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Larvicidal activity of a few botanical extracts against leaf roller in the mulberry

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Abstract
Leaf roller *Diaphania pulverulentalis* damage is one of the major problem in mulberry growing areas which affect both quality and yield of mulberry leaf. The leaf extracts of *Lantana camara* (11%), *Azadirachta indica* (20%), *Vitex negundo* (25%), cloves of *Allium sativum* (15%) and rhizome *Zingiber officinale* (15%) have been evaluated against second and third instar larvae of leaf roller. The results revealed that, by 144 hr. the methanol extract of the botanicals exhibited maximum mortality of second (85-95%) and third instar larvae (85-95%) compared to aqueous extract (68-82%) and (58-76%) respectively. Larvicidal effect of the extracts on second and third instar larvae of leaf roller have been investigated in a dose dependent manner for every 24 hr. including the mortality of pupae, adults, deformed pupae and moths also. Also the high percent of mortality of pupae (2-16%) and adult (2-6%) was noticed in case of the larvae treated with aqueous extracts compared to methanol extracts treated larvae. Among the extracts tested, the aqueous extract of lantana at lower concentration i.e. 11% has recorded the mortality percent of second and third instar larvae of leaf roller up to 69% and it was found to be better in controlling the leaf rollers compared to other extracts and safe for silkworm without any harm.

Keywords: Botanical extracts, Leaf roller *Diaphania pulverulentalis*, Larvicidal activity

1. Introduction
Mulberry (*Morus spp*.) is a perennial, deep rooted, fast growing and high biomass producing foliage plant. It is used as a food for the silkworm, *Bombyx mori* L. It plays an important role in the quantity and quality of silk production, contribute to 38.20% for the success of cocoon crop [1]. Mulberry foliage is prone to depredation by pests and diseases. Among the several pests known to attack mulberry, the lepidopteron leaf roller, *Diaphania pulverulentalis* (Hampson) (family; Pyralidae) observed as a serious pest of mulberry in Karnataka state since 1995 [2] and spread to the Tamil Nadu and Andhra Pradesh. It attacks the crop throughout the year, more in January to October. It caused 66% reduction in the leaf yield of M5, followed by MR2 (65%), V1 (52%) and S54 (30%) mulberry varieties [3]. The early larval stage inhabits the apical part (unopened leaves) of the mulberry shoot and feed by scraping the tender leaf tissue, resulting in drying of the terminal portion of the plant. The pest-infested plants show stunted growth resulting in considerable decline in the leaf yield [4]. The damage caused by the leaf roller to mulberry is to an extent of 20 to 40% in some traditional regions of Karnataka [5]. Since mulberry is the sole food plant of the silkworm *Bombyx mori* L., application of chemical pesticides to manage the pests of mulberry is not advisable. Recently non-chemical avenues like botanicals acted as an efficient alternative for the pesticides in mulberry garden [6]. The use of plant extracts and plant products is gaining attention due to proven specificity, biodegradability, low toxicity to non target organisms and low residual toxicity in the ecosystem [7]. Therefore, a study has been taken up to know the efficacy of botanical extracts in controlling the leaf roller and its infestation.

2. Materials and Methods
2.1 Collection Leaf roller larvae
Leaf roller *Diaphania pulverulentalis* is a major pest of mulberry (Family:Pyralidae) and lay about 80–150 eggs on tender apical leaf buds of mulberry and hatch within 2-3 days. The larval stage causes severe damage to apical portion of the plant and the larval span completes 8–12 days. The period of pupa, adult of male and female takes 7–9 days, 7–12 days and 9–14 days respectively. The second and third instar larvae of leaf roller were collected and utilized to study the efficacy of botanicals on control of the pest.

2.2 Collection of botanicals
The leaves of lantana (*Lantana camara* Linn.), neem (*Azadirachta indica* Juss.), vitex (*Vitex negundo* Linn.), cloves of garlic (*Allium sativum* Linn.) and rhizome of ginger (*Zingiber officinale* Rose.) were selected for the study to assess its larvicidal property.

2.3 Aqueous extraction of lantana, neem and vitex leaves
The extracts of the lantana, neem and vitex was prepared by homogenizing 10 gm of plant material (leaf) in 100 ml of distilled water. The homogenate of each botanical extract was filtered through three layered muslin cloth. The resulting aqueous extract was used as a stock solution to prepare the required dose [8].

2.4 Aqueous extraction of garlic and ginger
50 g Garlic was taken and ground well to obtain a paste form. Then 25 ml of distilled water is added to the paste and shaken well. The mixture was kept for three days; strained using a clean muslin cloth and the volume was made to 50 ml by adding further 25 ml of water, shaken well and stored in a dark bottle. Similar procedure was used to prepare the ginger extract also.

2.5 Soxhlet extraction
Similarly the extracts of the botanicals under study were obtained using the soxhlet extraction method. This was carried out by using the following procedure; 20 grams of was washed, dried and powdered plant part were taken and placed in a thimble made up from the thick filter paper, which was loaded into the main chamber of the Soxhlet extractor. The extractor was placed onto a round bottom flask containing the extraction solvent i.e. distilled water. The Soxhlet was then equipped with a condenser. The solvent was heated at 90 °C to reflux. As the solvent vapour travelled up a distillation arm into a condenser, the condensed vapours dripped back down into the chamber housing the solid material. The chamber containing the solid material slowly filled up with warm solvent. When the Soxhlet chamber was almost full, the chamber was automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. After extraction, the solvent was removed by means of a rotary evaporator. The extracted compound was collected. The non-soluble portion of the extracted solid in the thimble was discarded. Similarly soxhlet extraction of garlic and ginger was also done and obtained the stock solution.

2.6 Larvicidal activity
The larvae of leaf roller were collected from the mulberry field in and around Bangalore University. Before the larvae were treated with botanical extracts, the leaf roller was kept starved. The aqueous and methanolic extracts prepared from the botanicals were utilized to prepare the concentrations viz. 11% lantana, 15% garlic, 15% ginger, 20% neem and 25% vitex in order to evaluate the larvicidal activity on leaf roller.
Leaf-dipping method was used to evaluate the larvicidal activity of the botanical extracts against second and third instar larvae of leaf roller by keeping three replications per treatment i.e. 20 larvae/ replication. At the time of treatment, second/third instar larvae were transferred on control (untreated) as well as botanical extracts treated mulberry leaves placed in petri dishes. The mortality of the larvae was recorded at every 24 hr. intervals.

2.7 Statistical Analysis
The data were subjected to multivariate analysis to study how the plant extracts related to one another and how it works on the pest and its infestation to distinguish between the cases on which the observations are made to determine the percent reduction of second and third instars larvae of leaf roller after the treatment.

3. Results
All the tested plant extracts indicated the presence of larvicidal activity against larvae of the leaf roller. Dose dependent effect of methanol and aqueous extract of botanicals on the mortality percentage of second and third instar larvae of leaf roller was noticed.

3.1 Evaluation of botanical extracts against second instar larvae of leaf roller
The methanol extract of botanicals exhibited the maximum mortality of second instar larvae compared to aqueous extract. Among the extracts tested, the methanol and aqueous extract of 11% lantana recorded the mortality percentage of 0-87% and 0-69% respectively from 24-144 hr. Whereas the range of mortality recorded by the methanol and aqueous extract of 15% garlic, 15% ginger was 10-88% and 5-74%, 10-85% and 3-68% respectively. In case of neem, the mortality percent recorded was 19-93% and 14-81% respectively. However, the maximum mortality percent recorded by the methanol and aqueous extract of 25% vitex was 15-95% and 6-82% respectively. Few larvae treated with botanical extracts expressed the toxicity at the pupal and moths' stage and resulted in complete mortality with abnormalities (Fig.1-3). The details on the mortality percent of larvae, pupae and adults recorded at every 24 hr. intervals by the selected concentrations of the plant extracts are presented in Table-1.
The critical difference (CD) values indicated that the mortality of the second instar larvae of leaf roller recorded at each and every concentrations of the plant extracts are found to be significant compared to control at every 24 hr. intervals in both methanol and aqueous extracts.

3.2 Evaluation of botanical extracts against third instar larvae of leaf roller
Further the larvicidal efficiencies of the selected extracts were evaluated against third instar also. Here the percentage of mortality was less in third instar larvae compared to second instar. Increased mortality percent of the larvae was noticed at higher concentration of the extracts especially with methanol extracts compared to aqueous extracts. In case of lantana at 11%, the range of mortality percent recorded by the methanol and aqueous extract was 0-86% and 0-68% respectively. Whereas the maximum mortality recorded by the methanol and aqueous extract of 25% vitex was 8-95% and 5 – 76% respectively. The mortality recorded by methanol and aqueous extracts of 15% garlic, 15% ginger and 20% neem from 24 - 144 hr. was depicted in Table-2.
The mortality of pupae and moths at different concentrations of methanol and aqueous extract of botanicals was found to be inversely proportional. The mortality percent of pupae and moths with deformation was noticed at lower concentration viz.11% Lantana, 15% garlic and 15% ginger. No mortality of the pupae and moths was recorded at higher concentrations of the methanol extract and the reason might be due to maximum mortality noticed in the larval stage of leaf roller itself. In case of control, the mortality percentage was 0-3%.

The result indicated that it may be due to dose response relationship of the botanical extracts. Moreover the effect of botanical extracts against second and third instar larvae of leaf roller revealed the better performance of lantana at lower concentration compared to other botanical extracts.

Table 1: Effect of different concentrations of the plant extracts on second instar larvae of leaf roller *Diaphania pulverulentalis*

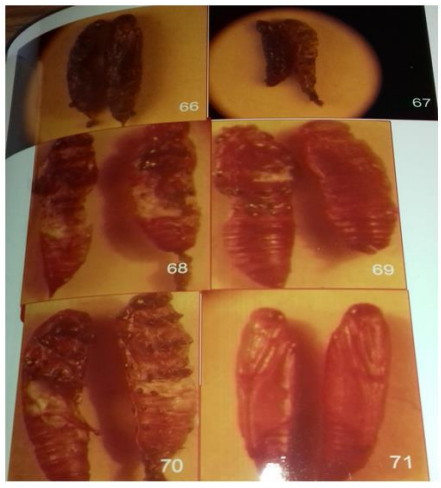
Plant Extract/ Conc.	Percent mortality in methanol extract								Percent mortality in aqueous extract							
	24h	48h	72h	96h	120h	144h	pupae	adults	24h	48h	72h	96h	120h	144h	pupae	adults
11% Lantana	0	26	50	64	74	87	7	2	0	18	43	50	59	69	13	5
15% Garlic	10	31	53	66	84	88	2	0	5	27	43	55	63	74	5	3
15% Ginger	10	30	50	67	80	85	2	2	3	21	39	54	61	68	5	4
20% Neem	19	31	50	67	80	93	2	0	14	30	47	62	71	81	4	2
25% Vitex	15	36	58	63	86	95	0	0	6	29	47	61	66	82	2	2
Control	0	0	1	2	3	3	0	0	0	0	1	2	3	3	0	0
CD @ 5%	4.65	8.39	11.8	8.30	8.90	8.10	5.06	2.96	5.44	10.8	12	12.2	11.6	8.49	7.38	5.73

Table 2: Effect of different concentrations of the plant extracts on third instar larvae of leaf roller *Diaphania pulverulentalis*

Plant Extract / Conc.	Percent mortality in methanol extract								Percent mortality in aqueous extract							
	24h	48h	72h	96h	120h	144h	pupae	adults	24h	48h	72h	96h	120h	144h	pupae	adults
11% Lantana	0	24	50	63	72	86	6	2	0	18	40	49	58	68	16	6
15% Garlic	4	27	47	66	76	86	0	0	0	23	38	50	58	67	4	2
15% Ginger	4	23	44	61	72	85	2	0	7	18	28	52	51	58	2	2
20% Neem	13	21	38	60	78	89	0	0	5	26	34	48	63	72	2	0
25% Vitex	8	24	43	64	85	95	0	0	5	29	34	46	60	76	0	0
Control	0	0	1	1	2	3	0	0	0	0	1	1	2	3	0	0
CD @ 5%	6.64	9.88	10.9	12.36	11.46	7.16	4.00	2.53	6.82	10.5	10.9	12.1	9.12	7.33	7.21	4.74

60- 11% Lantana; 61 - 15% Garlic; 62- 15% Ginger; 63- 20% Neem; 64 - 25% Vitex; 65 - Control

Fig 1: Effect of botanical extracts on larvae of leaf roller



66 - 11% Lantana; 67 - 15% Garlic; 68 - 15% Ginger; 69 - 20% Neem; 70 - 25% Vitex; 71 - Control

Fig 2: Effect of botanical extracts on pupae of leaf roller

72 - 11% Lantana; 73 - 15% Garlic; 74 - 15% Ginger; 75 - 20% Neem; 76 - Vitex; 77 - Control

Fig 3: Effect of botanical extracts on moths of leaf roller

4. Discussion

Among the botanical extracts tested, the methanol and aqueous extracts of lantana at lower concentration viz. 11% recorded the range of mortality percent of leaf roller larvae by 144 hr. was 69-87%. Whereas the other plant extracts viz. 15% garlic, 15% ginger, 20% neem and 25% vitex at higher concentration recorded the mortality of above 80%. This is in agreement with the findings of few indigenous plants viz. *Ocimum basilicum*, *Ocimum santum*, *Azadirachta indica*, *Lantana camera*, *Vitex negundo* and *Cleome viscose* [9]. Moreover the extracts/ essential oils of *Lantana* and their constituents possess varying degrees of pest controlling properties [10]. The above discussed effects of crude aqueous and methanolic extract of *L. camara* could be due to the presence of the active toxic group like Lantadene in the leaves [11]. Even the leaf applications of crude aqueous extract of *L. camara* leaves are found to be highly effective in controlling the lepidopteran pest *S. litura* [12]. Moreover the methanol

extracts of the selected botanicals recorded higher mortality percent of the leaf roller larvae compared to aqueous extracts. Even the deformation was observed in the pupae as well as moth's stage. Also these findings are in accord with the plant extracts tested against insect varies according to the mode of action, the tested part of the plant and the method of application [13].

Garlic extracts have shown a considerable toxicity to a number of species of different insect orders and to different developmental stages [14, 15]. In garlic, there are many secondary metabolites such as saponins, tannins, alkaloid steroids and glycosides play as a role in antifeedancy [16]. Similarly, the garlic extracts were found to act as antifeedants against different insect orders, for example Lepidoptera and Hemiptera [17, 18]. The findings of the present study also revealed the higher larvicidal efficacy against second and third instar larvae by the methanolic and aqueous extract of 15% garlic and 15% ginger. Effect of ginger against against *Aedes aegypti* L., *Culex quinquefasciatus* and adult beetle *Oryzaephilus surinamensis*, was evaluated [19, 20]. The findings of the study showed that the significant mortality with methanolic and aqueous extract of 20% neem. This could be due to the presence of azadirachtin in the neem leaves. Though these extracts were found effective in controlling the leaf roller, due to its persistant odour on mulberry after treatment, the consumption by silkworm *Bombyx mori* was less and it affects the cocoon yield.

Further, the treatment of the pest with 25% methanolic and aqueous extract of *Vitex negundo* was recorded 50% mortality significantly. This is in agreement with the findings that the leaf extract of *V. negundo* was found to be toxic against *Plutella xylostella* [21]. The presence of toxic compounds like terpenes, cinol, sabeniene, sesque terpenes in *Vitex negundo* extract might be the reason for its higher repellency property [22, 23]. Hence these extracts may not be advisable to use as a spray at higher concentration in the mulberry field since the silkworm depends only on mulberry for food. The findings on the study revealed that, using the botanical extracts in controlling the pests created way for natural control of the leaf roller.

5. Conclusion

Based on the results, the aqueous extract of *Lantana camara* was found to be most effective at lower concentration i.e. 11% in controlling the leaf roller and also it is safe for silkworm rearing. This in turn may increase the leaf yield of the mulberry as well as the cocoon production of silkworm *Bombyx mori* L without any harm.

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