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Substrate mobilization in *Sitophilus oryzae* (L) (Coleoptera: Curculionidae) and host pulse, *Cicer arietinum* (L) treated with *Clitoria ternatea* (L) leaf powder

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Abstract
The present study was to make a substrate mobilization in *Sitophilus oryzae* larvae reared in Bengal gram treated with *Clitoria ternatea* leaf powder to understand intrinsic biological factors related to the propagation and sustenance of the pest. In the present analysis shows the protein level was much lower in the tissues of *S. oryzae* larvae. The mobilization of the various metabolites led to a reduction in the amount of protein in the tissues with a corresponding increase in the concentration of carbohydrates and lipids. At the same time the Protein level was higher when compared to Carbohydrates and Lipids level of *S. oryzae* adults. The objective of this study was to use botanical derivatives to minimize the severe damage caused by insect pests, the traditional use of plant products, *C. ternatea* is proved to be highly effective against stored product insect *S. oryzae*. As these substance are not only of low cost, but also have less environmental impact in terms of insecticidal hazard.

Keywords: Substrate mobilization, *Sitophilus oryzae, Cicer arietinum, Clitoria ternatea*

1. Introduction
The rice weevil, *Sitophilus oryzae* (L) (Coleoptera: Curculionidae) is one of the most wide spread pests and causes heavy losses of stored grains both quantitatively and qualitatively throughout the world [14]. The rice weevil is classified as a primary pest as it can bore the grain kernel to feed as well as lay its eggs inside the kernel. Most insects have four development stages: namely eggs, larva, pupa and adults [11]. Different biological activities of plant derivatives have demonstrated for the control of stored grain pests [16].
Plants have developed for 400 million years and have acquired successful defence mechanisms that ensure endurance under rough environmental conditions and in the presence of natural enemies. Besides a number of morphological protective mechanisms, plants have developed subtile chemical defence mechanisms against insects and other organisms. These defence mechanisms do not generally produce direct death but do affect common biochemical and physiological functions [15].
C. ternatea is a very bioactive plant and used in various diseases as folklore medicine [4]. Another study showed that root of *C. ternatea* has anti-inflammatory, analgesic and anti pyretic properties [2]. Many bioactive compounds have been isolated from different parts of *C. ternatea.* Recent study showed that mylonated flavonol glycosides were isolated from the petals of *C. ternatea* [6]. Studies done with synthetic or natural foods have shown that Carbohydrates, Protein and Lipids were the main foods in the various physiological activities of organisms [19]. It has also be estimated that the quality and quantity of foods affect various biological activities such as the developmental time, larval stages, pre adult death rate, longitivity, adult size, fecundity and sex ratios in insects [1]. Management of *S. oryzae* had been a very challenging area. As an alternative to the chemical pesticide, a number of botanicals were effectively used in the control of stored grain pest like *S. oryzae*. In this study the substrate mobilization of *S. oryzae* larvae and adult reared on *C. ternatea* leaf powder treated Bengal gram was study to understand their infestive ability of a notorious pest. A contribution in any small measure towards better control and management of *S. oryzae* had been the ultimate aim this investigation.

2. Materials and Methods

2.1 Procurement of test insects

The *Sitophilus oryzae* were collected from the local farm fields and maintained in the Laboratory in the sterilized glass jars. After adult emergence the adults of *S. oryzae* were identified and isolated. The freshly emerged *S. oryzae* were sexed and grouped into 6x6 paired weevils and placed in sterilized jars for examining the development and life history traits of the species. Each jar was provided with fresh, clean and infestation free Bengal gram (*Cicer arietinum* (L) covered with fine mesh nets, under laboratory environmental conditions, weevil were removed from the jars eight days after introduction, eggs were incubated until hatching and the grubs were allowed to complete their maturity. Larvae that hatched out or the feasible eggs and the weevil that emerge from the seeds were used as the suitable initial stock for further studies. Since this pulse was easy to handle, eggs could be visibly observed and the grains contained sufficient nutrition to support larval development.

2.2 Plant Material

Green and fresh leaves of *Clitoria ternatea* (Fabales: Fabaceae) were collected and washed thoroughly air dried and ground with fine powder by an electric grinder. The dry powers obtained were kept at room temperature until use.

2.3 Experimental design

Small 1 litre air tight cylindrical plastic containers served as the exposure system. The substrate concentration of the whole body tissues were estimated in the larvae and adult of *S. oryzae* raised in the bengal gram treated with *C. ternatea* leaf powder at the concentration of 2%, 5% and 10% respectively. Each exposure system was marked for a particular type of treatment. First set of experiment containing *S. oryzae* larvae developing inside grains kept inside flour treated with different concentrations of *C. ternatea* leaf powder untreated grains served as control. The substrate mobilizations were estimated using the method of Lowry *et al.*, (1951) [8] for protein. Anthrone method for Carbohydrate and (Knigh *et al*., 1972) for lipid.

2.3.1 for the determination of total Proteins and total Carbohydrates. A known weight of larvae and adult and grains was homogenate in two milliliters of 10% saline solution using a homogenizer for about two minute. The tubes were centrifuged and the supernatant was used to determine total proteins and total carbohydrates. The remaining stock solution was stored in freezer until used [8].

2.3.2 Preparation of samples for the determination of total lipids. A known weight of larvae and adults were homogenate in two milliliters of 1:1 chloroform methanol using a homogenizer for about two minutes. The tubes were centrifuged and the supernatant was taken and heated until evaporation of the solvents. The precipitate was redisolved in 2 ml ethanol and used to determine total lipids [7].

3. Results and Discussion

Sitophilus oryzae is a major pest of stored grain. The adverse nature of some established methods makes a constant search for new means of controlling insects in stored grains; Insect pests attacking stored food grains cause substantial losses. The mode of fluctuation of different nutrients during the post embryonic development of *S. oryzae*, distinctly corroborates the heterogenic morphogenetic requirements in the later phases of grub development [18].

Relatively high level of almost all components (except lipid) in the last grub instar which represents a transitional phase between grub and pupal stage, may be due to the storage of nutrients for the non feeding pupal stage and for the provision of energy required for reconstruction of the important imaginary organs in the pupal stage and is surely indicative of the synthesis of these components in this stage.

The tissue carbohydrate, Protein and Lipid content (mg/g) of *S. oryzae* larvae developing inside grains kept inside flour containing 2%, 5%, 10% of *C. ternatea* leaf powder was represented in table, (1) and, 2% of *C. ternatea* leaf powder was 294 ± 2.77 in 10 day old larvae (table 1), whereas the 349.16 ± 6.46 for control (untreated grain rearing larvae) was recorded. The concentration of 5 % *C. ternatea* shows maximum in 4 day old larvae 196. 5 ± 5.20 was observed (tables 1). When 10% concentrated plant extract treated with the larvae the carbohydrate content were decreased very low in 10 days 168. 33 ± 5.16 (table 1). The tissue proteins and lipid content of S. oryzae larvae developing inside grains kept inside flour containing 2%, 5%, 10% of *C. ternatea* leaf powder was 141.16±8.61 (10 days) and 8.34±0.47 (10 days) respectively (table1). From the mentioned results, it is evident that the toxic groups of *C. ternatea* used causes significantly decrease the total carbohydrate, Protein, lipid content of the larvae.

Table 1: Tissue Carbohydrate, protein and *oryzae* lipid content (mg/gm) in S. larvae developing inside grain kept inside flour containing 2%,5%,10% C. ternatea leaf powder.

Treatments	Concentration of leaf powder (%)								
	Tissue substrate concentration (mg/ml)								
	2%			5%			10%		
Days	Protein	Carbohydrate	Lipid	Protein	Carbohydrate	Lipid	Protein	carbohydrate	Lipid
(Control in 5 days)	220.16±3.12	349.16±6.46	15.02±.74	220.16±3.12	349.16±6.46	15.02±.74	220.16±3.12	349.16±6.46	15.02±.74
4	187.5±5.78	232.16±3.92	10.58±0.59	120.5±7.42	196.5±5.2	7.45±0.45	89±3.22	112.16±3.37	5.66±0.42
6	201.16±5.30	266.16±2.9	12.27±0.39	159.16±7.93	205±7.45	8.15±0.22	109.33±3.88	125±3.89	7.24±0.35
8	210±4.73	279±1.9	14.01±0.52	164.16±9.15	221.16±4.4	8.56±0.48	120.8±3.86	156.16±4.7	7.09±0.35
10	213.5±4.8	294±2.77	14.49±0.41	1.78±8.5	271.83±3.4	9.10±0.62	141.16±8.61	168.33±5.16	8.34±0.47

The tissue carbohydrate content (mg/g) in *S. oryzae* adult developing inside grains kept inside flour containing 2 %, 5%, 10% of *C. ternatea* leaf powder was represented in table (2) and containing 2% of *C. ternatea* leaf powder was 111 ± 2.09 in 20 days old adult weevil (table 10), where as the 123.83 ± 2.40 for control (Untreated grain rearing larvae) was recorded. The concentration of 5% *C. ternatea* shows higher in 15 days old adult weevil 112.16 ± 5.45 was observed in (table 10) when 10% concentrated plant extract treated with the adult the Carbohydrate content were decreased very low in 30 days

45.83 ± 2.48 (table 2).The toxic groups of *C. ternatea* leaf powder used causes significantly decrease the total Carbohydrate, Protein, Lipid, content of the adult weevil. The level of total Carbohydrate was more or less uniform throughout the grub stages, but the glycogen level exhibited fluctuating changes in different instars reaching its peak in sixth instar which is in consistence with the findings of [13]. The level of Carbohydrate in different instars as found here suggests that the grubs feed voraciously to provide substrate for glycogen synthesized during the developmental stages is deemed to be "a mobile reservoir" whose concentration depends upon the period of development, mode of nutrition and the energy requirement for development [17]. The larvicidal action of tested groups could be due to growth inhibition effects. It is more pronounced against early instar and there is delayed physiological process. The toxicity of the tested groups *C. ternatea* plant may be used as a part of integrated pest control of *S. oryzae.*

Table 2: Tissue Carbohydrate, protein and lipid content (mg/gm) in *S. oryzae* adult weevil developing inside grain kept inside flour containing 2%,5%, 10% *C. ternatea* leaf powder.

Treatments	Concentration of leaf powder (%)								
	Tissue substrate concentration (mg/ml)								
	2%			5%			10%		
Days	Protein	Carbohydrate	Lipid	Protein	Carbohydrate	Lipid	Protein	carbohydrate	Lipid
(Control in 5 days)	144±2.09	123.83±2.40	4.16±0.05	144±2.09	123.83±2.40	4.16±0.05	144±2.09	123.83±2.40	4.16±0.05
15	132.5±2.16	112.16±2.09	3.45±0.28	$102.8^{\pm 2.85}$	91±3.74	3.21±0.14	86.5±3.88	79.66±2.25	2.12±0.10
20	125.5±1.51	111±5.45	2.68±0.34	92.66±3.88	80±3.79	2.85±0.08	76.33±2.33	65.16±3.37	1.89±0.07
25	$113.1^{\pm 3.48}$	97±5.47	2.18±0.06	81.66±1.21	74±4.04	2.67±0.06	65.66±3.61	55±4.04	1.70±0.23
30	105±2.52	82.66±1.21	2.15±0.06	77.16±3.92	65.16±4.16	2.01±0.14	44.30±4.67	45.83±2.48	1.09±0.09

4. Conclusion

Substrate mobilization in S. oryzae larvae reared in bengalgram treated with *C. ternatea* leaf powder to understand intrinsic biologicl factors related to the propagation and sustenance of the pest. In the present analysis shows the protein level was much lower in the tissues of S. oryzae larvae. The mobilization of the various metabolites led to a reduction in the amount of protein in the tissues with a corresponding increase in the concentration of carbohydrate and lipid. At the same time the protein level was higher when compared to carbohydrates and lipid level of S. oryzae adults. The objective of this study was to use botanical derivatives to minimize the severe damage caused by insect pests, the traditional use of plant products is proved to be highly effective against stored product insects, as these substance are not only of low cost, but also have less environmental impact in terms of insecticidal hazard.

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