Annals of Plant and Soil Research 21(2): 125-129 (2019)

Anti-insect activities of Large Caltrops (*Pedalium murex* Roen ex. L.)

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Received: March, 2019; Revised accepted: May, 2019

ABSTRACT

Large caltrops (Pedalium murex L.– Family: Pedaliacae), a weed plant, which have been using as traditional medicine in India was tested for it's anti - insect properties against a polyphagous pest, Spodoptera litura Fab. (Noctuidae:Lepidoptera). 3rd instar larvae were exposed to various doses of water extracts by following poison food technique (Leaf disc method). The result showed that fruit extract of P. murex recorded highest mortality (77%) of the larvae at 2.5% concentration and the lowest mortality of 66% in 10% concentration. The whole - plant extract recorded highest mortality of 88% and lowest of 66% in 7.5% and 5% respectively. In addition to mortality, the survived larvae ended with pre – pupal and pupal malformations. No adult emergence was recorded from the treatments of higher concentrations where as in control 100% adult emergence evident.

Key words: *Pedalium murex,* phyto insecticides, *Spodoptera litura*.

INTRODUCTION

Increased environmental hazards and ill effects due to non judicial uses of synthetic insecticides urged the entomologist to select viable alternates. Pesticidal plants are considered as one of the effective alternates from the time immemorial; because of their valuble secondary metabolites. The complex secondary metabolities of plants and the multiple mode of action avoid fast resistance development in insect pests (Liu *et al.*, 2000) (Sivakumar *et al.*, 2013). The very common secondary metabolites having pesticidal properties are alkaloids, non-protein amino acids, steroids, phenols, flavonoids, glycosides, glucosinolates, quinones, tannins and terpenoids. Further, Phytochemicals proved to be insecticidal, antifeedants, oviposition deterrents, repellents, juvenile hormone mimics, growth inhibitors and antimoulting hormones (Sutherland *et al.*, 2002; Kathirvelu Baskar *et al.*, 2014). The problems faced due to adoption of western science renewed interest to revive the traditional knowledge and however, few out of more than 250,000 plant species tested for this purpose (Selvaraj and Sahayaraj, 2005; Adesokan *et al.*, 2008). Hence, the present study was undertaken to explore the anti insect activity of *Pedalium murex* belongs to the family Pedaliaceae. It is a glabrous annual succulent herb with rather fetid smelling slime secreting glands, occurring as a weed of waste lands.

MATERIALS AND METHODS

Culturing of test insect

The test insect, *Spodoptera litura* Fab. (Noctuidae: Lepidoptera), was cultured on castor (*Ricinus communis* L.) leaves up to second instar and then on bengal gram flour based semi-synthetic diet up to pupation under the controlled conditions of 25±1ºC and 70 ± 5 % RH. Culturing of *S. litura* was initiated with egg masses collected from the field in and around Annamalainagar. Larvae hatched out from the collected egg masses were transferred on to castor leaves and kept inside a plastic container of 5L. capacity @ 50 per container and covered with lid fitted with muslin cloth. Fresh castor leaves were supplied daily after cleaning the container. When caterpillars became third instar, they were transferred onto semi-synthetic diet. Five pieces of semi synthetic diet (approximately 20g in weight) were placed on cardboard sheets and kept inside the plastic containers which had a layer of sterilized sand (5-7cm. thick). Twenty-five larvae were released per container. Pupation took place in the sand layer provided. Collected pupae were washed with 1% formaldehyde solution, sexed and transferred into oviposition cages (1'×1'×1') at the rate of ten pairs per cage. After adult emergence, 10% honey solution fortified with vitamin E was provided as adult food in a cotton wig. A twig of *Nerium oleander* (petiole immersed in water in a

conical flask) placed inside the oviposition cage acted as substrate for egg laying. Eggs laid were collected daily, washed with 0.05 % sodium hypochlorite solution and incubated. The hatched neonates were transferred onto castor leaves and recycled (Arivudainambi, 2001).

Extraction of *Pedalium murex*

Whole plants of *P. murex* were collected with fruits, washed in running water and the roots were cut off. The plant and fruit were made into small pieces each separately and ground with the help of electric blender without adding water. Then the respective quantity of paste of whole plant and fruit were weighed and mixed with required amount water to get the concentrations such as 2.5, 5, 7.5 and 10%. Then filtered using gada cloth (double layered) twice.

Effect of *P.murex* on feeding behavior, survival and ovipositional behavior

Poison food bioassay (Leaf disc bioassay – No choice test)

Castor leaf discs of 3.8 cm diameter were cut with petiole and the respective concentration was smeared on the both adaxial and abaxial surfaces of the leaf disc. Treated leaf discs were air dried and the petiole inserted in to 10ml glass vial which contained water. Three pre starved (3h) larvae were introduced onto leaf discs and covered. All the vials were kept under controlled conditions of 25±2 °C temperature and 70 ± 5 % RH. The bioassay was carried out after 6.00 pm as the test insect is nocturnal. Untreated leaf discs served as absolute control.

Leaves were collected from the vials after 3½ hours and the leaf area unfed was measured in each treatment by using leaf area meter (Elico model). Percent leaf area protection over control was computed and the antifeedancy was rated as per the formula and scale given below.

Percent leaf area protection over control =

$$\frac{\text{Percent leaf area protection in treatment} - \text{Percent leaf area protection in control}}{100 - \text{Percent leaf area protection in control}} \times 100$$

The antifeedancy was rated as per the scale given (Table 1).

Table1: Antifeedancy scale

Scale (% antifeedancy)	Rate
>80	Strong inhibition (++++)
51 – 80	Medium inhibition (+++)
20 – 50	Weak inhibition (++)
< 20	Insignificant inhibition (+)

Further, the exposed larvae were reared on untreated castor leaves separately and observations were made on their survival. Each treatment was replicated three times. Observations were made on antifeedancy, larval mortality and malformations, prepupal mortalities and malformations, pupal death and malformations and adult deformities.

On ovipositional behaviour

Fresh castor leaf discs (4cm dia.) excised along with petioles were inserted in vials containing water. Both the sides of the leaves were smeared with 1mL of extracts at various concentrations each separately. After air – drying, the treated leaves were placed in a cage and five pairs of newly emerged adults were released inside the cage for egg laying. One per cent honey solution soaked in a cotton swab served as adult food. Each treatment was replicated 3 times and the deterrent index was computed.

Deterrent index = $\frac{B-A}{A+B} \times 100$

Where A = No. of eggs on the treated surface

B = Number of eggs on the control surface (Sharaby *et al.*, 1993).

RESULTS AND DISCUSSION

Effect of *P.murex* on feeding, survival and ovipositional behavior

The antifeedancy studies by using fruit and whole plant extracts of *P. murex* against *S. litura* (3rd instar) on castor leaf disc revealed that the fruit extract at 7.5% concentration recorded 83.92% leaf area protection over control and the same extract at 2.5 per cent concentration recorded 45.40% leaf area protection over control. The leaf area protection ranges from 45.40 to 86.01% in fruit extract and 64.24 to 85.89% in whole plant extract. The whole plant extract at 2.5% concentration and 7.5% concentration recorded 64.24 and 68.49% leaf area protection over control respectively (Table 1).

Rearing of exposed larvae continuously on untreated food revealed that 5% concentration of whole plant extract recorded 11.11% larval mortality. The 5% concentration of fruit extract recorded 7.40% larval mortality. The 2.5% concentration of both fruit and whole plant extract recorded 3.70% larval mortality. Both 2.5 and 5% concentration of whole plant extract recorded 14. 81% prepupal mortality and malformations. 10% concentration of whole plant extract and 2.5% concentration of fruit extract recorded 7.40% pre – pupal mortality and malformations. Whereas 5, 7.5 and 10% of fruit extracts and 7.5% of whole plant extract recorded 11.11% pre – pupal mortality and malformations respectively. Fruit extract at 2.5 and 5% concentrations recorded 14.81% pupal mortality in the bioassay. Whereas the whole plant extract at 2.5 and 5% concentrations recorded 11.11% pupal mortality. Fruit extract at 10% concentration and whole plant extracts at 7.5 and 10% concentrations recorded 7.40% pupal mortality and malformations. Regarding adult mortality, only 3.70% was recorded at 7.5% concentration of whole plant extract and all other concentrations and of both whole plant and fruit recorded zero per cent adult mortality (Table 2).

Table 2: Effect of *P. murex* extracts on the feeding of *S. litura*

Treatment no.	Treatments	Per cent leaf area protection over control*	Antifeedancy ratting
T_1	2.5% Fruit Extract	45.40 (42.364)^{d}	++
T_2	5.0% Fruit Extract	56.73(48.864)^{cd}	+++
T_3	7.5% Fruit Extract	83.92 (67.120)^{ab}	++++
T_4	10.0% Fruit Extract	86.01 (68.725)^{a}	++++
T_5	2.5% Whole Plant Extract	64.24 (53.427)^{cd}	+++
T_6	5.0% Whole Plant Extract	64.12 (53.266)^{cd}	+++
T_7	7.5% Whole Plant Extract	68.49 (56.374)^{bc}	+++
T_8	10.0% Whole Plant Extract	85.89 (67.991)^{a}	++++
T_9	Control	-	-
	CD (0.05)	11.45	

**Mean of three replications, Values in parenthesis are arc sin transformed, Values with different alphabets differ significantly*

The mean number of eggs laid over the control leaf disc was 657.66. The leaf discs treated with 2.5 and 5 per cent fruit extract received 267.66 and 266.33 mean number of eggs. 10 per cent concentration of both the fruit and whole plant extracts recorded no eggs on the treated surfaces. At 7.5 per cent concentration, fruit and whole plant extracts recorded 108.33 mean numbers of eggs (Table 3).

Table 3: Effect of *P. murex* extracts on the larval, pre pupal, pupl & adult survival

Treatment no.	Treatments	Per cent larval Mortality and Malformation	Per cent Pre pupal Mortality and Malformation	Per cent Pupal Mortality and Malformation	Per cent Adult Mortality and Malformation
T_1	2.5% Fruit Extract	3.70(9.347)	7.40(14.636)	14.81(22.771)	0.0 (4.058)
T_2	5.0% Fruit Extract	7.40(12.192)	11.11(17.481)	14.81(22.771)	0.0 (4.058)
T_3	7.5% Fruit Extract	0.0(4.058)	11.11(19.926)	11.11(17.481)	0.0 (4.058)
T_4	10.0% Fruit Extract	0.0(4.058)	11.11(17.481)	7.40(12.192)	0.0 (4.058)
T_5	2.5% Whole Plant Extract	3.70(9.347)	14.81(22.771)	11.11(17.481)	0.0 (4.058)
T_6	5.0% Whole Plant Extract	11.11(17.481)	14.81(22.771)	11.11(17.481)	0.0 (4.058)
T_7	7.5% Whole Plant Extract	0.0(4.058)	11.11(14.554)	7.40(14.636)	3.70(9.347)
T_8	10.0% Whole Plant Extract	0.0(4.058)	7.40(14.636)	7.40(14.636)	0(4.058)
T_9	Control	0.0	0.0	0.0	0.0
CD (0.05)	NS	NS	NS	NS	

**Mean of three replications, Values in parenthesis are arc sin transformed, Values with different alphabets differ significantly*

From the results of poison food bioassay, it is known that both the extracts *P. murex* (whole plant and fruit) were found to possess antifeedant effect. The per cent leaf area protection falls between 45.4 and 86.1 in the treatments. While rating the antifeedancy it was found that fruit extracts at 7.5 and 10% and whole plant extract at 10% exerted strong antifeedancy (++++) which were more than 80% of leaf area protection over control. Medium antifeedancy (+++) were noted in all other concentrations of whole plant and fruit extracts except 2.5% fruit extract where weak antifeedancy (++) was recorded. However 50% leaf area protection were seen at lower concentrations tested. This proves the antifeedant properties of *P. murex* clearly; though it was dose dependent. Statistical analysis showed that 10% concentration of whole plant and fruit extracts and 7.5% of fruit extract were on par. Even though some degree of differences absorbed among the treatments, most of them are statistically on par.

From the observations, it was understood that the lower concentrations, which were allowed the larvae to feed more leaf area (around 40 to 50%) when compared with the higher doses, recorded larval mortality. However, the mortality percentage was not considerable. The maximum of 11.11 per cent larval mortality was exhibited. Pre pupal and pupal mortalities were seen in all treatments. But it was low (the maximum of 14.81%). Further the larvae successfully pupated; were emerged as adults without any problem because adult mortality was zero in the treatments. Larval, prepupal, pupal and adult death and deformities recorded in the treatments were not found promising. The death and deformities varied from 0.0 to 14.82%.

Table 4: Effect of *P. murex* extracts on the ovipositional behaviour of *S. litura* (Choice test)

Treatment no.	Treatments	*No. of eggs laid on the leaf disc	Oviposition Deterrent Index
T_1	2.5% Fruit Extract	267.66(16.37)^{c}	42.14
T_2	5.0% Fruit Extract	266.33(16.33)^{c}	42.35
T_3	7.5% Fruit Extract	108.33(10.42)^{d}	71.71
T_4	10.0% Fruit Extract	0.0 (0.701)^{e}	100.0
T_5	2.5% Whole Plant Extract	275.00(16.59)^{bc}	41.02
T_6	5.0% Whole Plant Extract	286.33 (16.93)^{b}	39.33
T_7	7.5% Whole Plant Extract	108.33 (10.42)^{d}	71.71
T_8	10.0% Whole Plant Extract	0.0 (0.701)^{e}	100.0
T_9	Control	657.66 (25.65)^{a}	-
CD(0.05)		0.360	

**Mean of three replications, Values in parenthesis are $\sqrt{}$ transformed, Values with different alphabets differ significantly*

The extracts of *P. murex* acted as antifeedant rather than insecticidal and growth regulation at the concentrations tested. In other way it can be inferred that the treated food ingested by the larvae might not be enough to cause insecticidal or growth regulatory action. The extracts of *P. murex* at the concentrations of 10% showed cent per cent ovipositional deterrent action. As the concentration degreases the deterrent action also decreases. It proves that the deterrent action is also dose dependent. Thus it is concluded that the whole plant and fruit extracts *P. murex* registered higher level of antifeedancy at higher concentration. The lower concentrations of whole plant and fruit extracts allowed the larvae to feed on treated surface but considerable mortality and malformations were recorded. However, the data related to their activity in the physiology of the larvae would support the plant to develop into potential botanical insecticide.

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