Biochemical studies of cotton pest *Sylepta derogata Fab.* *by Econeem, Acorus calamus* and *Piper longum* extracts

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Abstract- Many plant extracts or allelochemicals show a broad spectrum of activity against pests and such products have long been touted as attractive alternatives to synthetic chemical pesticides for pest management because they pose little threat to the environment or to human health. Cotton is the major cash crop in India. So we have to protect the cotton plant from pest organisms and to control the pests biologically instead of Chemical pesticides for safe to humans and non-target organisms. Many plant species produce substances that protect them by killing or repelling the insects that feed on them. Cotton leaf-roller S*ylepta derogata Fab* is a sporadic pest of cotton and belong to the family Pyralididae. The pest is a polyphagous insect and attacks agricultural crops like *Gossypium hirsutum, Abelmoschus esculentus, Hibiscus rosasinensis, Urena lobata, Althaca rosea, Sida cordifolia, Malvastrum tricuspidatum* and other Malvaceous plants. In my present study showed that the protein and glucose content of larval haemolymph of *Sylepta derogata Fab*. against three medicinal plant extracts of Econeem, *Acorus calamus and Piper longum*.

Index Terms- *Sylepta derogata*, haemolymph, Protein and Glucose content

I. INTRODUCTION

In many countries cotton (Gossypium spp.) is one of the most important fibre producing plants. Cotton crop not only provides fibre for the textile industry, but also plays a role in the feed and oil industries with its seed, rich in oil (18 – 24%) and protein (20 – 40%). An estimated 350 million people are engaged in cotton production either on-farm or in transportation, ginning, baling and storage. China consumes 40% of the world's raw cotton. Australia and Egypt produce the best quality cotton in the world. Cotton is a major export revenue source for Burkina Faso, Benin, Uzbekistan, Mali, Tajikistan, Ivory Coast, Kazakhstan, Egypt and Syria. The world's lowest cost cotton producers are Australia, China, Brazil and Pakistan**.** India is one among the mega species diversity country in the world which consists of wide variety of fauna and flora. Several plants and plant products have long been used as insecticides, repellents, antifeedants, sterilants and ovicides in insect control. Use of plant extracts is one of the possible methods of pollution free technology in insect control. Promising results have been achieved towards attaining this goal by treating the eggs, larvae, nymphs and adult insects with the extracts of total plants, leaves, roots, fruits and seeds.

STATUS OF THE PEST *(SYLEPTA DEROGATA)*

Cotton leaf-roller S*ylepta derogata Fab* is a sporadic pest of cotton in India (Sohi, 1964) and belong to the family Pyralididae. The pest occurs in India, Pakistan, Bangladesh, Burma, Australia, Africa, China, Japan, Sri Lanka and other parts of the world. The pest is active from the month of September to November.

The pest is a polyphagous insect and attacks agricultural crops like *Gossypium hirsutum, Abelmoschus esculentus, Hibiscus rosasinensis, Urena lobata, Althaca rosea, Sida cordifolia, Malvastrum tricuspidatum* and other Malvaceous plants.The damage caused by the caterpillars whose feeds on the leaves and young buds. The larva rolls the leaf and feeds on the green tissue in the early stage and eats up a large portion of the leaf as it grows. Flowering and fruiting are poor and yield declines considerably due to this pest. The webbing and withering of leaves could be the symptoms of this pest infection.

As a result of its attack the whole leaf has been eaten up or big holes are made into it and finally the infected leaves were dropped. Severe infection indicates the presence of a large number of leaf rolls and ultimately the plants become stunted.The caterpillars roll up the leaves from sides and bind the roll with silk spun by the spinnerets located near the mouth and the leaf tissues from inside. There may be 5 or 6 but sometimes 32 young caterpillars are seen in each infected leaf.

PIPER LONGUM

The Indian long pepper *(Piper longum.L)* is otherwise known as Pippali, Pipal, Tippli, Pihal, Dried catkins, is a climbing shrub is indigenous spices. The species *Piper longum* is of South Asian Origin (Deccan Penisular) and also cultivated in tropical countries such as India, Sri Lanka along with other crops. Fruits are used in the diseases of respiratory tract infection.

Fruits as well as roots are attributed with numerous medicinal properties and are used for diseases of respiratory tract viz., cough, bronchitis, asthma etc. as counter irritant and analgesic when applied locally on muscular pains and inflammation. Properties and uses of *Piper longum* are similar to those of black pepper.

Piper longum contains essential oil consisting of long chain hydrocarbons. The root contain the substance consisting of Piperine, Piper longuminine, Sylvatine, Guineesine sitosterol , Methyl piperate and Methyl - 3,4,5 trimethoxy cinnamate. The content of Piperine is slightly higher (about 6%) than in black pepper.

C_{17} H_{19} O_3 N

ACORUS CALAMUS

Acorus calamus or sweet flag has long been known for its medicinal value and is cultivated in Asia for this reason *Acorus calamus* contain aromatic oil that has been used medicinally, since ancient times and has been harvested commercially. This plant consider to possess anti – spasmodic, carminative and anthelmintic properties and also used for the treatment of epilepsy, mental ailments, chronic diarrhoea, dysentery, bronchial catarrah, intermittent fever, glandular and abdominal tumours. They are also employed for kidney and liver troubles , rheumatism, sinusitis and eczema. Other virtues of this plant include its mature leaves, which act as an insect repellant when cut up and stored in dry foods.

There are 47 constituents were identified from *Acorus calamus* leaf extract. The important compounds like Asarone, Linalool, Bornyl acetate (GC), Galgravin** (PB) Retusin** (PB) were seen in *Acorus calamus* oil.

NEEM

The neem tree *(Azadirachta indica A.juss),* Katpoora viruchum for centuries in the Indian subcontinent belongs to the family Meliaceae. Neem trees are native to India and is grown in various countries throughout the world including India, Burma, Sri Lanka and Australia. The different parts of the neem tree is used for production of grains from stored pest and woolen cloths was an ancient practice in India. Neem oil is a broad spectrum botanical insecticide, miticide and fungicide which derived on the seeds of the neem tree. Neem products are associated with many agricultural and medicinal uses. It is also used in many cosmetics and pharmaceutical products as well as pest control in agriculture and home gardens.

Azadirachtin is a highly oxidized tetranor-tri-terpenoid comprising an enol ether, acetal, hemi acetal and tetra oxirane. More than a hundred terpenoid compounds have been identified from different parts of the neem tree. Azadirachtin is the most active of these, several types of Azadirachtin have been isolated, the most important of which is Azadirachtin A.

C_{35} H_{44} O_{16}

As there is a paucity of literature on the toxicity of Econeem, *Acorus calamus* and *Piper longum* against the cotton pest *Sylepta derogata*, the present study aimed to find out the efficacy of these botanicals will open new vitas of research in the field of pest management.

II. MATERIALS AND METHODS

BIOLOGY OF EXPERIMENTAL ANIMAL

Biology of *Sylepta derogata Fab.* is studied by various workers like Sidhu (1979), Fadare and Amusa (2003). During the Month of March – October the moth *Sylepta derogata* lays 200 eggs on the under surface of the cotton leaves. The eggs are minute, scale like and brown or pale white in colour. The eggs hatch into larvae (First instar) in about 4-6 days, after hatching,they begins to roll the leaves and live inside the rolls feeding on the leaf tissue. The total larval period lasts 15-20 days. A full grown caterpillar is about one and a quarter inch long, greenish in colour with dark brown head. At the end of the larval period, as indicated by cessation of feeding, they undergo pupation. The pupation takes place either in rolled leaves or amongst the fallen plant debris in the soil. The pupa is generally reddish brown in colour. The period of pupation is about 6-12 days. The adult moth is white with yellowish wings bearing many fine lines. Sex could be distinguish at the adult stage (Nayer **et al.,** 1986). After copulation, the female lays eggs and the male dies. There are 3-4 generations in a year and the life cycle completed within 25-30 days.

COLLECTION AND MAINTENANCE OF THE ANIMAL

For experiments, freshly moulted *Sylepta derogata* larvae of the second instar were collected during the months of March – October from the nearby the cotton fields of Coimbatore. In order to avoid genetic and size variations, larvae were collected from the same locality and host plant for each experimental series. They were weighed and reared individually in plastic containers. During this period they were fed with fresh cotton leaves. The larvae were acclimatized to 30°±1°**C**, 75± 10% **rh** and 10 **h** photoperiod.

METHODS FOR PLANT EXTRACT PREPARATION
PREPARATION OF *PIPER LONGUM* EXTRACT

The extract of *piper longum* was prepared by the method of Ikan (1970). 10g of powdered pepper was mixed with 150 ml of 95% ethanol and kept for 3 hours. The extract was filtered and concentrated by adding 10ml of 10% alcoholic KOH. The residue was discarded and the supernatant was allowed to settle 12hrs. The yellow residue obtained after 12 hours, it was dissolved in 100ml of ethanol (Stock solution).

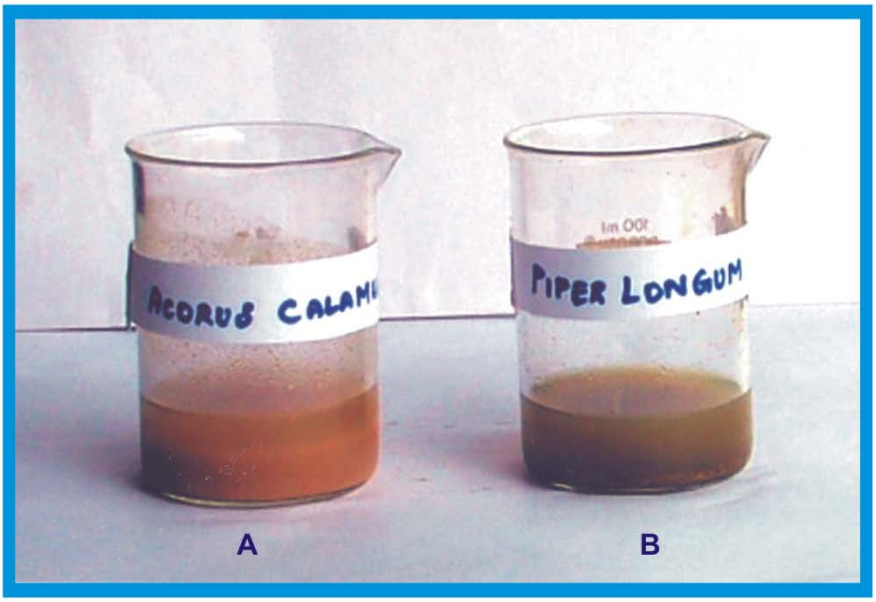
PREPARATION OF *ACORUS CALAMUS* EXTRACT

Acorus calamus extract was prepared by the plant extraction method of Harbone 1973. *The Acorus calamus* powder 10 g. was mixed with 100 ml of 10% acetic acid and kept for 4 hours. This was concentrated to one third of its volume by evaporation at room temperature. The Ammonium hydroxide (conc) was added drop by drop to precipitate the extract centrifuged and washed with 2ml of 1% NH_4OH. After this, the supernatants was discarded and the remaining residue was dissolved in 100 ml of ethanol and used as stock solution.

ECONEEM

The Botanical insecticide Econeem was brought from local pesticide shop which is manufactured by Margo Private Limited Tumkur.

The chemical constituents of this Pesticide are Azadirachtin 1% w/w; Solvent 54%, w/w; Neem oil 30%w/w; Emulsifier 15% w/w; Total 100% w/w.



A. Extract Of *Acorus calamus* B. Extract of *Piper longum .L*

C. Botanical Insecticide - Econeem

SAMPLE PREPARATION FOR BIOCHEMICAL ANALYSIS

The haemolymph for biochemical studies was drawn into a capillary tube from a puncture made on the cervical region and forelegs of the larvae. The obtained homogenate were centrifuged at 5000 rpm for 20 min and the supernatant was used for the biochemical analysis.

ESTIMATION OF TOTAL PROTEIN

The total protein concentration of the samples were estimated by the method of Lowry **et al.,** (1951).

PRINCIPLE

In alkaline solutions, Protein forms a complex with copper ions and this copper Protein complex reacts with Folin-Ciocalteace reagent to give a blue colour due to the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of the colour is proportional to the concentration of protein.

PROCEDURE

For plotting the standard curve, a set of standard solution of Bovine Serum Albumin (BSA) containing 0.1mg., 0.3mg., 0.5mg, 0.8mg, 1.0mg and 1.5mg of standard solutions were taken in a series of test tubes. The volume in each tube was made upto 1 ml with distilled water, 5 ml of alkaline copper reagent was added, mixed and allowed to stand for 10 minutes at room temperature. 0.5 ml Folin-ciocalteau phenol reagent was then added to each tube and shaken well. The blue colour developed was read at 720 nm after 20 min., against a reagent blank in a spectrophotometer. The standard graph was drawn by plotting the concentration of the standard solution of the ordinate and the optical density on the abscissa.

For the estimation of tissue protein, 0.01 ml, of the extracts were taken and it was made upto a final volume of 1 ml with distilled water. The same procedure was followed as described for the standard. The amount of protein present in 0.01 ml of sample was calculated by referring to the standard curve obtained. The protein concentration was expressed in mg/g tissue.

ESTIMATION OF GLUCOSE FROM LARVAL HAEMOLYMPH

The Glucose levels in larval haemolymph of *Sylepta derogata* were estimated by Glucose oxidase method.

PRINCIPLE

Glucose oxidase catayses the oxidation of alpha-D-glucose to D-glucono-1, 5 lactone (gluconic acid) with the formation of hydrogen peroxide. The oxygen liberated from hydrogen peroxide by peroxidase reacts with the O-dianisidine and oxidizes it to a red chromophore product.

Glucose

$$\text{Glucose} + O_2 \xrightarrow[\text{Oxidase}]{\text{Glucose}} H_2O_2 + \text{Gluconic Acid}$$

$$H_2O_2 + \text{O} - \text{dianisidine} \xrightarrow{\text{Peroxidase}} \text{Red-coloured product}$$

MATERIALS

Glucose Oxidase Peroxidase Reagent: Dissolve 25 mg O-dianisidine completely in one ml of methanol. Add 49ml of 0.1ml phosphate buffer (pH 6.5). Then add 5mg of peroxidase and 5mg of glucose oxidase to the above prepared O-dianisidine solution.

STANDARD: Dissolve 100mg glucose in 100ml water. Dilute 10ml of this stock to 100ml to obtain the working standard.

PROCEDURE

1. To 0.5mLof larval haemolymph was added with 0.5mL distilled water and 1mL glucose oxidase-peroxidase reagent.
2. Into a series of test tubes pipette out 0 (blank), 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard glucose solution and make up the volume to 1.0mL with distilled water. Then 1mL of glucose oxidase-peroxidase reagent to as added.
3. All the tubes at 35º C for 40 minutes were incubated.
4. Addition of 2mL of 6N-HCl to terminate the reaction.
5. The intensity of the colour developed were read at 540 nm.

POLYACRYLAMIDE GEL ELECTROPHORETIC SEPERATION OF LARVAL HAEMOLYMPH PROTEIN

COLLECTION OF HAEMOLYMPH FOR ELECTROPHORESIS

The control and treated larvae were punctured on the cervical region and fore legs with the help of microneedle and then pressed gently. The syringe was used to collect the haemolymph. Then the haemolymph was suspended in a centrifuge tube and centrifuged at 1000 rpm for 5 min. The haemolymph, thus tapped in the centrifuge tubes was utilized for electrophoretic separation of protein.

PRINCIPLE

SDS is an anionic detergent which binds strongly to, and denatures, proteins. The number of SDS molecules bound to a polypeptide chain is approximately half the number of amino acid residues in that chain. The protein – SDS complex carries net negative charges, hence move towards the anode and the separation is based on the size of the protein.

MATERIALS

- Stock Acrylamide Solution

Acrylamide 30%	30g
Bisacrylamide 0.8%	0.8g
Water to	100 ml.

- Separating Gel Buffer

- 1.875 M Tris – HCI 22.7g
  pH 8.8

Water to 100 ml.

- Stacking Gel Buffer

0.6M Tris – HCI 7.26g
pH 6.8
Water to 100 ml.

- Polymerising Agents

a) Ammonium 0.5g/10ml, prepare freshly before use
Persulphate 5%
b) TEMED fresh from the refrigerator.

- Electrode buffer

0.05 M Tris 12g
0.192M Glycine 28.8 g
pH 8.2 – 8.4
0.1% SDS 2G
No adjustment
Water to 2 L
required

PREPARATION OF SLAB GELS:

1. Thoroughly clean and dry the glass plates and assemble them in gel casting assembly. Seal the two glass plates with the help of tygon tubing, clamp them and place the whole assembly in an upright position.

2. Mix various components of resolving get as indicated in the above table except for SDS, APS and TEMED. Degas the solution for 1 min using a water pump and then add the above remaining components of the gel.

3. Mix gently and pour the gel solution into the mould in between the clamped glass plates taking care to avoid entrapment of any air bubbles. Overlay distilled water on the top as gently as possible and leave for 30 min for setting of the gel.

4. When the gel has polymerized, remove the water layer and rinse the gel surface with stacking gel buffer.

5. Mix the stacking gel components in the same way as described above for the resolving gel.

6. Pour the stacking gel and immediately insert the supplied plastic comb in the stacking gel. Care should be taken that no air bubbles are entrapped. Allow the gel to polymerize for about 20 min.

7. After the stacking gel has polymerized, remove the comb without distorting or damaging the shapes of the wells. Clean the wells by flushing with electrode buffer using a syringe.

8. Remove the tygon tubing and install the gel plate assembly into the electrophoretic apparatus. Pour reservoir buffer in the lower and upper chambers. Remove any trapped bubbles at the bottom of the gel.

ELECTROPHORESIS OF SAMPLE:

9. Load 10-20 µl sample (100-200 µg protein) in the sample wells. Also load molecular weight marker proteins in one or two of the wells.

10. Switch 'ON' the current maintaining it at 10-15 mA for initial 10-15 min until the samples have traveled through the stacking gel. Then increase the current to 30mA until the bromophenol blue dye reaches near the bottom of the gel slab. This may require 3-4h.

11. After the electrophoresis is complete, turn 'OFF' and disconnect the power supply and carefully remove the gel slab from in between the glass plates.

12. Place the gel in a trough containing staining solution for 3-4 h or it can be kept for staining overnight. Destain the gel with destaining solution , till a clear background of the gel is obtained.

13. Record the distance traveled by the dye and various protein hands and calculate R_m values .

R_m Value was calculated by the following formula

$$R_m = \frac{\text{Distance traveled by the protein fraction}}{\text{Distance traveled by the Bromophenol blue}}$$

III. RESULTS

EFFECT OF *PIPER LONGUM* ON THE PROTEIN CONTENT OF THE LARVAE AND PUPAE OF SYLEPTA DEROGATA.

Results on the influence of piper longum on total heamolymph protein of larvae and pupae are presented in Table

1. The protein content of the III instar control larva was (0.906 mg/ml) and it slightly increased (0.907 mg/ml) on the IV instar control larva and significantly increased 1.318 mg/ml during the V instar stage in Table 1. When compared to the larvae, and pupae showed decreased level (0.901 mg/ml) of protein Table 1. After sublethal treatment of *Acorus calamus* extract, the III instar larvae showed increased level of protein content (7.9% Fig.1). The same pattern of increased level of protein was observed in the IV instar 0.982% and the V instar 1.304% for its respective controls. When compare to the control and treated pupae showed significantly increased level of protein 6.7% (Fig.1).

S.No.	Larval Stage	Protein content mg/ml		Glucose content mg/ml	
		Control	Treated	Control	Treated
1.	III Instar	0.971 ± 03	0.978 ± 02 *	54.50 ± 2.28	49.20 ± 1.74 *
2.	IV Instar	0.968 ± 03	0.973 ± 02 * *	55.20 ± 2.28	51.80 ± 0.96 * * *
3.	V Instar	1.338 ± 00	1.302 ± 03 *	56.10 ± 2.28	52.10 ± 0.83 * * *
4.	Pupae	0.977 ± 03	0.997 ± 04 *	31.20 ± 2.28	29.30 ± 0.22 * * *

EFFECT OF *PIPER LONGUM* ON THE GLUCOSE LEVEL OF THE LARVAE AND PUPAE OF *SYLEPTA DEROGATA*.

Glucose concentration of the control larvae increased with the advancement of age, from the III instar 54.50mg/ml and it increased at the end of the feeding period 56.10 mg/ml Table 1 . In control pupae there was a gradual decline the glucose concentration 31.20mg/ml in *Piper longum* treated larva there was a gradual decrease of glucose in the III instar, followed by a IV instar 51.80mg/ml and marginal decrease in V instar 52.10mg/ml. Table 1. There was marginal decrease 29.30mg/ml in the glucose concentration in *Piper longum* treated pupae, when compared to control.

Table:1 Effect of *Piper longum* on the Protein and Glucose content of the larvae and pupae of *Sylepta derogata*

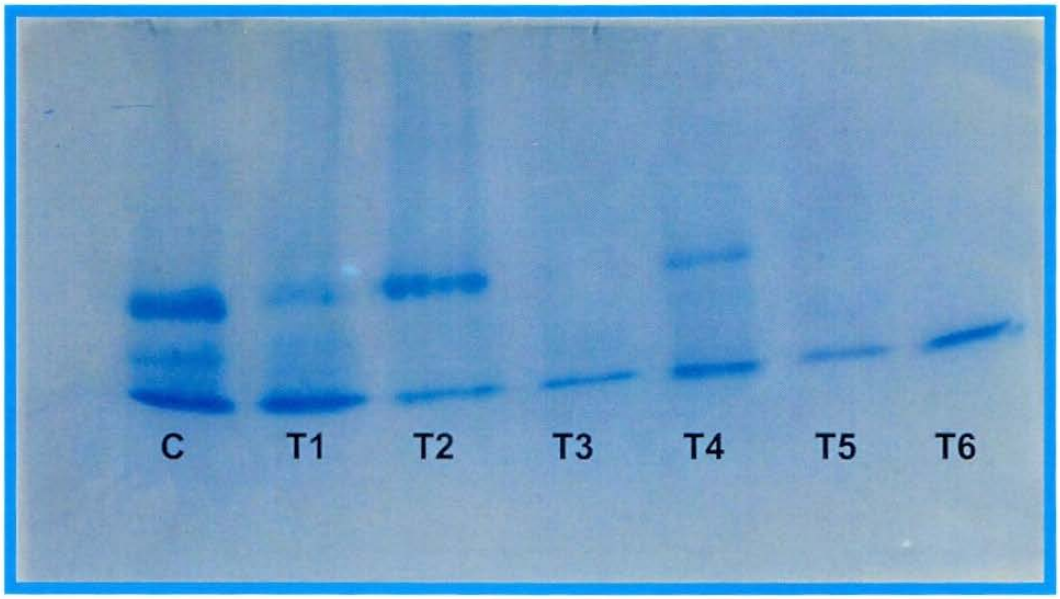
Each value represents the mean of 5 determinations.
Sign + or - represents percentage increase or decrease over the control.

P < 0.01 *
P < 0.05 **
NS = Non Significant * * *

EFFECT OF *ACORUS CALAMUS* EXTRACT ON THE PROTEIN CONTENT OF THE LARVAE AND PUPAE OF *SYLEPTA DEROGATA*

On the III instar stage, the control larvae showed the protein content (0.934 mg/ml) Table 8 and it was found to be (0.930 mg/ml) depleted on the IV instar control. The V instar control larvae exhibited increased level of protein content (1.338 mg/ml) Table 8, than the previous stage. Remarkable depletion on the protein content (0.9 mg/ml) was found in the control pupae. After *Acorus calamus* treatment, the III instar larvae showed significant raise in the protein content and it was continued all the stages of larvae 6.9%, 2.4%, and pupae 6.9% Fig.1.

SDS - PAGE of Haemolymph Protein Profile of V - instar Larvae of *Sylepta derogata*



C = Control

T1 = Econeem treatment - 48 h

T2 = Acorus calamus treatment - 48h

T3 = Piper longum treatment - 48h

T4 = Econeem treatment - 24 h

T5 = Acorus calamus treatment - 24h

T6 = Piper longum treatment - 24h

EFFECT OF *ACORUS CALAMUS* ON THE GLUCOSE LEVEL OF THE LARVAE AND PUPAE OF *SYLEPTA DEROGATA*.

The data on the effect of *Acorus calamus* treatment on the glucose level of larvae and pupae are presented in Table 2.The Glucose level of the control larvae of III instar showed 50.16 mg/ml with the advancement of age, it was decreased 50.40mg/ml on IV instar larvae. It was found that more glucose level 51.30 mg/ml in the control V instar larva and significantly

decrease 30.90 mg/ml was observed in the control pupae. When compared to control the glucose level had decreased significantly on III, IV and V instar in the *Acorus calamus* treated larvae. The changes in the haemolymph glucose level of the control and treated pupa had shown a more or less similar trend.

Table:2 Effect of *Acorus calamus* on the Protein and Glucose level of the larvae and pupae of *Sylepta derogate*

S.No.	Larval stage	Protein content mg/ml		Glucose content mg/ml	
		Control	Treated	Control	Treated
1.	III Instar	0.934 ± 03	0.994 ± 03 *	50.16± 2.32	45.85 ± 2.28 *
2.	IV Instar	0.930 ± 03	0.995 ± 02 * *	50.40± 2.28	46.30 ± 2.13 *
3.	V Instar	1.338 ± 00	1.305 ± 03 *	51.30± 2.28	44.80 ± 0.65 * *
4.	Pupae	0.932 ± 03	0.997 ± 03 *	30.90± 2.28	28.70 ± 0.4 * * *

Each value represents the mean of 5 determinations.
Sign + or - represents percentage increase or decrease over the control.

P < 0.01 *
P < 0.05 **
NS = Non Significant * * *

EFFECT OF ECONEEM ON THE PROTEIN CONTENT OF THE LARVAE AND PUPAE OF *SYLEPTA DEROGATA*.

The data on the effect of treatments on the III instar larva are present in Table 3. The larval haemolymph protein concentration of the control larvae increased with the advancement of age from initial value 0.97 mg/ml at the end of IV instar (0.97 mg/ml) followed by a increase 1.338 mg/ml in the V instar larvae. There after, there was a steep fall in the pupal (0.892mg/ml)stage.After Econeem treatment the larvae showed increased level of protein concentration 0.978 mg/ml Table 7 and there was steep increase 1.302 mg/ml at pre-pupal stage. There was a significant decrease in the protein concentration in Econeem treated pupae when compared to control pupae.

EFFECT OF ECONEEM ON THE GLUCOSE LEVEL OF THE LARVAE AND PUPAE OF *SYLEPTA DEROGATA*.

The control larvae of III instar showed the glucose level 44.30 mg/ml and it gradually decreased during IV instar 42.80mg/ml and marginal decline 42.50 mg/ml was noted in the V instar control larvae of *Sylepta derogata*. After Econeem treatment, the significant decline in the glucose level was observed 26.20mg/ml. The same pattern of glucose level observed in the treated IV instar and V instar larvae. Glucose level in the treated pupae has the marginal variation upto 29.70 %.

EFFECT OF ECONEEM AND PLANT EXTRACT ON THE GLUCOSE LEVEL IN THE LARVAE AND PUPAE

Literature on the physiological functions of glucose in insect is very vast. Glucose has a central place in carbohydrate metabolism but the amount of free glucose is quite little.(candy, 1984) in insects which may be incorporated into chitin for the cuticle (Bade and Wyatt, 1962, Candy and Kilby, 1962). Generally glucose takes part in the synthesis of trehalose via reverse glycolysis and in the production of energy via glycolytic pathway (Lipke ***et al.,*** 1965).

The results of present study displayed the toxicity of plant extracts which seemed to effect glycogenolysis which effect lower level of glucose. Toxicity of in larva heavily inflicted depression in the content of glucose. Venugopalan (1974) also observed similarly lower percentage of glucose in insects treated with tetramycin and sulphanilamide Mansing (1964) recorded that malathion toxicity enhanced the catabolism of glucose cock. The depletion in the level of glucose in the III instar larvae which specified it conversion either to synthesis of trehalose or its mobilization to glycolytic pathway.

In the present investigation when compared to control was significantly declined in the Econeem treated larvae probably shows the mobilization of glucose into glycolytic pathway in 1965order to produce energy which is needed for toxic stress as suggested by Lipke **et al.,.** The overall mean glucose concentration of the Econeem, *Acorus calamus* and *Piper longum* treated larvae was significantly lower in Econeem and marginal decrease in the *Piper* l*ongum* treatment when compare to control. The Econeem and *Acrous calamus* treatment caused heavy fall in the glucose level on the larvae and pupae.

EFFECT OF ECONEEM AND PLANT EXTRACTS ON THE HAEMOLYMPH PROTEIN PATTERN AND THEIR QUALITATIVE CHANGES IN THE LARVAE AND PUPAE OF *SYLEPTA DEROGATA*

The haemolymph protein pattern were observed during development in several insects (Wyati, 1961: Rajagopal and Basheer, 1993 and Archana and Nath, 1995). Proteins were supplied by diet or by synthesis by trans-amination (Mills and Cochran, 1963, Sasaki and Ishikawa, 1995). Twenty different aminoacids were synthesized from 20 different multi-enzyme sequences. The total amino acid level reflects. It was found that both the numbers and the concentrations of these proteins varied during development. According to Archana and Nath (1995) the concentrations of proteins increased during the larval stages and decreased during the pupal stage, because of the morphogenetic process of different organs taking place. Only few worker have studied the effect of insecticides on haemolymph protein in

Manduca sexta (Wongkobrat and Dablman, 1976). Generally protein promotes physiological processes, moulting , growth as well as ovulation (Beck,1950). Increase in the litre of protein is also reported just prior to pupation in *culex* species (chen,1959) and *Drosophila* (Chen,1966).

However no account is available for the impact of Econeem, *Acrous calamus* extract and *Piper longum* extract on *Sylepta derogata* keeping this in vies, the present work has been undertaken to study the effect of these extracts on haemolymph protein in larvae and pupae of *Sylepta derogata*. Jasmine **et al.,** (2002) reported that the total haemolymph protein had increased significantly in the *Bombyx mori* larvae when treated with diethylsitos. They also reported that, the appearance of new polypeptides in the protein profile of the haemolymph of *Bombyx mori* after treatment.

According to Padmaja and Rao (1999) the protein concentration of the control larvae of *Helicoverpa armigera* increased with the advancement of age, but significantly decreased after the treatment of a seratum oil.

Deformed Larva of *Sylepta derogata* Treated with *Acorus calamus*

Deformed Larva of *Sylepta derogata* Treated with *Piper longum .L*

Deformed Larva of *Sylepta derogata* Treated with Econeem

Deformed Pupa of *Sylepta derogata* treated with *Acorus calamus*

Deformed Pupa of *Sylepta derogata* treated with *Piper longum .L*

Deformed Pupa of *Sylepta derogata* treated with *Econeem*

EFFECT OF ECONEEM AND PLANT EXTRACT ON THE LARVAE OF *SYLEPTA DEROGATA* ON SDS –PAGE OF HAENCOLYMPH PROTEIN PROFILE

In control V instar larvae of *Sylepta derogata* four protein bands (0.36,0.41,0.53, 0.68) were observed during active feeding stage. In Econeem treated larva, similar number of protein bands (0.43,0.58, 0.66) were observed with uniform pattern and irregularities distribution.

At 48 hrs the protein profile of Econeem treated larvae showed the presence of four bands with increased (Rm range 0.36, 0.48, 0.56, 0.63) in one band. Some protein fractions (Rm range 0.5, 0.6) were observed in *Piper longum* treated larvae in the initial period, later on they slowly decreased in band and also the colour (Rm range 0.41, 0.48, 0.58) after 48 hrs were observed.

There are three protein bands were seen in the *Acrous calamus* treated larvae (Rm range 0.46, 0.53, 0.68) later on they slowly decreased and disappeared at the 48 hr treatment, at the end two bands were seen(Rm range 0.40, 0.53, 0.60,0.65). Formulation of new protein fraction also observed. The above results are corroborated with that of the work of Kulkarni and Mehrotra (1973). According to them, this new protein band may be new in reality and thought some of the native proteins in the band formed slightly in chromatographic analysis, there could be many exposure of insects to insecticides leads to the induction of microsomal enzyme which metabolism xenobiotics and a possible correlation has been made been the enzyme induction due to insecticide and protein biosynthesis (Wilbinson and Brattson, 1972). It is inferred or decrease in the haemolymph proteins of larvae are due to the toxic effect of Econeem and *Piper longum* and *Acorus calamus.*

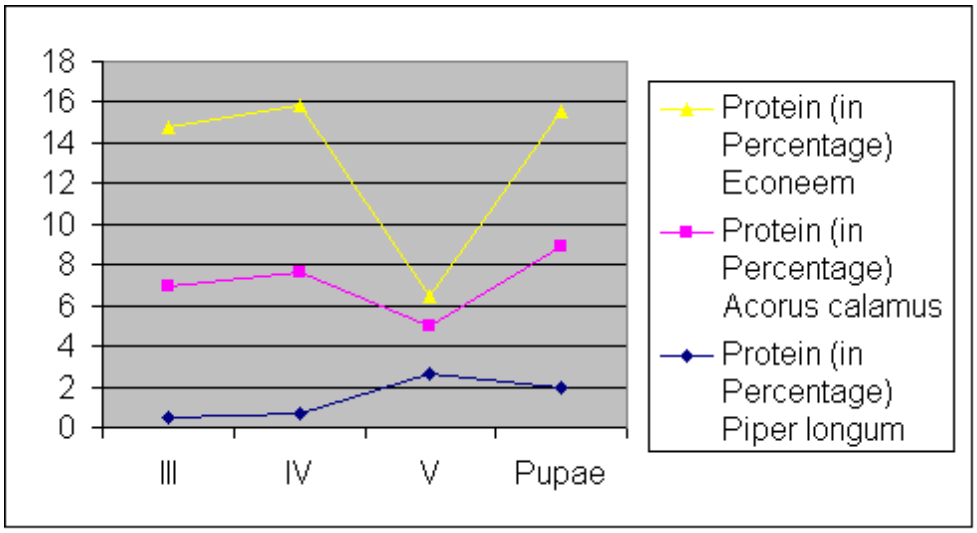
Table:3 Effect of *Acorus calamus* on the Protein and Glucose level of the larvae and pupae of *Sylepta derogata*

S.No.	Larval stage	Protein content mg/ml		Glucose content mg/ml	
		Control	Treated	Control	Treated
1.	III Instar	0.934 ± 03	0.994 ± 03 *	50.16± 2.32	45.85 ± 2.28 *
2.	IV Instar	0.930 ± 03	0.995 ± 02 * *	50.40± 2.28	46.30 ± 2.13 *
3.	V Instar	1.338 ± 00	1.305 ± 03 *	51.30± 2.28	44.80 ± 0.65 * *
4.	Pupae	0.932 ± 03	0.997 ± 03 *	30.90± 2.28	28.70 ± 0.4 * * *

Each value represents the mean of 5 determinations.
Sign + or - represents percentage increase or decrease over the control.

P < 0.01 *
P < 0.05 **
NS = Non Significant * * *

Fig:Percent variation of Protein content of *Sylepta derogata*



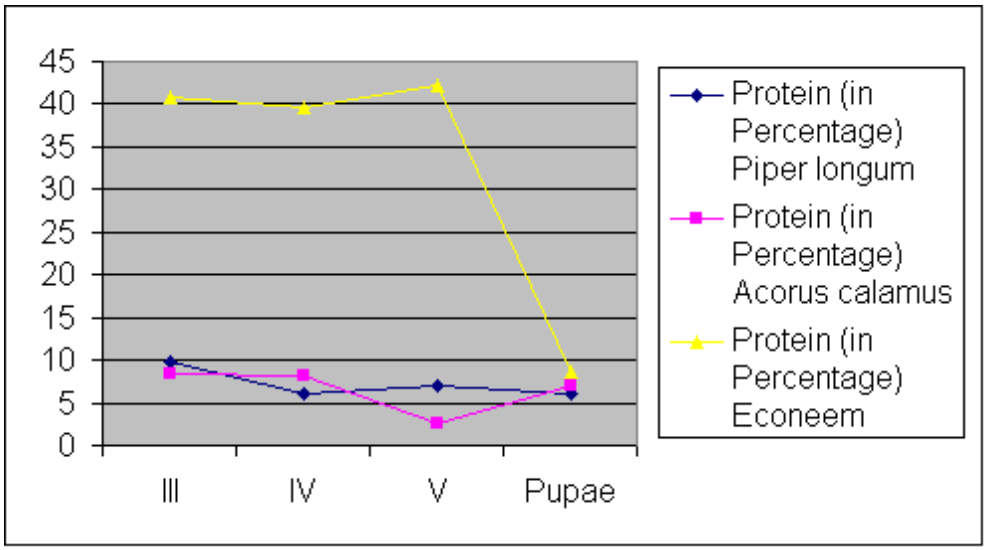


Fig: Percent variation of Glucose level of *Sylepta derogata*

IV. DISCUSSION

Literature on the larvicidal effect of chemical and Bio-pesticides in Lepidopteran insect is very vast. Fadare and Amusa(2003), reported that, the percentage of bollworm damage caused ranging from 12 to 13% after the post spray application of chemical pesticide.

The antifeedant, insecticidal and repellent action of *Acorus calamus* and other plant extract against several insect species were well reported by (Saxena and Srivastava, 1972) Edig and Davis, 1980 and Tripathi and Sing 1993 and chandel ***et al.,*** 2001). The products of neem have been studied for their efficacy against large number of insect pest (Mehrotra and Gujar, 1986, Schmutter 1990 and Gujar 1997). It has been found to be an antifeedant, chitin synthesis inhibitor and ecdysteriod inhibitor against a number of lepidopterous and hemipterous insect pests (Kubo ***et al.,*** 1983, Chockalingam ***et al.,*** 1990 and Krishnayya and Rao, 1995), Gopal and Senguttuvan (1997), Sarode (1998) and Vaish1998) who observed that *Helicoverpa armigera* was successfully managed in Chick Pea Crop by the use of bio-agents like, Azadirachtin, Btk, and Ha NPV.

It was found that the *Sylepta derogata,* stopped its spinneret and failed to produce tiny silken thread to roll the leaves after the Econeem and *Acorus calamus* treatement. Starvation in the larvae was noted when *Sylepta derogata* treated with *Piper longum* extract, when compared to other two botanicals, the *Acorus calamus* extract treatment caused lesser mortality in the larvae of *Sylepta derogata* and *Piper longum* find Econeem caused severe damage in the larvae. The results in the present investigation are similar to the findings of the earlier workers Saxena and Srivastava, 1972. When the high dosage of botanical extracts with increased duration caused significant mortality in the larvae of *Sylepta derogata.*

SUMMARY OF RESULTS

Toxicity of Econeem, *Acorus calamus* and *Piper longum* enhanced to arrest the spinneret and lead to arrest the silken thread formation.

1. Morphological deformities were observed at low dosages in all the three treatments.
2. The total protein content of larval haemolymph was significantly increased in Econeem. The non significant increase in the protein content was also noted V-instar larva treated with 25% of 1.304. The increase in the protein content after treatment might be synthesis of new protein or accumulation of new protein due to toxic effect to compared with the toxic stress and nullify the toxic effect.
3. The significant decline in the glucose level were observed in that treated larva when compared to the control. The significant increase was noted 44.30% in III instar larvae after Econeem treatment. The glucose might be either used for synthesis of trehalose, nor directly enter into the TCA cycle for production of energy. The utilization of more glucose level in the treated larvae than the control was clearly showed the effect of toxicity caused more stressed in the larva which is need for more energy.
4. The heamolymph protein profile showed the disappearance and reappearance of new protein fractions with varied peak length have been observed.

V. CONCLUSION

In the present investigation, the administration of Econeem, *Acorus calamus* and *Piper longum* promoted the larval morphological deformities in different larval stages at different doses at varies duration. The toxicity in the treated larvae enhanced the biochemical changes which altered the quantities of protein and glucose content. When compared to other two plant

extracts, the Econeem is more effective and the *Acorus calamus* extract is equally important to that of Econeem, the *Piper longum* extract also exhibited its potential to control the larva and altered the biochemical constituents, therefore from the above findings it is inferred that Econeem and *Acorus calamus*, *Piper longum* extracts may be used in the pest control management instead of hazardous chemical pesticides.

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