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RESEARCH ARTICLE

COMPARATIVE STUDIES OF ECONEEM AND *PIPER LONGUM* EXTRACTS AGAINST COTTON ROLLER *SYLEPTA DEROGATE FAB.*

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ABSTRACT

Cotton is a plant which is desirable to many pests. For this reason, many chemical and synthetic pesticides are used of which many are insecticides. Plant extracts aspest repellents is a very effective method of pest control. Plant extract is locally available and which is found least attractive to pests can be used as pest repellent in the crop. The cotton leaf roller *Sylepta derogata* is a key pest of cotton, irrespective of the use of conventional and organic management. The damage caused by the caterpillars whose feeds on the leaves and young buds. *Piper longum* is a slender aromatic climber and having repellent and insecticidal property. Neem products have been used in india for over two millennia for their medicinal properties. Neem oil can cause some forms of toxic encephalopathy. Neem is key ingredient in non-pesticidal management. One of the important effect of neem is the ovicidal effect or egg mortality against agricultural insect pests. ertain phytochemicals are known to be antifeedant compounds or reject ants which can deter insect pests from feeding plant tissues.

KEY WORDS: Sylepta Derogate, Piperlongum, Econeem, Larvicidal Effect, Protein and Glucose Level.

INTRODUCTION

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. Cotton plants, however, host a variety of herbivorous pests, and the lack of the proper adoption of pest control practices in organic cotton fields makes it difficult to produce a profitable crop. 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. Plants infested with cotton leaf roller Sylepta derogata exhibit reduced development and curled leaves, especially the young leaves driving the growth of the main stem and the leaves of the reproductive branches (Leclant; Deguine 1994). Botanical extractive, as non toxic bio rational substances are capable of bringing soil plant pest system into balance. This sequence leads to good crop health. Recently, attention has been given to the isolation and identification from plant source for various botanical compounds, possessing insecticidal properties (attracting, repeeling, feeding, deterring, growth inhibiting and reproduction steriling effects).

Sylepta derogate

Cotton leaf-roller S*ylepta derogata Fab* is a sporadic pest of cotton in tropical and subtropical regions (Sohi, 1964) and belong to the family Pyralididae.

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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 growsThe webbing and withering of leaves could be the symptoms of this pest infection. 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.

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 and is a flowering vine in the family *Piperaceae*. 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. Indian long pepper contains chemical called piperine. Piperine may be able to fight certain parasites that can infect people. 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.

Empirical formula: $C_{17}\ H_{19}\ O_3\ N$

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.

Empirical formula: $C_{35}\ H_{44}\ O_{16}$

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.

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.

Adult of *Sylepta derogata*

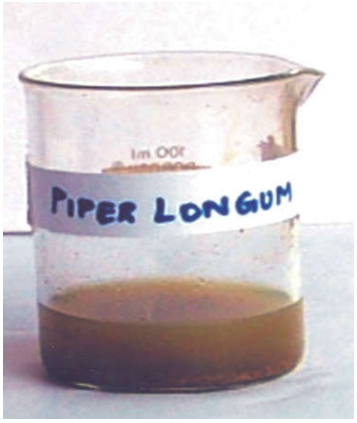
Methods for plant extract preparation

Plant materials

The plants were used in present study are *Azadiracta indica and Piper longum* were collected. The whole plants were air dried under natural laboratory conditions. Dried plants were ground using electric mill sieved and kept for extraction.

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).



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.

Larval mortality

The newly moulted I, II, III, IV and V instar larvae of *Syleta derogata* starved for 2 hours. After measuring the initial weight of the larvae. They were individually introduced into separate plastic containers. The different concentrations of plant extracts of *Acorus calamus* (5%, 10%, 15%, 20%, 25%) *Piper longum* (5%, 10%, 15%, 20%, 25%) were prepared from the stock solution by adding 80% acetone were prepared by using distilled water. The different concentration of Econeem (5%, 5%, 10%, 15%, 20%) were prepared by using distilled water . The I, II, III, IV and V instar larvae of *Sylepta derogata* were treated topically with different concentration of Econeem and *Acorus calamus and piper longum* extracts used fresh cotton leaves soaked for 10 seconds. Control larvae fed with fresh cotton leaves soaked in acetone for 10 seconds and 20 larvae of *Sylepta derogata* are used for each experiment. The experiment was repeated 5 times and after 24 hours and 48 hours the number of death larvae were noted.. The percentage of mortality was calculated by using the following formula.

The results were corrected for control mortality by using Abbots (1925) formula.

$$\text{Percentage Mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

The results were corrected for control mortality by using Abbots (1925) formula.

$$n_t = \frac{n_o - n_c}{100 - n_c} \times 100$$

Where

n_t = Corrected percent mortality
n_o = Observed mortality in treatment
n_c = Observed mortality

Biological assay

For Biological assay, the percentage mortality data after corrections were subjected to probit analysis for calculating Median lethal concentration (LC 50) (Finney , 1991).From the Median lethal concentration, the physiological doses were selected for biological and biochemical studies. The II - instar larvae of *sylepta derogata* were treated with safe sublethal doses (Econeem 0.8%, *Acorus calamus* 0.9% and *Piper longum*1.4%) for 48h and the larvae and has been allowed to fed untreated leaves. After the sublethal treatment the morphological changes of larvae has been studied till pupation.

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.

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

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

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

- To 0.5mLof larval haemolymph was added with 0.5mL distilled water and 1mL glucose oxidase-peroxidase reagent.
- 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.
- All the tubes at 35º C for 40 minutes were incubated.
- Addition of 2mL of 6N-HCl to terminate the reaction.
- The intensity of the colour developed were read at 540 nm.

Preparation of standard graph

The standard graph for Glucose were plotted by using the values of the concentration against optical density. The Glucose level of the samples were calculated from standard graph.

Statistical analysis

Results obtained in the present investigation has been subjected to the following statistical analysis.

- Standard Deviation
- Standard Error
- 't' Test

STANDARD DEVIATIONS (± SD)

$$SD = \sqrt{\frac{\sum d^2 \left[\sum d^2\right]}{n \quad n}}$$

where d = deviation taken from assumed mean
n = number of observation

STANDARD ERROR (SE)

$$SE = \frac{SD}{\sqrt{n}}$$

SD = Standard Deviation

't' TEST

$$t = \frac{X_1 - X_2}{\sqrt{\frac{S_1^2}{n_1 - 1} + \frac{S_2^2}{n_2 - 1}}}$$

X_1, X_2 = Means of Sample data
S_1, S_2 = Standard Deviation
n_1, n_2 = Total no.of values

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
- Ammonium 0.5g/10ml, prepare freshly before use
- Persulphate 5%
- 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

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. 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. 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.When the gel has polymerized, remove the water layer and rinse the gel surface with stacking gel buffer. Mix the stacking gel components in the same way as described above for the resolving gel. 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. 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. 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

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

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

RESULTS

Effect of econeem on the larvae of sylepta derogata

I-instar larvae

Amount different concentration of Econeem treated on first instar larvae of *Sylepta derogata.*

At the end of 24hrs (5%) econeem treatment, the larvae showed 62.97% mortality and increased upto 61.90% after 48hrs. The Econeem concentration increased to (10%) the percentage of larval mortality also increased. The mortality rate is found to be 68.39% at 24hrs and 72.82% in 48hrs . The (15%) of Econeem treatment exhibited 75.09% mortality at 24 hrs. and correspondingly increased to 78.48% at the end of the 48 hrs. At (20%) of Econeem treatment, the larval mortality rate 78.48% in 24hrs and 83.66% in 48hrs. The high degree of larval mortality was observed at (25%) level of 24 hrs. 91.41% and 48hrs has 72.82% of larval mortality.

II- Instar larvae

The second instar larvae exhibited the mortality 37.64% rate at (5%) concentration at the end of 24 hrs. The same concentration influenced the higher mortality 52.49% after 48 hrs. The (10%) Econeem formulation caused 57.67% mortality during 24 hrs and it elevates the mortality rate upto 61.90% after 48 hrs. The considerable elevation in the percentage mortality was found at (15%) level for 24 hrs 61.90% and 48 hrs 72.82%. The (20%) Econeem concentration exhibited 66.23% mortality at 24 hrs and it was increase 75.09% after 48 hrs. The significant mortality rate was observed during the 24 hrs 85.44% and 48 hrs 90.20 at (25%) level.

III- Instar larvae

After 24 hrs. treatment, the larvae showed the mortality rate at 5% level 31.97% and 48 hrs. 37.64%. The larval Mortality in 10% strength after 24 hrs 37.64% and 48 hrs. 52.49% were observed. The 15% of Econeem formulation caused 52.49% mortality during 24 hrs. and it elevates the mortality rate upto 62.97% after 48 hrs. The concentration of 20% Econeem exhibited 59.81% Mortality and 24 hrs and it was oincrease 68.39% after 48 hours. At 24 hrs 25% Econeem treatment, the larvae showed 68.39% and upto 72.83% after 48 hrs.

IV- Instar larvae

Results reveal that among the different concentration of Econeem applied on fourth instar larvae of *Sylepta derogata*. At (5%) level it has give 36.69% mortality after 24 hrs and 46.43% were observed in 48 hrs treatment. The mortality rate 37.64% at (10%) concentration at the end of 24 hrs and 52.49% at 48 hrs treatment. The (15%) concentration of Econeem caused 46.43% mortality during 24 hrs and the death rate upto 57.67% during 48 hrs were observed. The effect of (20%) econeem against fourth larval instars were observed in 24 hrs and 48 hrs in 59.81% and 71.83% mortality. At 25% concentration in 24 hrs treatment, exhibited 68.19% mortality and after 48 hrs has given 78.48% of larval mortality

V- Instar larvae

When the fifth larval instars of *Sylepta derogata* are treated with Econeem (5%) larval mortality is 31.97% after 24 hrs treatment and 48 hrs treatment, it has given 37.64% mortality. After 24 hrs the mortality rate is 36.69% and 48 hrs the rate of mortality is 48.45% at (10%) concentration. The effect of Econeem at (15%) after 24 hrs and 48 hrs is 46.43% and 57.67% Econeem (20%) have shown 57.67% and 68.19% larval mortality at 24 hrs and 48 hrs. After 24 hrs treatment, the larvae showed the mortality rate at (25%) level 59.81% and 72.82%.

Effect of *piper longum* extract on the larvae of *Sylepta derogata*

I- Instar larvae

Application of *piper longum* extract on the First instar larvae showed, significant mortality level at 24 hrs 36.69% and 48 hrs 52.49% at (5%) treatment.

The (25%) extract formulation caused 66.33% mortality during 24 hrs and it elevates the mortality rate upto 90.20% after 48 hrs.

II- Instar larvae

The second instar larvae exhibited the mortality 29.20% rate at (5%) concentration at the end of 24 hrs.

Table 1. Effect of econeem on the larvae of sylepta derogata

	Larva	% larval mortality														
		First Instar			Second Instar			Third Instar			Fourth Instar			Fifth Instar		
		24hr	48hr	mean	24hr	48hr	mean	24hr	48hr	mean	24hr	48hr	mean	24hr	48hr	mean
5%	20	61.90	62.97	62.43	37.64	52.49	45.06	31.97	37.64	34.80	36.69	46.43	41.56	31.97	37.64	34.80
10%	20	68.39	72.82	70.60	57.67	61.90	59.78	37.64	52.49	45.06	37.64	52.49	45.06	36.69	48.45	42.57
15%	20	75.09	78.48	76.78	61.90	72.82	67.36	52.49	62.97	57.73	46.43	57.67	52.05	46.43	57.67	52.05
20%	20	78.48	83.66	81.07	66.23	75.09	70.66	59.81	68.39	64.10	59.81	71.83	65.82	57.67	68.19	62.93
25%	20	91.41	91.41	91.41	85.44	90.20	87.82	68.39	72.83	70.60	68.19	78.48	73.33	59.81	72.82	66.31

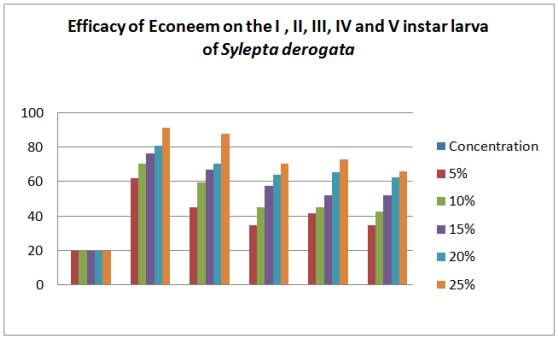


Table 2. Efficacy of *Piper longum* on the I , II, III, IV and V instar larva of *Sylepta derogata*

Conc	Larva	% larval mortality														
		First Instar			Second Instar			Third Instar			Fourth Instar			Fifth Instar		
		24hr	48hr	mean	24hr	48hr	mean	24hr	48hr	mean	24hr	48hr	mean	24hr	48hr	mean
5%	20	36.69	52.49	44.59	29.20	73.64	33.42	28.27	36.69	32.48	23.73	35.75	29.74	17.70	36.69	21.17
10%	20	48.48	78.48	63.48	37.64	52.49	45.06	31.97	37.64	34.80	31.97	37.64	34.80	28.27	37.64	32.48
15%	20	50.57	70.79	60.68	46.43	55.61	51.02	36.69	52.49	44.59	37.64	52.49	45.06	31.97	57.67	34.80
20%	20	60.88	85.44	73.16	57.67	68.19	62.93	46.43	62.97	54.70	46.43	57.67	52.05	46.43	64.06	52.05
25%	20	66.33	89.06	78.26	68.19	78.48	73.33	52.49	71.83	62.16	57.67	71.83	64.75	57.67	68.39	60.86

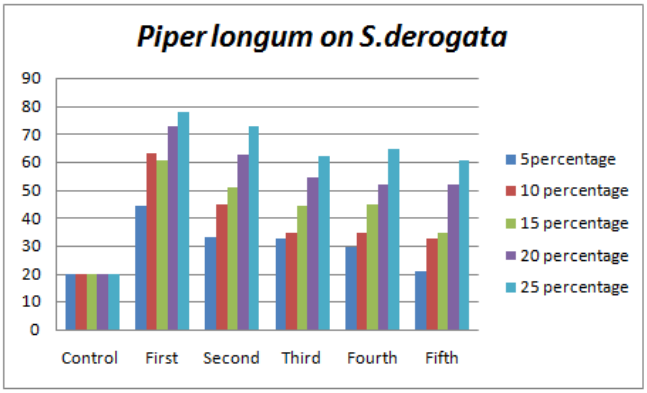


Figure 2.

The administration of *piper longum* extract (10%) at the end of 24 hrs in 48.48% and 48 hrs in 78.48% level of mortality. The (15%) of extract treatment exhibited 50.57% at 24 hrs and correspondingly increased to 70.79% at 48 hrs. At (20%) of *Piper longum* extract treatment, the larval mortality rate 60.88% in 24 hrs and 85.44% in 48 hrs.

The same concentration influenced the higher mortality 37.64% after 48 hrs. The *Piper longum* extract concentration increased to (10%) the percentage of larval mortality also increased. The mortality rate is found to be 37.64% at 24 hrs and 52.49% is 48 hrs. At (15%) the percentage of larval mortality at 24 hrs and 48 hrs 46.43% and 55.61% were

observed. The concentration of (20%) in 24 hrs 57.67% and 48 hrs 68.19% were noted. *Piper longum* extract (25%) has recorded 68.19% of larval mortality in 24 hrs and 78.48% in 48 hrs.

III- Instar larvae

Among the different concentration of *Piper longum* extract applied on third larval instar of 5% level has given 28.27% mortality in 24 hrs and 36.69% in 48 hrs. At 10% concentration in 24 hrs 31.97% and 37.64% in 48 hrs *Piper longum* extract 15% concentration, the larval mortality is 36.69% in 24 hrs and 52.49% in 48 hrs. The 20% extract formulation caused 46.43% mortality during 24 hrs and mortality rate upto 62.97% in 48 hrs. The high concentration of *piper longum* extract 25% in 24 hrs 52.49% and 48 hrs 71.83 was noted.

IV- Instar larvae

The fourth instar larvae exhibited the mortality 23.73% rate at (5%) concentration of 24 hrs, the same concentration influenced higher mortality 35.75% at 48 hrs. At (10%) extract formulation caused 31.97% in 24 hrs and 37.64% in 48 hrs. The considerable elevation in the percentage mortality was found at (15%) level for 24 hrs 37.64% and 52.49% in 48 hrs. The (20%) concentration of *Piper longum* extracts after 24 hrs and 48 hrs in 46.43% and 57.67%. The higher mortality obtained in (25%) concentration of extract after 24 hrs in 57.67% and 48 hrs in 71.83%.

V- Instar larvae

When fifth larval instars of *Sylepta derogata* are treated with *Piper longum* extract at (5%) concentration is 17.70% after 24 hrs and 24.65% after 48 hrs. At (10%) extract formulations were treated, the results obtained after 24 hrs is 28.27% and after 48 hrs is 36.69%. The (15%) concentration of *Piper longum* extracts were applied, the mortality rate was obtained after 24 hrs and 48 hrs is 31.97% and 37.64%.

The administration of *piper longum* extract (20%) at the end of the 24 hrs in 46.43% and 48 hrs in 57.67% were obtained. At higher concentration (25%) has given higher larval mortality after 24 hrs and 48 hrs in the percentage of 57.67% and 64.06%. Different concentration of Econeem resulted better mortality rate on I larval instar of *Sylepta derogata*. From these results, it is observed when the concentration of Econeem increased, correspondingly the mortality rate also increased. The mortality effect of Econeem is more effective than *Piper longum* extract.

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 7. 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

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

Deformed Larva of *Sylepta derogata* Treated with Econeem

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 *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 9. 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 9. When compared to the larvae, and pupae showed decreased level (0.901 mg/ml) of protein Table 9. 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.

Table:3 Effect of Econeem on the level of Protein and Glucose of the larvae and pupae of *Sylepta degrogata*

	Larval stage	Protein content mg/ml		Glucose content mg/ml	
		Control	Treated	Control	Treated
1.	III Instar	0.906 ± 03	0.978 ± 02 *	44.30 ± 2.28	26.20 ± 0.14 *
2.	IV Instar	0.907 ± 03	0.982 ± 03 *	42.80 ± 2.28	25.80 ± 0.17 *
3.	V Instar	1.318 ± 02	1.304 ± 03 * *	42.50 ± 2.28	24.60 ± 0.29 *
4.	Pupae	0.901 ± 03	0.962 ± 03 *	32.50 ± 2.28	29.70 ± 0.15 * * *

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 * * *

Table 4. Effect of *Piper longum* on the Protein content 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	0.971 ± 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 * * *

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 * * *

Table 5. The Relative Mobility (RM) values of haemolymph, Protein fractions in control and treated V-instar larvae of *Sylepta derogata*

S.No.	Name of the Treatment	Rm values treated with 24 hrs.		Rm values treated with 48 hrs.	
		Control	Treated	Control	Treated
1.	Econeem	0.36 (D)	0.43 (L)	0.36 (D)	0.36 (L)
2.	Econeem	0.41 (D)	0.58 (M)	0.41 (D)	0.48 (M)
3.	Econeem	0.53 (M)	0.66 (D)	0.53 (M)	0.56 (D)
4.	Econeem	0.68 (L)	--	0.68 (L)	0.63 (D)

D = Dark bands; M = Medium bands; L = Light bands

Table 6. The Relative Mobility (RM) values of haemolymph, Protein fractions in control and treated V-instar larvae of *Sylepta derogata*

S.No.	Name of the Treatment	Rm values treated with 24 hrs.		Rm values treated with 48 hrs.	
		Control	Treated	Control	Treated
1.	P. longum	0.36 (D)	0.5 (L)	0.36 (D)	0.41 (M)
2.	P. longum	9.41 (D)	0.6 (L)	9.41 (D)	0.48 (M)
3.	P. longum	0.53 (M)	--	0.53 (M)	0.58 (D)
4.	P. longum	0.68 (L)	--	0.68 (L)	--

D = Dark bands; M = Medium bands; L = Light bands

When compare to the control and treated pupae showed significantly increased level of protein 6.7% (Fig.1).

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 12.

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 12. There was marginal decrease 29.30mg/ml in the glucose concentration in *Piper longum* treated pupae, when compared to control.

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 plant extracts 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 (Mebrotra 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 treatement. Starvation in the larvae was noted when *Sylepta derogata* treated with *Piper longum* extract, when compared to other botanicals, *Piper longum* find Econeem 91.41% 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.*

The result of the present study showed that more than 88 percentage of the adults were dead within 24hrs and it was increased 92% at 48 hrs. The population of the larvae originally introduced was reduced due to the phagodetteresnt and the insecticidal action. Starvation of the larvae is also evident with pupal formation on VI instar treatment after 6 days. The econeem treatment also effective in the *Sylepta derogate* larvae which caused 91.7% mortality in the first instar after 48hrs. The *Piper longum* extract caused lesser damage in the larvae which is about only 80%. Therefore it is concluded Econeem is highly effective which caused severe damage in all stages of larvae when compare to *Piper longum* extract.

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 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, 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 *Piper longum* treatment caused heavy fall in the glucose level on the larvae and pupae.

Effect of econeem and *Piper longum* on the haemolymph protein pattern and their qualitative changes in the larvae and pupae of *sylepta derogate*

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, 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.

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. 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.*

Summary of results and Conclusion

Toxicity of Econeem, and *Piper longum* enhanced to arrest the spinneret and lead to arrest the silken thread formation.Morphological deformities were observed at low dosages in all these treatments.High percentage mortality 91.41% was observed in Econeem at 25% level. *Piper longum* showed low mortality rate 90.20%. 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.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. The heamolymph protein profile showed the disappearance and reappearance of new protein fractions with varied peak length have been observed.

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