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Impact of Selected Plant Oils on Haemolymph Protein in the Different Instar Larvae of *Pericallia ricini* (Fab) (Lepidoptera:Arctidae)

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Abstract: Now-a-days scientist and planners are giving more emphasis on organic farming and sustainable agriculture that is the demand of the time and that can manage the pest population with the minimum use of efficient and effective biocides, which are eco-friendly, do not leave any toxic residue on agriculture products and also cost effective. Plant oils are well known to act as biopesticides. The potential losses caused by pest damage worldwide contribute to a loss of nearly 50%. In tropical regions like India, the loss is estimated to be even higher than this. Synthetic pesticides though have played a major role in controlling the Lepidopteron pests, could not control much. This has led to the formulation of several synthetic chemicals, such as organochlorines, organophosphates, carbamates, pyrethroids, and more. Major breakthrough of such synthetic pesticides has triggered research on the use, which in turn has caused an irretrievable damage to the environment. *Pericallia ricini* is a polyphagous wooly bear moth. It is a serious pest on *Ricinus communis* commoly called castor plant. In this work an attempt has been made to study the effect of different concentration of selected plant oils namely Neem Oil (*Azadirachta indica*), Eucalyptus Oil *(Eucalyptus globulus*) and Lemongrass Oil (*Cymbopogon citratus*) on haemolymph protein in III, IV and V instar larvae of *P. ricini.* Results showed that when increasing concentration of plant oils, there was decrease in the protein content of the insects. Based on our findings, it is recommended to compact the agricultural pest *P.ricini* using the above said plant oils with an ecofriendly strategy.

Keywords: *Pericallia ricini, Ricinus communis, Azadirachta indica, Eucalyptus globules*, *Cymbopogon citrates*, Pest, Instar, Larvae

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Introduction

The main economically important crops in Tamil Nadu are cotton, sugarcane, oilseeds, coffee, tea, rice, maize, jowar, bajra, ragi, and pulses. The amount of food production has witnessed a fall

due to pest and diseases that cause crop damage either directly by causing economic losses to crops in the field or indirectly by causing disease and reducing crop yield. To feed the growing population there is a need to double the food production that warrants a threefold increase in the annual use of fertilizers and pesticides. Modern Agriculture involves the use of commercially available pesticides to combat a variety of insects, weeds, and other agricultural pests. Some of these pesticides are considered to be toxic to human-beings and pose potentially serious health risks to non-target organisms (Mendki *et al.*, 2001).

Essential oils (EO) and plant extracts represent alternatives to pesticide for pest control (Machial *et al.*, 2010; Fouad *et al.*, 2014) with the advantage of high diffusion rate in the environment because of their low molecular weight and high vapor pressure (Bakkali *et al*., 2008). Essential oils are cost effective and have lower risk on the environment and beneficial organisms (Matos Neto *et al.*, 2002; Alagawany *et al.*, 2021; El-Tarabily *et al.*, 2021), beside interfering with the biology, physiology, and nervous system of the insect (Mikhaiel, 2011, Mann and Kaufman, 2012). Consequently, use of essential oils may allow a more sustainable agricultural practice (Isman, 2006).

The azadirachtin is nowadays one of the most important natural insecticides due to a secondary metabolite produced by the neem tree (*Azadirachta indica* A. Juss.). Many formulations of neem seed oil exhibit insecticidal properties like antifeedant, ovicidal, larvicidal, insect growth regulatory, and repellent activity in a wide variety of insect taxa, including Lepidoptera (Chaudhary *et al.*, 2017).

Eucalyptus, neem, lemongrass, citronella, ginger grass and palmarosa provided a total protection to groundnut pods by inhibiting ovipisition of bruchid, *Caryedon serratus* (Mishra and Dash, 2009). Different concentrations of neem oil were highly effective against IV instar of *P. ricini* (Revathi and Kingsley, 2008).

Pericallia ricini Fab. (Lepidoptera:Arctiidae) commonly known as hairy caterpillar is the major pest of castor, gingelly, cotton, country bean, brinjal, drumstick, banana, calotropis, sunflower, oleander, tea, sweet potato and pumpkin (David and Ananthakrishnan, 2004). In the present study an attempt has been made to use Neem Oil (*Azadirachta indica*), Eucalyptus Oil *(Eucalyptus globulus*) and Lemongrass Oil (*Cymbopogon citratus*) aganist the different instars of *P. ricini*.

Materials and Methods

Culture of the insect:

The eggs and freshly emerged I instar larvae of *P. ricini* were collected from the castor plants cultivated in the vicinity of Madurai and kept in the laboratory at room temperature of 29±1˚C throughout the period of study. The larvae were fed with fresh castor leaves and allowed to hatch to moths. These moths were fed with 10% sucrose solution soaked in a small piece of cotton. Male and female moths were kept in separate container for mating. Castor leaves were introduced and the moths were allowed to lay eggs. The egg hatching was completed on the leaf itself and the freshly emerged I instar larvae were collected and separated. These individuals were maintained in the laboratory for the experimental purpose. The glasswares used were often sterilized with 0.1% mercuric chloride solution. III, IV and V instar larvae were separated for experimental studies and were weighed on a mono-pan balance sensitive to 0.01 mg.

Plant oils used:

Three plant oils namely Neem Oil (*Azadirachta indica*), Eucalyptus Oil *(Eucalyptus globulus*) and Lemongrass Oil (*Cymbopogon citratus*) were selected for this study. Three different concentrations (25%, 50%, and 100%) of each of the tested plant oils were prepared from the stock solution by diluting with acetone in volumetric flasks to desired concentrations.

Method of application:

The susceptibility of the III, IV and V instar larvae

of *P. ricini* to the above mentioned plant oils was tested by the use of leaf spraying technique as follows: Fresh castor bean leaves, *Ricinus communis* were sprayed with different concentrations of selected plant oils, and then the leaves were left until dryness before being offered to the larvae for feeding on it.

Biochemical determination:

In each treatment, castor leaves sprayed with (LC_{50}) of selected plant oils (Neem oil, Eucalyptus oil and Lemon grass oil) were offered to five replicates of III, IV and V instar larvae. Control groups were also maintained simultaneously. Each replicate contains ten larvae in wide–mouth plastic containers. Newly treated leaves were offered to the larvae every day; after three days the haemolymph was collected from treated larvae for the analysis of biochemical constituents.

Haemolymph collection:

Haemolymph was collected from the survived III, IV and V instar larvae by cutting one or two prothoracic legs and drown into a test tube containing a few milligrams of phenylthiourea to prevent inhibitory effect of tyrosinase (Abou El-Ghar *et al*., 1996). Samples thus obtained were centrifuged at 2500 rpm for 10 min at 4°Ć. The supernatant was used for further investigation.

Estimation of Protein content:

The protein content of control and experimental samples was determined spectrophotometrically by Lowry *et al.* (1951) method using bovine serum albumin (BSA) as standard.

Results

Protein metabolism is considered as most important in the insect physiology because of its vital role in the determination of chemical characters of insect proteins. Haemolymph play a major role in protein metabolism and they are the important sites of biocide toxicity. In the present study significant variations are observed in the protein metabolism of III, IV and V instar larvae of *P. ricini* on exposure to selected plant oils at different concentrations (0, 25, 50 and 100%).

Effect of neem oil on protein content:

The total protein content in treated larvae was reduced from 79.8 ± 0.84, 84.2 ± 0.9 and 91.6 ± 1.7 in the control to 48.6±1.0, 51.2±1.2 and 61.8±1.3 treated at the concentration of 25%, 41±1.4, 44±1.4 and 51±1.04 treated at the concentration of 50%, and 36.4±1.0, 38.4±1.0 and 47±1.3 at the concentration of 100%, respectively in III, IV and V instar larvae of *P. ricini.* Data depicted that neem oil is effective against the selected insect pest at varying concentration caused maximum effect in the III and IV instar stage of the insect larvae 54.4 per cent for 100% followed by 47.7 and 39.2 per cent for 50 and 25 % treatments and 54.3 per cent for 100% followed by 48.6and 29.2 percent for 50and 25% respectively in IV and III instar stage (Table 1).

Effect of Eucalyptus oil on protein content:

When *P. ricini* was treated with *Eucalyptus* oil at varying concentrations, it was observed that, on increasing concentration of plant oil the protein level declined. 59.4±1.4 (III instar), 61.4±1.0 (IV instar) and 69.6±1.1 (V instar) of protein was found to be declined at 25%, whereas at 50% 44.6±1.0 (III instar),50±1.4 (IV instar) and 53±1.0 (V instar) reduction in the protein level was observed. At 100%, the III instar is 35.1±1.4, the IV instar is 39.8±0.7, and the V instar is 41.6±1.1 was recorded in experimental larvae. Inevitably, when the percentage effectiveness of eucalyptus oil against the selected insect pest at varying concentration was calculated, the maximum effect was recorded in the IV instar stage of the insect larvae 52.7 per cent for 100 % followed by 40.6 and 27.0 per cent for 50 and 25 % treatments, respectively (Table 2).

Effect of Lemon grass oil on protein content:

Table 3 shows protein content in the haemolymph of *P. ricini* treated with three concentration of Lemon grass oil. The protein content in the haemolymph of *P. ricini* was higher in the untreated insect when compared to treated ones. There was a gradual change in the protein content of Lemon grass oil treated *P. ricini* at the

Table 1: Effect of neem oil on total content of haemolymph protein in III, IV and V instar larvae of *P. ricini*

Concentration of Neem oil (%)	Larval Instars		
	III	IV	V
Control	79.8±0.84	84.2±0.9	91.6±1.7
25	48.6±1.0 (29.2)	51.2±1.2 (39.2)	61.8±1.3 (32.5)
50	41±1.4 (48.6)	44±1.4 (47.7)	51±1.04 (44.3)
100	36.4±1.0 (54.3)	38.4±1.0 (54.4)	47±1.3 (48.6)

Data represents the average of five replicates; Mean ± Standard error (Mean ± SD); Values in the parenthesis represents percentage reduction in content from its respective control

Table 2: Effect of eucalyptus oil on total content of haemolymph protein in III, IV and V instar larvae of *P. ricini*

Concentration of Eucalyptus oil (%)	Larval Instars		
	III	IV	V
Control	79.8±0.84	84.2±0.9	91.6±1.7
25	59.4±1.4 (25.4)	61.4±1.0 (27.0)	69.6±1.1 (24.0)
50	44.6±1.0 (44.1)	50±1.4 (40.6)	53±1.0 (42.1)
100	35.1±1.4 (56.1)	39.8±0.7 (52.7)	41.6±1.1 (54.5)

Data represents the average of five replicates; Mean ± Standard error (Mean ± SD); Values in the parenthesis represents percentage reduction in content from its respective control

Table 3: Effect of lemon grass oil on total content of haemolymph protein in III, IV and V instar larvae of *P. ricini*

Concentration of Lemon grass oil (%)	Larval Instars		
	III	IV	V
Control	79.8±0.84	84.2±0.9	91.6±1.7
25	63.8±0.83 (20.0)	70.8±0.7 (15.9)	73±0.6 (20.3)
50	56.8±0.8 (28.8)	67±0.8 (20.4)	68±0.7 (25.7)
100	49.8±0.84 (37.5)	60.8±0.8 (27.8)	62±1.1 (32.3)

Data represents the average of five replicates; Mean ± Standard error (Mean ± SD); Values in the parenthesis represents percentage reduction in content from its respective control

concentration of 25% from 79.8 ± 0.84, 84.2 ± 0.9 and 91.6 ± 1.7 in the control to 63.8 ± 0.83, 70.8±0.7 and 73±0.6 in III, IV and V instar larvae of *P. ricini*, respectively. The protein content in larvae in stages III, IV, and V was determined to be 56.8±0.8, 67±0.8, and 68±0.7 at a 50% concentration, respectively. At 100% concentration, the highest effect was observed, and the

protein content in larvae in stages III, IV, and V was determined to be 49.8±0.84, 60.8±0.8, and 62±1.1, respectively. While calculating the effectiveness of lemongrass oil against the *P. ricini* at varying concentrations, maximum effect was recorded in the III instar stage of the insect larvae 37.5 per cent for 100% followed by 28.8 and 20 per cent for 50 and 25% treatments, respectively.

Discussion

Larvicidal activity mainly depends on the presence of toxic materials in plants extracts and mortality may be due to reduction in the total protein content which is a major component for metamorphosis of the larval instars (Elanchezhiyan *et al*., 2015). In the present study, the larvae of *P. ricini* treated with neem oil exhibited decrease in the total haemolymph protein content of the insects with the increase in the concentration of Neem Oil. Maximum effect was at 100 %. Maximum effect was recorded in the III instar larvae after exposure to neem oil on insect-pest.

Reduction in the body protein content of treated insects might have been due to inhibition of further synthesis or protein degradation due to the stress (Baskar *et al*., 2011). When treated with Eucalyptus Oil similar results were observed--with the increase in the concentration of Eucalyptus Oil, there was decrease in the haemolymph protein content of the insect. While, application of essential oil from *C. citratus* at varying concentrations showed decrease in the total haemolymph protein content of the insects. Data depicts that maximum effect was recorded in the III instar stage of insect larvae 37.5 per cent for 100%. This result is in accordance with findings of Krishnayya and Rao (1995), who have noticed that application of Plumbagin reduced the protein content in *Helicoverpa armigera*.

It has been well established that protein is necessary for many physiological processes including growth, moulting, and egg development. Ayyangar and Rao (1990) showed that azadirachtin injected to the final instar larvae of *P. ricini* significantly reduced the protein content. Reduction in the body protein content of *P. ricini* has also been reported when the insects were treated with juvenoids like methoprene (Kranthi, 1991), and extracts of *Argemone mexicana and Nerium odorum* (Tripathi, 2003).

P. ricini treated with plant extract showed reduction in protein content. Decrease in protein content was observed in the larvae fed with treated wheat bran than the control. Reduction in protein content is a common phenomenon in insects after treatment with toxic compounds (Nathan *et al.,* 2008). However, it is likely that the insect degrades proteins to resultant amino acids in order to let them enter into the TCA cycle to compensate energy loss caused by stress (Nath *et al*., 1997). Results obtained in the present study coincide with other reports where toxicity of essential oil lead to reduced protein content. Bouayad *et al.* (2013) reported that the protein content was severely affected in *Plodia interpunctella* larvae fed on *Centaurium erythraea*, *Peganum harmala* and *Cistus ladanifer* treated diets.

Krishnaveni *et al.* (2013) have reported biochemical changes due to Pongam and neem oil in the V instar larvae of *Spodoptera litura*. The total protein content of post treated larvae were generally decreased (535 ± 3.45 µg/g f.w.) as compared to control. Abou El-Ghar *et al.* (1996) studied the biochemical changes in *Agrotis ipsilion* treated with extracts of *Melia azedarach.* The present study is in conformity with the findings of Padmaja and Rao (2000) who treated *Helicoverpa armigera* with extracts of *Artemisia annua, Ageratum conyzoides* and *Azadirachta indica.*

Medhini *et al.* (2012) reported highest reduction in protein content of the larvae of *Spodoptera litura* when treated with *C. olficinalis* extracts. The level of protein content in the body of larva depends upon the rate of synthesis, the breakdown of proteins and even water movement between tissues. Further, it has been pointed out that haemolymph of an insect can also account for change in protein level. The reduction may be due

to increased breakdown of proteins to detoxify the active principles present in the pongam oil. The decrease in protein level observed in present investigation may be due to their degradation and possible utilization for metabolic purposes. Elumalai *et al.* (2010) reported that neem oil showed significant insecticidal activity. It may be due to reduction in the total protein content which is a major component for the metamorphosis of the larval instars and adult *Spodoptera litura*. Decreased protein content might also be attributed to the destruction or necrosis of cells and consequent impairment in protein synthesis machinery. The quantity of protein depends on the rate of protein synthesis or on the rate of its degradation (Pathak and Pandey, 2011).

The total protein reduction caused due to plant extract treatment in *P. ricini* and other insects, studied by earlier authors revealed a complete agreement in the metabolism of *P. ricini*; this in turn will cause reduction in the organic constituents in the insects body tissues which indirectly affect the hormonal regulation of insects. It is likely that irrespective of variation in plant extracts concentration, the toxicant effected alteration in metabolic pathways withstand the stress imposed by toxicants.

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