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An Assessment of Antifeedant Potential and Cidal Activity of Plant Extracts on Fifth Instar Nymphs of Red Cotton Bug, *Dysdercus koenigii* Fabricius (Heteroptera: Pyrrhocoridae)

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Abstract
Antifeedant and Insecticidal properties of ethanol extracts of *Catharanthus roseus, Ocimum sanctum,* and *Lantana camara* were assessed by 'seed dip method' on *Dysdercus koenigii*. Significant differences were observed in the feeding response of fifth instar nymphs on seeds treated with the ethanol extract of *Catharanthus roseus, Ocimum sanctum,* and *Lantana camara*. It was reported that *Ocimum sanctum,* and *Lantana camara* extract were more effective than *Catharanthus roseus* in restraining the feeding. The effect of all the three extracts was dose dependent and in positive correlation with the concentration of the extracts. It was also reported that number of insects feeding on the extracts increased with time interval. This reflected possibility of the presence of antifeedant in the extracts. Although the cidal effects of the plant extracts were not much pronounced after 24 hr of exposure in all the three experimental plant extracts, a significant decrease in the survival of treated insects was observed specially during later part of their life. On seventh day of the exposure In comparison to *Ocimum and Lantana*, *Catharanthus* was most effective in reducing the percent survival of the treated fifth instars. Ethanol extracts of the plants also had impact on the longevity of the *Dysdercus*. There was reduction in the longevity of the treated nymphs and the adults developed from the treated fifth instar nymphs. The effects were most conspicuous with the *Catharanthus.*

Keywords: *Catharanthus roseus, Ocimum sanctum, Lantana camara, Dysdercus koenigii* Antifeedant, Survival, Longevity

1. Introduction
Dysdercus koenigii commonly known as 'Red Cotton Bug' is a polyphagous heteropteran insect [1, 2]. It inflicts considerable damage to standing crops of cotton and other malvaceous plants [3] and is a serious pest of cotton in India and many parts of the world [4].
Pesticides are widely used in various sectors of the agriculture to avoid or reduce losses by pests to improve yield [5, 6]. Despite their popularity, the extensive uses of pesticides have developed serious concerns about health risks to agricultural labour [7, 8]. Often, many times pesticide applications prove counterproductive on account of pest resurgence, environmental contamination and non-target interaction [9]. Therefore, indiscriminate use of pesticide has raised serious concerns to human health and to wildlife and sensitive ecosystems [10]. To combat the problems associated with the insecticides, an alternate strategy is to reduce the dependence on synthetic insecticides and encourage the use of botanical insecticides which are considered safe to environment [11].
Role of botanicals in insect pest management is well documented. The plant products are highly potent and versatile in influencing survival and longevity of insects [12]. The majority of commercially produced botanical insecticides utilize the secondary plant metabolites, which are toxic to insects [13]; phytochemicals often possessed antifeedant, growth-inhibiting and antiovipositional activity [14]. These chemicals have the potentials to reduce the load of harmful insect pests significantly.
Catharanthus roseus, a plant of medicinal importance and popularly known due to its anticancer and antitumor properties which are attributed to the presence of the alkaloids vincristine and vinblastine in its leaves [15]; *Ocimum sanctum* also known as queen of herbs, possess insecticidal properties and marked repellent action against mosquitoes [16]. *Lantana camara* is an obnoxious weed and have wide potential in insect pest management [17]. Potentials of these plants as an antifeedant and insecticidal action on insect pests have

less extensively investigated. Therefore, a schematic research pertaining to importance of *Catharanthus roseus, Ocimum sanctum* and *Lantana camara* on feeding behaviour, survival and longevity of *Dysdercus koenigii* was undertaken in the present research work.

2. Materials and Methods

2.1 Rearing and Maintenance of Insect Culture

A culture of *Dysdercus koenigii* was maintained in an insectary and BOD incubator in sterilized conditions under ambient conditions of temperature 28.0±2.0 ºC, relative humidity 70.0±5.0%, and 12hr. light: 12hr. dark, photoperiod regimen to obtain insects having sustained quality with respect to proper growth and adult viability. The insects were provided with cotton seeds which were previously washed in running water and dried on filter paper. Sterilized cotton swabs soaked in water were provided as a source of water. The insects were kept in rearing jars covered with fine muslin cloth. Individual mating pairs of the bugs were placed in small rearing jars (200ml) whereas 5-10 mating pairs were kept in the jars of large size (500ml). Generally, the female lays eggs in batches beneath the cotton seeds. These eggs were separated within 12 hr. of oviposition. The eggs were incubated in small sterilized glass hatching vials (1x5 cm) in a BOD incubator at high humidity (80% to 90%). Normally eggs hatched in 5-6 days. The newly emerged nymphs were transferred to glass jars containing food and water, and were reared as stock culture. For all experimental purposes newly emerged fifth instar nymphs were isolated from the stock culture. Male and female fifth instar nymphs were identified on the basis of their relative sizes, as female nymphs were broader and stouter than the male nymphs [18].

2.2 Plants used in Study

Leaves of *Catharanthus roseus, Ocimum sanctum* and *Lantana camara* were the plants used in the present investigations. These plants grow frequently in Deshbandhu College campus, New Delhi, India, under natural conditions. The leaves of the plants of same age group were collected during specific months of the year for preparation of extract for the experimental purpose.

2.3 Preparation of Ethanol Extract

The ethanol extracts of plants were prepared by 'cold extraction method' described by several researchers [19, 20, 21]. The concentrated extracts were dissolved in ethanol (1gm/ml) and subsequently, diluted with dilution fluid (1: 9, ethanol and distilled water) to obtain 10% stock solution of the extract. Few drops of 0.2% Tween 80 were added to the stock solution to stabilize the extract. The stock solution was serially diluted and 5%, 2.5% and 1.25% extracts were used in experiments.

2.4 Treatment of the seeds with Ethanol Extract

Fresh dried seeds of cotton were treated with ethanol extract by 'seed dip method' [22]. 1gm. of the seeds was soaked in the ethanol extract of desired concentration for one hr. The seeds were subsequently dried for a period of 2 hr.

2.5 Treatment of the Insects

Newly emerged fifth instar nymphs were separated from the stock culture. The nymphs were starved but kept on water for a period of 24 hr. Subsequently, they were provided with seed dipped in the extracts and feeding behaviour of the nymphs was recorded after every 15 min. for a period of 1 hr. Feeding in the insects was also confirmed by observing their excreta. The experiments were continued to find out the doses exhibiting no significant aberration in feeding response of the insects. These doses were used to study effect of ethanol extract on the survival and longevity of *Dysdercus.*

Fifteen insects were kept in each experimental set up. Control experiments were conducted along with the test for each concentration. All the experiments were replicated five times. The results were statistically analyzed using IBM SPSS 19.1 software. Validity of hypothesis was confirmed by one way ANOVA followed by TUKEY test at 95% of confidence limit [23].

3. Results

The results summarized in Table 1 indicated that ethanol extract of *Catharanthus* had adverse effect on feeding activity of fifth instar nymphs of *Dysdercus*. Although, the number of insects showing feeding response on treated cotton seeds was increased as a sequel of time, it was significantly lesser than the control. After 15 min. of experimentation 9.33% and 12% insects were observed feeding on the cotton seeds soaked in 10% and 5% *Catharanthus* ethanol extract, while after 60 minutes the number of insects showing feeding response increased to 32% and 37.33% respectively (Table 1). The results also indicated that the number of insects feeding on seeds treated with 2.5% was significantly less than control especially during first 30 minutes of observations. 1.25% concentration of *Catharanthus* extract was least effective for antifeedant activity (Figure 1). It was observed that ethanol extract of *Ocimum sanctum* was most effective in showing antifeedant activities. At 10% almost all the fifth instar nymphs avoided the feeding for first15 minutes and 90.67 after 60 minutes. When fed on the cotton seeds soaked in 5% ethanol extract the feeding response after 15 min and 60 min was 1.33% and 40% respectively. At 2.5% and 1.25% the number of insects feeding after 15 minutes was 18.67and 14.67 and after 60 minutes 45.33% and 52% respectively which was less than control and was statistically significant (Table 1). *Lantana camara* was least effective in reducing the feeding response of *Dysdercus koenigii* fifth instar nymphs in comparison to *Catharanthus* and *Ocimum*. At 10% almost no insect was found feeding after 15 minutes; the number of feeding insects increased to 10.67% after 60 minutes. The 5% ethanol extract of *Lantana* was also effective and the reduction in feeding in percent was 86.33 and after 60 minutes. The lower concentration of *Lantana camara* was less effective and the percent feeding on the cotton seeds treated with 2.5% and 1.25% ethanol extract was 13.33 and 37.33 after 15 minutes. It was increased to 73.33 and 78.67 respectively after 60 minutes of observation. The results indicated that the number of insects showing feeding response increased with the time in all the experimental set ups (Figure 1).

Our results showed that 10% and 5% ethanol extract of the three test plants showed strong antifeedant activities and insects did not feed on the seeds; therefore these concentrations were not used in study of survival and longevity. Results pertaining to influence of ethanol extracts of *Catharanthus*, *Ocimum* and *Lantana* on the survival of treated fifth instar nymphs of *Dysdercus* are presented in Figure 2. The data indicated that the three extracts had no significant effect on the survival of the fifth instar nymphs of *Dysdercus* after 24 hr. of feeding. On the contrary, number of insects survived decreased significantly on the seventh day of

feeding in all the experimental set ups. It was reported that at the two concentrations, *Catharanthus* was most effective (survival rate 77.33% and 52%); followed by *Ocimum* (survival rate 81.33% and 74.67%). *Lantana* ethanol extract was least effective with survival percent 92 and 90.67 respectively on seventh day of treatment (Figure 2). The difference in the results of survival on seventh day in treated and control experiments was statistically significant. The results presented in Figure 3 indicated that there was profound effect of ethanol extracts of *Catharanthus* and *Ocimum* on the survival of the adults emerged from the treated nymphs. It was reported that when treated with 1.25% and 2.5% ethanol extracts of *Catharanthus*, 61.33% and 45.33% survived after 7 days of emergence. Similarly treatment with *Ocimum* extract at these concentrations reduced the survival to 61.33% and 56% on seventh day. *Lantana* ethanol extract reduced the percent survival of the adults to 78.67% and 77.33% respectively.

The longevity of *Dysdercus* as influenced by *Catharanthus*, *Ocimum* and *Lantana* ethanol extracts was studied in two segments of life. The first segment was a period of seven days of fifth instar nymphs, a time interval from newly emerged fifth instar nymphs to emergence of adults. The second segment was a period of 7 days after adult emergence. The two segments included both the nymphal as well as adult life. The data presented in Table 2 indicated that ethanol extracts of the plants tested did not have conspicuous effect on longevity of the fifth instar nymphs. *Catharanthus* ethanol extract was most effective among the three plants tested. The results were statistically significant only in the treatment with 2.5% *Catharanthus* (Table 2). Longevity of the adults *Dysdercus* developed from treated instar nymphs with 1.25% and 2.5% *Catharanthus* was 6.57, 6.47 days respectively (Table 3). Parallel results were observed after treatment with *Ocimum* and *Lantana ethanol extract.* The results were statistically significant at $p < 0.05$ (Table 3).

Table 1: Effect of ethanol extracts of *Catharanthus roseus, Ocimum sanctum* and *Lantana camara* on feeding behavior of fifth instar nymphs of *Dysdercus koenigii*.

Plants tested	Concentration of ethanol extracts	Number of insects showing feeding response (Mean ± SE)*			
		15 min.	30 min.	45 min.	60 min.
Catharanthus	Control	$45.33^{a} \pm 1.30$	$77.33^{a} \pm 0.89$	$78.67^{a} \pm 1.09$	$94.67^{a} \pm 0.44$
	1.25%	$41.33^{ab} \pm 2.86$	$65.33^{ab} \pm 3.89$	$77.33^{a} \pm 3.50$	$85.33^{a} \pm 0.83$
	2.5%	$16.00^{bc} \pm 1.81$	$40.00^{bc} \pm 2.12$	$58.67^{a} \pm 1.64$	$80.00^{a} \pm 3.08$
	5%	$12.00^{c} \pm 2.16$	$32.00^{c} \pm 0.83$	$33.33^{b} \pm 1.22$	$37.33^{b} \pm 1.34$
	10%	$9.33^{c} \pm 2.60$	$20.00^{c} \pm 1.41$	$26.67^{b} \pm 1.41$	$32.00^{b} \pm 2.16$
Ocimum	Control	$45.33^{a} \pm 1.30$	$77.33^{a} \pm 0.89$	$78.67^{a} \pm 1.09$	$94.67^{a} \pm 0.44$
	1.25%	$14.67^{b} \pm 0.83$	$25.33^{b} \pm 2.16$	$41.33^{b} \pm 3.03$	$52.00^{b} \pm 1.78$
	2.5%	$18.67^{b} \pm 1.48$	$41.33^{b} \pm 2.16$	$41.33^{bc} \pm 2.16$	$45.33^{b} \pm 2.77$
	5%	$1.33^{c} \pm 0.44$	$1.33^{c} \pm 0.44$	$20.00^{bc} \pm 3.6$	$40.00^{b} \pm 3.00$
	10%	0.00^{c}	$2.67^{c} \pm 0.89$	$5.33^{c} \pm 1.30$	$9.33^{c} \pm 1.14$
Lantana	Control	$45.33^{a} \pm 1.30$	$77.33^{a} \pm 0.89$	$78.67^{a} \pm 1.09$	$94.67^{a} \pm 0.44$
	1.25%	$37.33^{a} \pm 2.70$	$50.67^{a} \pm 3.20$	$62.67^{a} \pm 2.07$	$78.67^{a} \pm 2.38$
	2.5%	$13.33^{b} \pm 2.54$	$52.00^{a} \pm 4.96$	$66.67^{a} \pm 2.82$	$73.33^{a} \pm 3.24$
	5%	$2.67^{b} \pm 0.54$	$5.33^{b} \pm 0.83$	$10.67^{b} \pm 1.14$	$13.33^{b} \pm 1.22$
	10%	0.00^{b}	$4.00^{b} \pm 0.54$	$6.67^{b} \pm 0.70$	$10.67^{b} \pm 0.54$

Means followed by the same letter in a column are not significantly different at $P<0.05$ (ANOVA followed by Tukey test).
*Average of five replicates, 15 insects per replicates.

Table 2: Influence of ethanol extracts of *Catharanthus roseus, Ocimum sanctum* and *Lantana camara* on the longevity of fifth instar nymphs of *Dysdercus koenigii*.

Concentration of the ethanol extracts (%)	Longevity of the fifth instar nymphs*		
	Catharanthus **(Mean ± SE)**	***Ocimum*** **(Mean ± SE)**	***Lantana*** **(Mean ± SE)**
Control	$7.00^{a} \pm 0.00$	$7.00^{a} \pm 0.00$	$7.00^{a} \pm 0.00$
1.25	$6.11^{ab} \pm 0.18$	$6.33^{a} \pm 0.34$	$6.69^{a} \pm 0.14$
2.5	$5.07^{b} \pm 0.26$	$6.26^{a} \pm 0.19$	$6.74^{a} \pm 0.14$

Means followed by the same letter in a column are not significantly different at $P<0.05$ (ANOVA followed by Tukey test).
*Average of five replicates, 15 insects per replicates.

Table 3: Influence of ethanol extracts of *Catharanthus roseus, Ocimum sanctum* and *Lantana camara* on the longevity of the adults developed from treated fifth instar nymphs of *Dysdercus koenigii*.

Concentration of ethanol extracts (%)	Longevity of the adults developed from treated fifth instar nymphs*		
	Catharanthus **(Mean ± SE)**	***Ocimum*** **(Mean ± SE)**	***Lantana*** **(Mean ± SE)**
Control	$7.00^{a} \pm 0.00$	$7.00^{a} \pm 0.00$	$7.00^{a} \pm 0.00$
1.25	$6.57^{b} \pm 0.12$	$6.28^{b} \pm 0.13$	$6.30^{b} \pm 0.18$
2.5	$6.47^{b} \pm 0.14$	$6.37^{b} \pm 0.20$	$6.33^{b} \pm 0.07$

Means followed by the same letter in a column are not significantly different at $P<0.05$ (ANOVA followed by Tukey test).
*Average of five replicates, 15 insects per replicates.

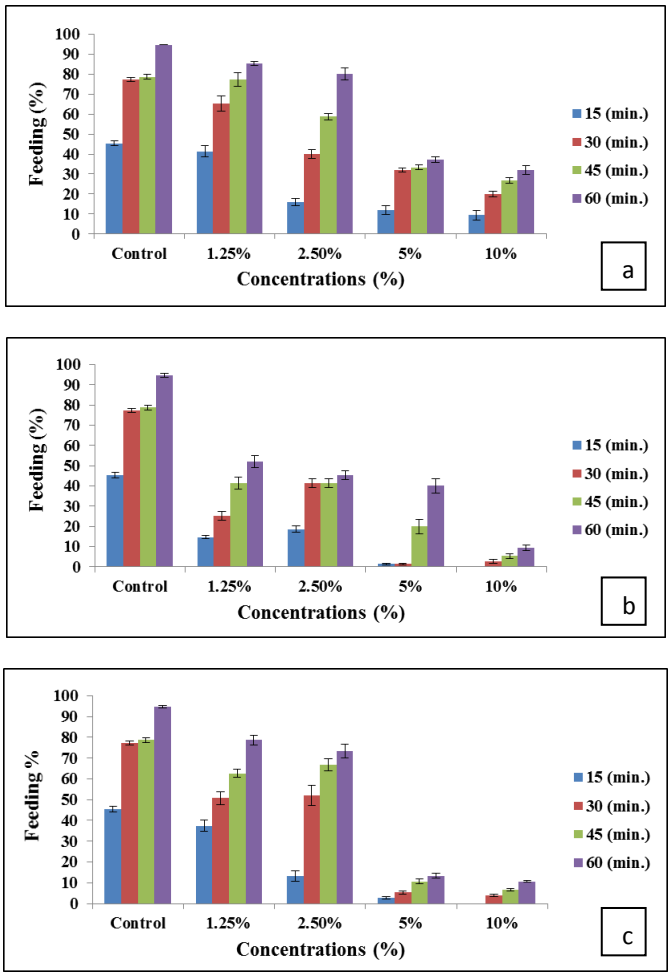

Fig 1: Effect of ethanol extracts of (a) *Catharanthus roseus*, (b) *Ocimum sanctum* and (c) *Lantana camara* on feeding behavior of fifth instar nymphs of *Dysdercus koenigii*.

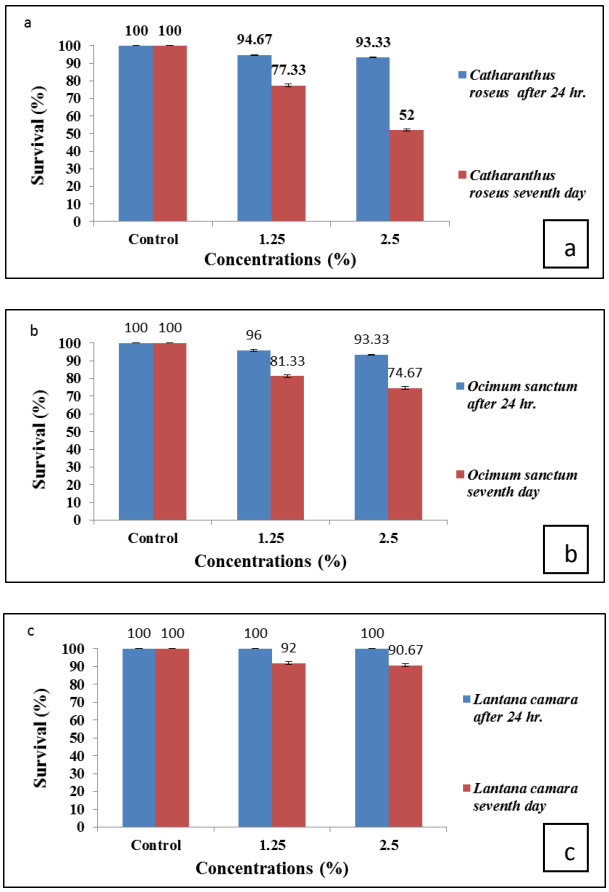

Fig 2: Influence of ethanol extracts of (a) *Catharanthus roseus*, (b) *Ocimum Sanctum* and (c) *Lantana camara* on the survival of the fifth instar nymphs of *Dysdercus koenigii* after 24 hr. and seventh day of treatment.

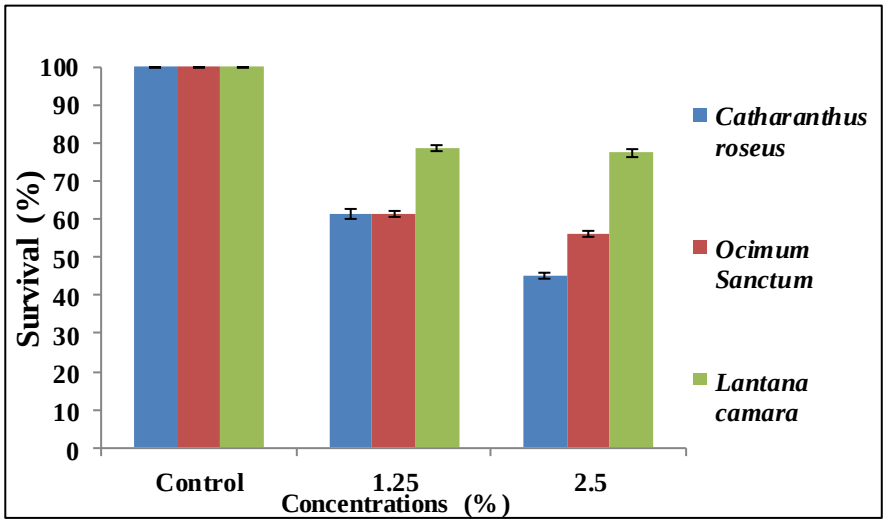

Fig 3: Influence of ethanol extracts of *Catharanthus roseus*, *Ocimum sanctum* and *Lantana camara* on the survival of the adults developed from treated fifth instar nymphs, after seventh day of adult emergence.

4. Discussion

The nymphs of *Dysdercus koenigii* displayed a series of behavioural pattern in order to initiate feeding. A nymph performed random movements; this brings it in the vicinity of the food. Subsequently the nymph takes test ingestion; presence of phagostimulant in the food permits the insect to continue feeding. Presence of antifeedant in the food results in termination of feeding activity. Our studies indicated that ethanol extracts of *Catharanthus*, *Ocimum* and *Lantana* impaired the feeding behaviour of fifth instar nymphs of *Dysdercus* to the varied extant. It was evident from the studies that ethanol extract of *Ocimum sanctum* was highly effective. Treatment with 10% and 5% extract resulted in complete inhibition of the feeding of the fifth instar nymphs when observed after 15 min. However, number of insects showing feeding activity increased during subsequent time intervals. Similar trends were observed with *Catharanthus*. Our findings were in agreement with Summarwar and Pandey [24]. They studied antifeedant properties of *Ocimum sanctum* and *Catharanthus roseus* against fourth instar larvae of *Spodoptera litura* and observed maximum antifeedant activity at higher concentration. Anshul *et al.* [25] reported antifeedant activities of *Ocimum sanctum* against VI instar larvae of gram pod borer *Helicoverpa armigera*. Ethanol extract of *Catharanthus roseus* showed moderate antifeedant activity on the fifth instar nymphs. However, the results were profound during early period especially at higher concentrations. The antifeedant activities of *Catharanthus* were reported against the larvae of gram pod borer *Helicoverpa armigera* [24]. *Catharanthus* leaves when extracted with methanol, petroleum ether, methanol fraction and ethyl acetate fraction and tested at a concentration of 1,000 ppm showed antifeedant activity. The antifeedant activity of *Lantana* ethanol extract on *Dysdercus* was conspicuous only at higher concentrations after 15 min; significant reduction in the percent feeding was observed at higher concentrations i.e. 10% and 5%. Antifeedant properties of the plant extracts have been described by many researchers [26, 27, 28]. Neemjeevan a Neem based product showed antifeedant activities against the fourth instar nymphs of *Dysdercus koenigii* [29]. Nathala and Dhingra [27] reported antifeedant activity in the seed extracts of *Melia azedarach* against *Helicoverpa armigera*. *Abutilon indicum, Achyranthus aspera, Aerva lanata, Albiziz amara, Andrographis paniculata, Cardiospermum halicacabum, Cassia tora, Catharanthus roseus, Datura metal* and *Tribulus terrestris* had antifeedant activities against the sixth instar larvae of gram pod borer *Helicoverpa armigera* [30]. Ethanol extracts of the plants tested had no significant adverse effect on the survival of fifth instar nymphs at least after 24 hr. of exposure; however, significant reduction in the survival was observed after seventh day of exposure. It was reported that the mortality in the fifth instar nymphs was dose dependent. Maximum toxicity was observed in the insects treated with *Catharanthus* extracts. *Lantana* ethanol extract was least effective against fifth instar nymphs of *Dysdercus*. The influence of ethanol extracts was more prominent in the adults developed from these treated fifth instar nymphs. The continued reduction in the survival was observed in the adults emerged. The insecticidal properties of *Catharanthus roseus* have been reported against various insects such as *Dysdercus cingulatus* [31]; *Amsacta moorei* [32]; *Pthroimaea operculella* [33]; and *Spodoptera littoralis* [44]. Singh and Mehta [35] reported toxicity and insect growth regulatory activity of acetone extract of *Catharanthus roseus* against the larvae of *Spodoptera litura* and *Helicoverpa armigera*. Kumar *et al.* [36] reported that root extracts of *Catharanthus roseus* affected the larval survival of *Spodoptera litura*. *Catharanthus roseus* also had moderate biopesticidal activity against larvae of *Helicoverpa armigera* and mosquitoes [35]. Essential oil of *Lantana camara* leaves showed insecticidal properties against newly emerged fifth instar nymphs of *Dysdercus similies* [37] and *Helicoverpa armigera*, [38]. The reports also indicates that the essential oil from the leaves of *Lantana camara* had insecticidal properties against *Aedes aegypti*[39], *Culex quinquefasciatus, Anopheles culicifacies, Anopheles fluvialitis* and *Anopheles stephensi* [17]. Extracts of *Lantana camara* leaves were also effective against adult termite workers [40]. These finding supports our results for the insecticidal and antifeedant activities in the *Catharanthus*, *Ocimum* and *Lantana*. Treatment with ethanol extracts of all three plants was not effective in reduction of the longevity of treated fifth instar nymphs. However, the extracts were effective in decreasing the average longevity of the adults developed from these treated fifth instar nymphs. The decrease in the mean lifespan was related to an increase in the mortality and the reduction in the lifespan. These findings were in agreement with previous report on many insects [41]; methanol extract of *Ulva fasciata* and *Ulva lactuca* reduced longevity of the

adults of *Dysdercus cingulatus.* Sahayaraj and Jeeva [42] also reported decrease in the longevity of adult *Dysdercus cingulatus* after treatment with the extracts of sea weed *Sargassum tenerrimum*.

5. Conclusion

Our results suggested the significant and variable efficiency of ethanol extracts of *Catharanthus roseus, Ocimum sanctum and Lantana camara* leaves as antifeedant and insecticidal against *Dysdercus koenigii*. Different types of the phytochemicals present in these plant extract may be responsible for the variability. These plants are available in the tropical region; commercial exploitation of these plants may be used as cost-effective, sustainable, and ecofriendly alternative of insect control.

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