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Effect of ethanol extract of *Catharanthus roseus*, *Ocimum sanctum* and *Lantana camara* on fecundity and fertility of red cotton bug, *Dysdercus koenigii* Fabricius (heteroptera: pyrrhocoreidae)

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Abstract
Bioefficacy of ethanol leaf extract of *Catharanthus roseus*, *Ocimum sanctum* and *Lantana camara* was evaluated on fecundity and fertility of the red cotton bug, *Dysdercus koenigii*. The Phytoconstituents of each plant were extracted individually with ethanol by 'cold extraction method'. The seeds were soaked with the 2.5% and 1.25% extracts. The newly emerged fifth instar nymphs were fed on the treated seeds for 24 h. The oviposition behaviour, fecundity and fertility of the females emerged from the treated nymphs were assessed. Our study indicated that the *Dysdercus koenigii* showed marked reduction in their reproductive success in response to the plant extracts. The treated females laid lesser number of egg batches and eggs in their life span. *Catharanthus* leaf ethanol extract was most effective in suppression of oviposition among the plants extracts tested. The eggs laid by the mated females were fertile indicating insemination of the mated females; however, the percent hatchability of the eggs laid by the females was less. The eggs laid by the females treated with *Ocimum* leaf ethanol extract were least fertile. It was surmised that some of compounds present in the extracts individually or synergistically alter fecundity and fertility of *Dysdercus koenigii.*

Keywords: *Catharanthus roseus, Ocimum sanctum, Lantana camara, Dysdercus koenigii* Fecundity, Fertility

1. Introduction
One of the important attributes of insects is their immense reproductive potential. Insect reproduction includes mating behaviour, oviposition behaviour, fecundity and fertility. Phytoconstituents present in many plants can easily challenge any of these aspects of insect reproduction [1, 2]. Many of the secondary metabolites present in plants adversely alter the fecundity and fertility of insect pests [3, 4, 5]. Therefore, studies pertaining to influence of plant extracts in insect reproduction are important in integrated pest management *Catharanthus roseus* (Apocyanaceae) grows naturally in warmer regions of the world, including India. It has various uses in folk medicine [6]. Potentials of phytochemicals present in the *C. roseus* as insecticide were widely studied against a variety of insects. Exposure to aqueous, ethyl acetate and methanol extracts of *C. roseus* have been reported to induce the larval and pupal mortality in *Anopheles stephensi* and *Culex quinquefasciatus* [7]. Panneerselvam, *et al*. [8] explored the activity of *Catharanthus roseus* leaf extract against the malarial vector *Anopheles stephensi* and observed larvicidal effects after 24 h of exposure.
Ocimum sanctum Linn., commonly known as 'Tulsi' is one of the most popular herb of family Lamiaceae grown all over the world. *Ocimum sanctum* has been used for thousands of years in Indian traditional medicine systems like Ayurveda for its diverse healing properties. Because of its popularity, it is often referred as "Queen of the herbs [9]. The leaf oil of *O. sanctum* possesses insecticidal properties. It has marked repellent action and insecticidal activity against various insect pests [10, 11]. Manzoor *et al*. [11] also found that hexane, butanol, chloroform, methanol, ethyl acetate and water extracts of inflorescences, leaves, root and stem extract of *O. sanctum* possessed insecticidal activity against termites *Heterotermes indicola*.
Lantana camara is a native of the tropical and subtropical America. A few taxa are originally from tropical Asia and Africa. Although an obnoxious weed, the plant has various medicinal uses.

The toxic effects of the leaves have been attributed to a series of pentacyclic triterpenes, of which Lantadenes A and B are typical members. Due to the presence of a great diversity of secondary metabolites, *Lantana* is practically resistance to attack by herbivores; some species of *Lantana* are utilized as biopesticides against a variety of insects [12, 13, 14].

Dysdercus koenigii Fabricius (Hemiptera: Pyrrhocoridae) commonly known as 'Red Cotton Bug' is a polyphagous heteropteran insect of paramount economic importance. The pest inflicts considerable damage to standing crops of cotton and other malvaceous plants in many parts of the world including India. Both, the nymphs and the adults suck sap from the developing seeds, which thus lose the oil contents and germinating capacity. The pest is also responsible for spreading the internal boll disease of cotton, 'Stigmatomycosis' which causes staining of lint [15, 16]. *Dysdercus koenigii* has high reproductive potential. The females lay eggs in batches throughout their reproductive life; a single female may lay up to seven egg batches. Each egg batch contains about 100-150 eggs [17].

Previous studies on *Catharanthus roseus, Ocimum sanctum* and *Lantana camara* extracts were restricted mainly to their larvicidal activity. Adverse effects of the plant extracts on fecundity and fertility of *D. koenigii* were less extensively explored. Moreover, the solvents used for the extraction in the earlier studies were highly toxic. Therefore, the present study was initiated to evaluate the bioefficacy of ethanol leaf extract of *Catharanthus roseus, Ocimum sanctum* and *Lantana camara* extracts on fecundity and fertility of *Dysdercus koenigii*. The studies are of particular relevance in understanding the role of plant extracts in aberration of reproduction of *Dysdercus koenigii*.

2. Materials and Methods

2.1 Rearing and Maintenance of Insect Culture

A culture of *Dysdercus koenigii* was maintained in BOD incubator 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 kept in rearing jars to provide water to the insects. Individual mating pairs of the bugs were placed in small rearing jars (200 mL) whereas 5-10 mating pairs were kept in the jars of large size (500 mL). The eggs were kept in small sterilized glass hatching vials (1x5 cm) in a BOD incubator at high humidity (80% to 90%) for incubation. The nymphs were transferred to glass jars containing food and water, and were reared as stock culture. Newly emerged fifth instar nymphs were used for the experimental purpose.

2.2 Plants used in Study

Leaves of *Catharanthus roseus, Ocimum sanctum* and *Lantana camara* were used in the present investigations. These plants grow frequently in natural conditions in Deshbandhu College campus, New Delhi, India (28.5403° N, 77.2544° E). The leaves from 4-5 months old plants were collected during the month of April and May in order to ensure uniformity of the phytoconstituents in the extracts throughout the experimental work [18].

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 leaves of the plants were washed thoroughly and dried for two weeks; the dried leaves were ground into fine powder. The powders of the leaves were mixed with five times ethanol (weight to volume) in conical flasks separately, and were extracted for 24 h. The mixtures were then filtered and the residues were extracted again twice in the same way. The extracts were pooled and desiccated in 'Vacuum Rotary Evaporator' (Buchi type) at speed 120 rpm and 40 °C temperature. After complete desiccation the extract was stored at -20 °C in separate bottles. The concentrated extracts were dissolved in ethanol (1gm/ml) and subsequently, 10% stock solution was prepared by diluting it with 10% ethanol. Few drops of 0.2% Tween 80 were added to the stock solution to stabilize the extract. The stock solution was serially diluted to prepare 5%, 2.5% and 1.25% solutions for experimental purpose.

2.4 Treatment of the Seeds with Ethanol Extract

The dried seeds of cotton were treated with the ethanol extract of each plant by 'seed dip method' [22]. 1 gm. of the seeds was submerged in the ethanol extract of desired concentration for two hr. The seeds were subsequently dried under electric fan for 2 h.

2.5 Treatment of the Insects

Newly emerged fifth instar nymphs were starved but kept on water for 24 h. The fifth instar nymphs were fed on the seeds treated with 2.5% and 1.25% ethanol extracts of *C. roseus, O. sanctum* and *L. camara*, as described by Kodandaram *et al*. [22] Feeding in the insects was confirmed by observing their excreta; after confirming feeding, the males and females developed from the treated fifth instar nymphs were kept in separate jars for a period of 72 h for sexual maturation [23]. Five mating pairs were used for each experimental set up. Total number of eggs, egg batches, and number of eggs in each egg batch was analyzed in each experimental set up. Hatchability of egg was assessed to analyzed fertility of the females. In control experiments, the adult males and females, developed from the nymphs fed on the seed soaked in 10% ethanol for 24 h were used. Subsequently, these insects were provided with normal food and water. Separate control experiments were conducted along for each test. All the experiments were replicated five times. The results were statistically analyzed using IBM SPSS 19.1software. Validity of hypothesis was confirmed by one way ANOVA followed by TUKEY test at 95% of confidence limit [24].

3. Results

Effect of ethanol extracts of *Catharanthus roseus, Ocimum sanctum* and *Lantana camara* on fecundity and fertility of *D. koenigii* was studied on the adults emerged from the fifth instar nymphs fed on the seeds soaked with the extract. Feeding was inhibited on the seeds treated with 10% and 5% extracts of these plant extracts [23]. Therefore, the present studies were conducted on 2.5% and 1.25% extracts only. The results summarized in Table 1 indicated that the ethanol extracts of all the three plants tested in the present study were effective in suppression of the number of eggs laid by a female in its entire lifespan. There was reduction in the egg output upto 50% in the treated females. *C. roseus* and *O. sanctum* were more effective than the *L. sanctum*. However, the difference in the fecundity of the control and the females treated with 2.5% *L. camara* extract was also statistically significant. The difference in the number of eggs laid at

treatment with two concentrations i. e. 2.5%, and 1.25% of *O. sanctum* and *C. roseus* was not statistically significant (Table 1). The results also indicated that the treatment with extracts also reduced the number of egg batches laid by the females in its reproductive life. The effectiveness of the extracts decreased in the order *C. roseus* > *O. sanctum* > *L. camara*. The difference in the number of egg batches laid by the females treated with 2.5% and 1.25% extracts was not statistically significant (Table 2). The ethanol extract also influenced the number of eggs in one egg batch laid by the treated females (Figure 1). Treatment with 2.5% concentration was effective in reduction in the number of eggs in an egg batch. *O. sanctum* was most effective (Figure 1B) and *L. camara* was least effective (Figure 1C).

Results of influence of ethanol extracts of *C. roseus*, *O. sanctum* and *L. camara* on fertility of the treated females are presented in Table 3. The percent hatching success of the eggs laid by the treated females was significantly lesser than the control. There was dose dependent difference in the egg hatchability. *L. camara* was most effective in suppression of the fertility at the concentration of 2.5%. At concentration, 1.25% *O. sanctum* was most effective among the three plants tested.

Table 1: Effects of ethanol leaf extracts on total number of eggs laid by females, developed from treated fifth instar nymphs of *Dysdercus koenigii*.

Concentration of ethanol extracts	Total number of eggs laid per female* (Mean ± SE)		
	Plants Tested		
	Catharanthus roseus	*Ocimum sanctum*	*Lantana camara*
Control	233.32^{a} ± 11.82	245.64^{a} ± 13.64	245.32^{a} ± 14.76
1.25%	143.00^{b} ± 22.01	141.04^{b} ± 14.12	189.04^{ab} ± 27.89
2.5%	119.32^{b} ± 26.99	114.00^{b} ± 24.73	149.92^{b} ± 11.39

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, five females per replicates

Table 2: Effects of ethanol leaf extracts on number of egg batches laid by the females, developed from treated fifth instar nymphs of *Dysdercus koenigii*.

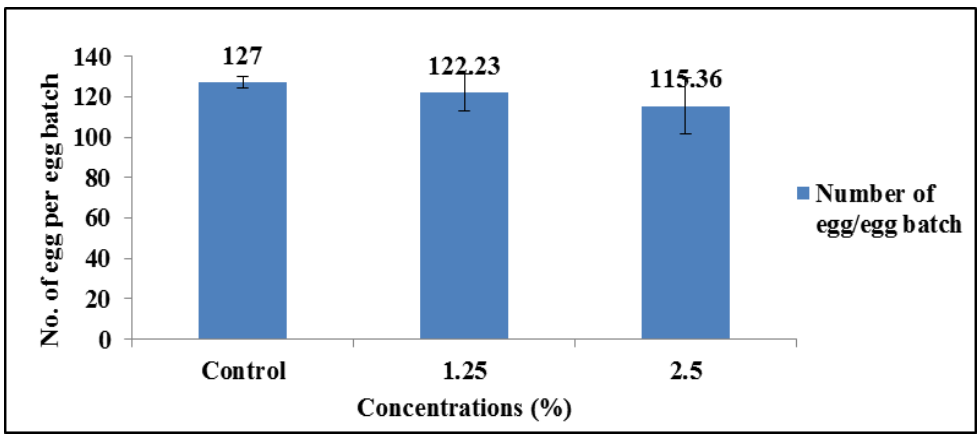
Concentration of ethanol extracts	Number of egg batches laid per female* (Mean ± SE)		
	Plant Tested		
	Catharanthus roseus	*Ocimum sanctum*	*Lantana camara*
Control	1.84^{a} ± 0.09	1.88^{a} ± 0.10	1.96^{a} ± 0.09
1.25%	1.16^{b} ± 0.17	1.20^{b} ± 0.10	1.44^{ab} ± 0.21
2.5%	1.00^{b} ± 0.20	1.12^{b} ± 0.22	1.32^{b} ± 0.12

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, five females per replicates

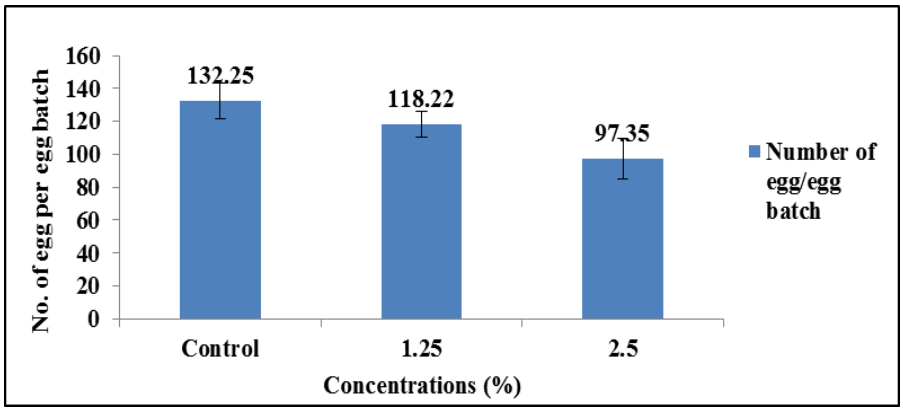
Table 3: Effects of ethanol leaf extracts on hatchability of eggs laid by the females, developed from treated fifth instar nymphs of *Dysdercus koenigii*.

Concentration of hexane extracts	Percent hatchability* (Mean ± SE)		
	Plants Tested		
	Catharanthus roseus	*Ocimum sanctum*	*Lantana camara*
Control	77.91^{a}± 0.91	78.20^{a}± 1.91	75.93^{a}± 1.95
1.25%	69.00^{a}± 4.61	61.94^{b}± 3.24	58.90^{b}± 3.92
2.5%	48.09^{b}± 3.36	48.18^{c}± 2.96	52.30^{b}± 3.58

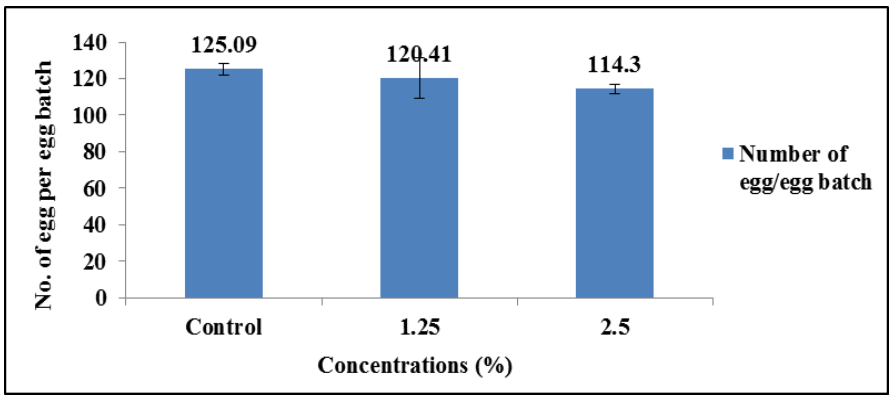
Means followed by the same letter in a column are not significantly different at $P<0.05$ (ANOVA followed by Tukey test).
*Average of five replicates, five females per replica



(a)



(b)



(c)

Fig 1: Effect of ethanol extracts of (a) *Catharanthus roseus*, (b) *Ocimum sanctum* and (c) *Lantana camara* on number of eggs per egg batch laid by females, developed from treated fifth instar nymphs of *Dysdercus koenigii*.

4. Discussion

In our earlier study (Kayesth and Gupta) [23] the antifeedant activity of ethanol extracts of *Catharanthus roseus*, *Ocimum sanctum*, and *Lantana camara* was assessed on *Dysdercus koenigii* and significant differences were observed in the feeding activity of fifth instar nymphs. The extracts were also shown to possess the cidal activities; consequently, a significant decrease in the survival of treated insects was reported specially during later part of their life. Ethanol extracts of the plants 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 [23].

Ethanol extracts of *Catharanthus*, *Ocimum* and *Lantana* extracts had significant effects on fecundity as reflected by the number of eggs and egg batches laid by the treated females. The extracts also affected fertility as a result hatching success of the eggs laid by the treated females was reduced. The effects were dose dependent. The number of egg batches laid per the females treated with 2.5% and 1.25% *C. roseus* ethanol leaf extract was 45% and 36% lesser than control. A significant difference in the treated and the control was also observed in the number of eggs laid by the female. The ethanol extract of *C. roseus* affected the percent egg hatchability; a reduction of 38% in hatchability of the eggs was observed, when the female treated with 2.5% extract. *Catharanthus* extracts have been reported to influence the fecundity and fertility of the *Dysdercus* [25]. These results were almost parallel to our studies. The alkaloid components of the leaf extracts of *Catharanthus roseus* has been reported to influence the egg production and fertility in *Gryllodes sigillatus*, in a dose-dependent manner [26]. Kumar *et al.* [27] showed influence of *Catharanthus roseus* root extracts on fecundity and fertility of *Spodoptera litura*. The adults showed reduced fecundity and hatching success of eggs. Our studies were also in agreement with Guddewar [25]. They reported decreased fecundity and fertility in the *Dysdercus koenigii* treated with acetone extracts of *Catharanthus roseus*.

The effects of ethanol extracts of *Ocimum sanctum* on the oviposition behaviour and percent egg hatchability of *Dysdercus* were prominent. Like *Catharanthus roseus*, the effects were dose dependent. A significant difference in the number of egg batches laid by the females was observed in treated and control females. The reduction in the number of 1.25% of *Ocimum sanctum* was 40% and 36% reduction in the number of egg batches was observed in the females treated with and 2.5% and 1.25% ethanol extract of *O. sanctum* than control experiment. Similarly, a significant difference was observed in fertility of the treated female as reflected by decrease in the hatching success of the egg laid. Effects of *Ocimum* extracts have also been reported on the fecundity and fertility of various insect pests including *Dysdercus.* Kiradoo and Srivastava [28] observed significant reduction in the number of eggs laid by *Callosobruchus chinensis* treated with *Ocimum* extracts. Similarly, the ether extracts of *O. Sanctum* was found effective against nymphs of *Dysdercus koenigii*; the adults had a decreased fecundity and fertility [25].

Ethanol extract treatment of *Lantana camara* also influenced the reproductive bioactivities of the adults. However, the effects were less intense in comparison to *Catharanthus* and *Ocimum*. The number of egg batches laid per female in treatments with 2.5% and 1.25% were 32% and 26% lesser than control. Similarly, the total number of eggs laid per female and the hatchability of the eggs laid by the treated females was less. Similar trends were reported when the

petroleum ether and methanol extracts of the aerial part of *Lantana camara* were applied on *Callosobruchus chinensis*, and marked reduction in fecundity and oviposition was observed [29].

Many plant other than *Catharanthus*, *Ocimum* and *Lantana* have been shown to influence fecundity and fertility of *Dysdercus.* The dose related changes in number of eggs laid, no of egg batches and percent egg hatching showed parallel trends shown in our study. Sontakke *et al*. [30] reported the effects of *Psoralea corylifolia* extracts on reproductive bioactivities of *Dysdercus cingulatus*. They reported the reduction in egg laying and hatching success in the treated females. *Eucalyptus* oil odour causes an adverse effect on the reproductive performance of the adults and egg yield and egg hatching of *Dysdercus koenigii* [31, 32]. Neem-based insecticides affected mating, fecundity and hatching of *Dysdercus koenigii* [33, 34]. Methanol Extract of *Ulva fasciata* possesses ovipositional deterrent activity, reduced fecundity, and hatchability against *Dysdercus cingulatus* [35].

The phytoconstituents present in the ethanol extracts of *Catharanthus*, *Ocimum* and *Lantana* plant extract can be explored for the management of other hemipteran insect pests by hampering their reproductive potentials.

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Contributions made by each listed author:

KKG conceived and designed research. SK conducted experiments, collected data and performed statistical analysis and prepared the manuscript. MS and SK helped in preparation of extracts, statistical analysis and in preparation and editing the manuscript.

All authors have read and approved the manuscript.

Conflict of Interest: The authors declare that they have no conflict of interest.

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