Indian J.Sci.Res. 19(1): 80-84, 2018

ISSN: 0976-2876 (Print)
ISSN: 2250-0138(Online)

ANALYSIS OF COMPARATIVE EFFICACIES OF VARIOUS PLANT EXTRACTS AS BIO-PESTICIDES AGAINST *Rhynchophorus ferrugineus*

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ABSTRACT

Experiments were carried out to evaluate the insecticidal potential of three medicinal plants - *Ocimum sanctum, Saraca asoka* and *Asparagus racemosus* against the serious coconut pest *Rhynchophorus ferrugineus (*Red Palm Weevil*)*. Plant extracts were prepared using solvents like acetone, ethanol and water. Different doses of these plant extracts were applied to the fourth instar larvae *of R.ferrugineus*. The results showed the plant extracts had direct toxic effect on red palm weevil larvae and among them aqueous extracts of *A.racemosus* showed significant morphological changes and moulting disturbances in treated insects and the effect was dose dependent. LD $_{50}$ value was assessed using probit analysis. The effect of the plant *A.racemosus* was found to be most significant causing highest mortality compared to other plants and sublethal concentration of this extract elicited significant changes with the amount of important biochemicals such as protein, glycogen, aminoacid and lipid in treated insects compared to control.

KEYWORDS: - *Rhynchophorus ferrugineus*, Biopesticide, Biochemicals, *Asparagus racemosus*

In Kerala Coconut production plays an important role in the state economy and culture. Kerala is actually named after the coconut tree with "Kera" meaning Coconut tree and "Alam" meaning land, so means "Land of Coconut Trees". By the late 1970s it accounted for about 68% of total production in India and at one stage about 899,198 hectares were reportedly under cultivation. Today Kerala accounts for roughly 45% of India's coconut production, with some 92% of total production lying in the southern Indian states. One problem which poses a major threat to production in Kerala is the attack by Red Palm Weevil.

Red Palm Weevil (RPW), *R.ferrugineus*, is a serious pest of palms throughout South and Southeast Asia, which are the native habitat of this pest. The female weevil lays its eggs in soft tissues of young palms and in the wounds present on the stem or leaf stalk. The grub tunnels its way into the trunk and feeds on the internal tissues. The larval period ranges from 36 to 78 days. The eggs are laid in the exposed soft tissues of crown and the grubs on hatching enter directly into the growing point resulting in the toppling of crown.

Around the world, residual chemical insecticides and fumigation are currently the method of choice for the control of insect pests. Their widespread use has led to serious environmental as well as health hazards. Plants are a promising source of secondary metabolites including alkaloids, terpenoids, phenolics, and flavonoids having insecticidal effect. They may disrupt major metabolic pathways and cause rapid death, act as attractants, deterrents, phago-stimulants, antifeedants or modify oviposition. They may also slow down or accelerate development. There is an urgent need to develop environment friendly alternatives with the potentials to replace the highly toxic chemicals. It has also been well recognized internationally that some plant derived insecticides can affect a limited range of insect pests and have no harmful effect on non target organisms and the environment. This study reports the insecticidal potential of aqueous extracts of *A.racemosus* on fourth instar larvae of *R.ferrugineus* as evidenced by assessing mortality rate and biochemical analyses.

MATERIALS AND METHODS

Insect Culture

The insect were collected from local affected coconut palms. The collected insects were paired and kept in small plastic containers for mating and provided with sugarcane pieces. After hatching, the newly emerged larvae were transferred in to the plastic bottle containing coconut husk. At the early stage of larval development only one larva was placed in each bottle to avoid cannibalism. Actively feeding 4th instar larvae were selected for the experiment.

Plants Selected For The Study

Three plants namely *Ocimum sanctum, Saraca asoka and Asparagus racemosus* were collected from local areas and identified at Botany Department, University college, TVPM.

Preparation Of Extracts

Fresh leaves of three medicinal plants were collected, washed and shade dried. The dried plant materials were ground to fine powder using a mechanical

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grinder and proceeded for soxhlet extraction for 8-10 hrs; according to Karmegam *et al.* (1997) method using different solvents such as ethanol, acetone and water. The weighed quantity of the plant material was reduced to a viscous dark green residue and the crude extracts were further evaporated.

BIOACTIVITY OF PLANT EXTRACTS ON *R. ferrugineus*

Newly moulted fourth instar larvae of *R.ferrugineus* were introduced in separate bottles. They were placed in bottles containing coconut husk dipped in different concentration of plant extracts which were dried in room temperature after soaking for 6 hours. The percentage of mortality was checked and the larvae were maintained till pupation. The untreated control was maintained for each treatment. Six replicates were maintained for each experiment.

EFFECT OF PLANT EXTRACTS ON BIOCHEMICAL PARAMETERS

Biochemical analysis can be analysed using appropriate protocol. Protein estimation by Lowry *et al*., 1951. Level of total free Amino acid was determined by using Lowry *et al*., 1951. Glycogen contents were measured according to the method of Dubois *et al*., 1956. Level of total lipids in haemolyph of *R.ferrugineus* was estimated according to method Folch *et al.,* 1957.

RESULT AND DISCUSSION

Mortality

The present study evaluated the larvicidal effect of A.racemosus in fourth instar larvae of *Rhynchophorus ferrugineus*. Treated larvae showed 73.33 % mortality (Figure 2) at concentration 6.978 (Figure 1) percentage in aqueous extracts of A.racemosus whereas acetone and ethanol extract of the same plant showed 43.33% (Figure 2) and 46.66% (Figure 2) mortality respectively. When fourth instar larvae were treated with different solvents of O.Sanctum, Aqueous (20%), acetone (26.6%) and ethanol (13.3%) extracts showed no significant mortality rate. When treated with S. Asoka aqueous extract showed a mortality rate of 23.3%acetone extract with a mortality of 20% and ethanol extract showed the least mortality rate of 16.66% (Table 1).

Biochemical Analysis

Fourth instar larvae treated with A.racemosus showed significant increase in the total body protein (6.8433) when compared to the control (5.633) [figure 3]. A significant decrease in the total body glycogen in the treated larvae (.3433) when compared to control (.6733) [figure 3]. The amound of amino acid in the treated larvae (2.3267) show an increased amount compared to control larvae (.6533) [figure 3]. A significant increase in lipid content (4.85) is observed in treated larvae compared to control (2.74)[figure 3] (Table 2).

The present study also attempted to shed some information on the influence of the different plant extracts on the, Protein, Glycogen and Amino acid. Haemolymph act as a storage organ for metabolites such as amino acid, carbohydrates and proteins; transport of nutrients between organs, transport of waste products to the organs of excretion and also transport of regulatory hormones from the organs of synthesis to their sites of action. Haemolymph also functions in the protection of the tissues from invading parasites and micro-organism (Arrese and Soulages, 2009: Smith, 1994). In the above study shows some significant difference in haemolymph protein of control over treated.

The result reveals that the treated insects show increased amount of amino acid. A significant increase in the total body glycogen was observed in treated larvae when compared to control insect larvae. Aqueous extracts of *A. racemosus* treated larvae showed a significant increase in the total body amino acid. Some amino acid obvious increase in the haemolymph of the larvae after treatment with different plant extracts. An increase in the amino acid content indicates either low transaminase activity or high proteolytic activity of enzymes. Moreover, this increase may be due to low food intake, reduction in protein synthesis or higher mobilization of protein. Same results were observed in tasar silkworm. A significant increase in lipid content is observed in treated larvae.

The aqueous extracts of Asparagus treated insects show typical moulting defects leading to high mortality. The mortality observed in the present study in most cases of treated larvae can be attributed to a malfunction in the moulting process by preventing them from shedding their old cuticle. (Ruscoe, 1972; Kubo *et al*., Koul ,*et al*.,2000).

Screening plant extracts for deleterious effects on in-sects is one of the approaches used in the search for novel botanical insecticides. Secondary plant compounds act as insecticides by poisoning or by production of toxic molecules after ingestion. These compounds also deter or possibly repel an insect from feeding. It is possible that the insecticidal property present in the selected plant extracts may arrest the various metabolic activities. The result of

this preliminary study thus indicates that leaf extract of *A. racemosus* is promising enough for further detailed research and can be incorporated into integrated pest management strategies.

Table 1: Table showing percentage mortality and LD_{50} values

	No. Of Larvae Control	No. Of Larvae Treated	After Treating No. Of Larvae Dead In Control	After Treating No. Of Larvae Dead In Treated	% Of Mortality	LC50
Ocimum sanctum						
Aqueous	30	30	0	6	20	15.189
Acetone	30	30	0	8	26.6	38.842
Ethanol	30	30	0	4	13.3	12.37
Asparagus recemosus						
Aqueous	30	30	0	22	73.33	6.987
Acetone	30	30	0	13	43.33	7.321
Ethanol	30	30	0	14	46.66	7.239
Saraca asoka						
Aqueous	30	30	0	7	23.33	24.313
Acetone	30	30	0	6	20	12.37
Ethanol	30	30	0	5	16.66	10.343

Table 2: Biochemical analysis of control and treated larvae of *Rhynchophorus ferrugineus*

		Protein	Amino Acid	glycogen	lipid
control	Mean ± Std. Deviation	5.6333 ± .03215	.6533 ± .02517	.6733 ± .02082	2.7367 ± .03786
treated	Mean ± Std. Deviation	6.8433 ± .02517	2.3267 ± .02082	.3433 ± .03512	4.8500 ± .04000

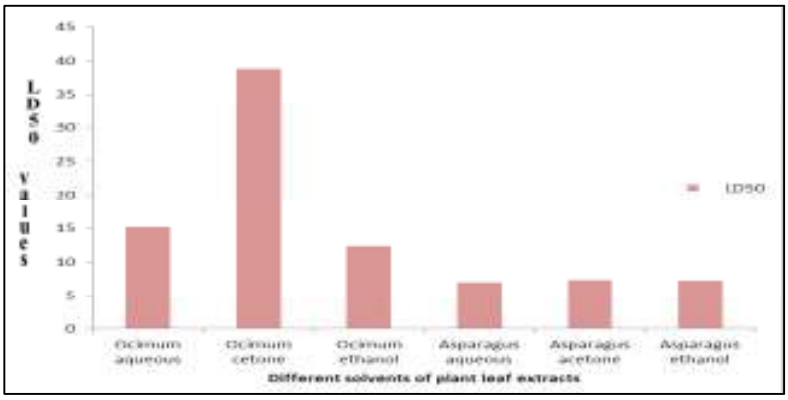

Figure 1: Showing the LD_{50} dose of different plant extracts

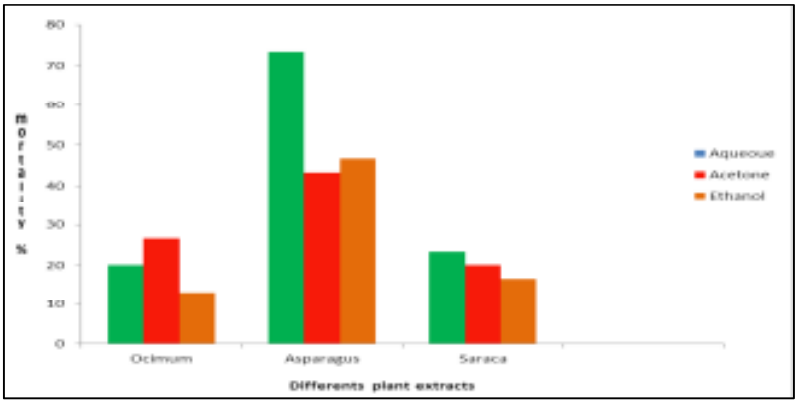

Figure 2: Mortality percentage on different plant extracts

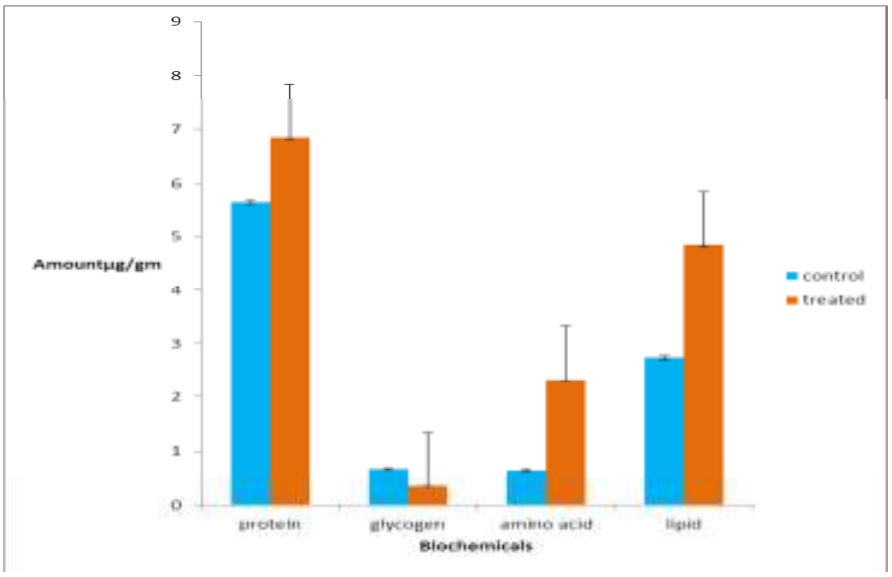

Figure 3: Biochemical analyses of control and trated larvae of *Rhynchophorus ferrugeneus*

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