recorded were not significant in the larval stages.

3.2.Pupal period :The first day of the pupal period (25th day of the life cycle) recorded a value of 0.625 ± 0.0006 mg/gm weight of the tissue. The recorded value was 0.653± 0.0007 mg/gm weight of the tissue on the 2nd day of the pupal period and it was 0.928 ± 0.0008 mg/gm weight of the tissue on the last day of the pupal period i.e 30th day of the life cycle. (Table 3). The recorded values clearly shows the decrease in the protein content in the ovaries of treated resultant pupae when compared with the control ones.

3.3.Adult stage: The protein content in the ovaries of the treated resultant adults on the first day of the adult life (31st day of the life cycle) recorded was 1.244 ± 0.0008 mg/gm weight of the tissue. It further decreased to 1.064 ± 0.0008 mg/gm weight of the tissue on the second day of the adult life to 0.148 ± 0.0004 mg/gm weight of the tissue on the 6th day of the adult life. (Table 3).

IV. Discussion

Cleistanthin-C treated resultants exhibited a decline in the protein content when compared with the control larvae.

In control insects, protein content in the ovaries rapidly rise from the 1st day of 3rd instar (10th day) to last day of the 5th instar (24th day) in fat body and haemolymph. Due to the morphological transformation the protein level gradually increases from the 1st day of the pupal stage to the adult stage. But this type of increase does not appear in the treated resultants.

The drop in the haemolymph protein concentration in the larva of the final instar is attributed to the fact, that the larvae prepares itself to larval-pupal transformation. The fat body diminishes its activity in intermediary metabolism of protein synthesis and changes to function chiefly in storing nutrients for adult development. At this stage the fat body protein concentration greatly increases. Our results are in conformity with that of (Chippendale , 1970 ; Raja et al., 1986 and DeenaVardhini , 1997).

A decline in fat body concentration and a concomitant rise in the protein concentration in ovaries is observed in the later stages. This is correlated to vitellogenesis and to the possibility that the excess proteins of the fat body are utilized by the growing oocytes, confirming the results of Prabhu and Nair 1971 and Raja et al., 1988 ;Anithaet al., 2000).

The synthesis of proteins in the fat body and its transport by haemolymph and uptake by the oocytes is the main factor on which the vitellogenesis of the insect depends. Different studies confirmed that biosynthesis and uptake of vitellogenin was under the hormonal control. The two larval hormones juvenile hormone and ecdysone plays a crucial role in the control of vitellogenin biosynthesis. (Kunkel and Nordin, 1985 ; Hagedorn, 1985).

V. Conclusion

Cleistanthin-C is responsible for the decline in the protein concentration in various tissues at different stages of the treated resultant C. chinensis and Cleistanthin – C might be influencing the hormonal activity in treated resultants. The treatment of the Cleistanthin – C influences protein synthesis ,Storage and uptake of proteins by the fat body, Haemolymph and ovaries resulting in formation of abnormal adults which shows low fecundity thus suppressing the population of Callosobruchus chinensis.

Table- 1 (a) Protein content in the Fat body of Callosobruchus chinenseis in control and treated (Cleistanthin-C) resultant insects

Larval Stage	Age in days	Control	Treated with Cleistanthin-C
III Instar	10 days	0.517±0.0006	0.517±0.0008 ^{NS}
	11 days	0.540±0.0006	0.534±0.0022 ^{NS}
	12 days	0.577±0.0008	0.540±0.0006
	13 days	0.622±0.0009	0.600±0.0005 ^{NS}
	14 days	0.656±0.0006	0.567±0.0007
IV Instar	15 days	0.697±0.0006	0.599±0.0008
	16 days	0.924±0.0008	0.643±0.0005
	17 days	0.979±0.0008	0.686±0.0008
	18 days	1.104±0.0007	0.740±0.0008
	19 days	1.365±0.0008	0.797±0.0008
V Instar	20 days	1.673±0.0007	0.820±0.0004
	21 days	1.893±0.0007	0.864±0.0007
	22 days	2.125±0.0008	0.894±0.0005
	23 days	2.349±0.0008	0.936±0.0006
	24 days	2.575±0.0008	0.987±0.0005

The values are expressed in mg of protein /gm of fat body.
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%).

Table-1 (b) protein content in the fat body of *callosobruchus chinenseis* in control and treated (cliestanthin-c) resultant insects.

Stage	Age in days	Control	Treated with Cliestanthin-C
Pupal period	25 days	2.427±0.0006	1.016±0.0008
	26 days	2.123±0.0007	0.956±0.0007
	27 days	2.029±0.0006	0.848±0.0006
	28 days	1.847±0.0005	0.796±0.0008
	29 days	1.715±0.0008	0.727±0.0008
	30 days	1.549±0.0005	0.652±0.0017
Adult period	31 days	1.225±0.0006	0.524±0.0008
	32 days	1.026±0.0006	0.485±0.0008
	33 days	0.986±0.0008	0.404±0.0006
	34 days	0.834±0.0008	0.356±0.0004
	35 days	0.727±0.0006	0.308±0.0003
	36 days	0.658±0.0005	0.256±0.0009

The values are expressed in mg of protein /gm of fat body.
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%).

Table-2 (a) protein content in the haemolymph of *callosobruchus chinenseis* in control and treated (cleistanthin-c) resultant insects.

Larval Stage	Age in days	Control	Treated with Cleistanthin-C
III Instar	10 days	0.682±0.0005	0.683±0.0006 ^{NS}
	11 days	0.713±0.0297	0.685±0.0006
	12 days	0.725±0.0006	0.686±0.0007
	13 days	0.779±0.0006	0.689±0.0008
	14 days	0.879±0.0005	0.696±0.0006
IV Instar	15 days	1.055±0.0007	0.714±0.0006
	16 days	1.470±0.0007	0.728±0.0003
	17 days	1.977±0.0006	0.745±0.0009
	18 days	2.328±0.0005	0.764±0.0008
	19 days	2.425±0.0006	0.777±0.0006
V Instar	20 days	2.797±0.0007	0.795±0.0010
	21 days	2.945±0.0008	0.823±0.0007
	22 days	3.213±0.0006	0.865±0.0008
	23 days	3.685±0.0006	0.894±0.0004
	24 days	3.843±0.0007	0.955±0.0009

The values are expressed in mg protein /ml of heamolymph.
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%).

Table-3 Protein content in the Ovaries of Callosobruchus chinenseis in control and treated (Cleistanthin-C) resultant insects.

Stage	Age in days	Control	Treated with Cleistanthin-C
V Instar	20 days	0.527±0.0008	0.524±0.0008 ^{NS}
	21 days	0.569±0.0006	0.540±0.0008
	22 days	0.613±0.0008	0.565±0.0008
	23 days	0.683±0.0006	0.579±0.0004
	24 days	0.796±0.0006	0.579±0.0003
Pupal period	25 days	0.896±0.0006	0.625±0.0006
	26 days	1.214±0.0004	0.653±0.0007
	27 days	1.448±0.0008	0.707±0.0006
	28 days	1.727±0.0008	0.775±0.0005
	29 days	2.315±0.0004	0.856±0.0009
	30 days	2.628±0.0008	0.928±0.0008
Adult period	31 days	3.128±0.0009	1.244±0.0008
	32 days	2.543±0.0008	1.064±0.0008
	33 days	2.147±0.0005	0.747±0.0008
	34 days	1.759±0.0008	0.328±0.0006
	35 days	1.347±0.0006	0.223±0.0008
	36 days	1.245±0.0008	0.148±0.0004

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%).

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