Bioefficacy of plant extract against cabbage butterfly, *Pieris brassicae* (Lepidoptera: Pieridae)

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***Abstract*:** Methanol extract of *Lantana camara* L. was tested for antifeedant, toxicity effects and Gonado Somatic Indices against *Pieris brassicae* (Linn.). Antifeedent index increased with increasing concentration of methanol extract. At 5 % concentration, AFI was 60 % in the 3rd instar larvae and 51 % in the 4th instar larvae. 100 % mortality of larval stages was observed with increase in concentration of dose and application time. A decrease in overall body weight, weight of testes and ovaries, Female Fecundity Index (FFI) and Male Fecundity index (MFI) was also observed in methanol extract treated adults of *P. brassicae* in comparison to control group.

***Index Terms:* *Pieris brassicae*, plant extract of *Lantana camara*, antifeedent effect, toxicity, Gonado somatic indices**

I. INTRODUCTION

Cole crops include vegetables cabbage, cauliflower, mustard, broccoli belonging to the cruciferous family. The cabbage (*Brassicae oleracea* var. *capitata*), cauliflower (*Brassicae oleracea* var. *botrytis*) and mustard (*Brassicae campestris*) originated in mediterranean introduced in India (Das, 1992; Khalid, 2006).

It has been estimated that 38 insect pests on cole crops cause more than 40 % of yield lost annually (Pajmon, 1999). Among them, cabbage butterfly, *Pieris brassicae*one of the most destructive pest, is distributed along the Himalayan range throughout the plain except the Southern plain, causing damage to all growing stages of a plant (Sachan and Gangwar, 1980; Lal and Ram, 2004; Raqib, 2004; Younas et al., 2004; Khalid, 2006; Ali and Rizvi, 2007; Sharma and Gupta, 2009; Hasan and Ansari, 2010; Ali et al., 2017). The young larvae feed gregariously on leaves, resulting in defoliation (Younas et al., 2004; Khalid, 2206; Hasan and Ansari, 2010). Moreover, this pest has became resistant against a few pesticides.

The indiscriminate use of chemical pesticides have resulted many serious problems like genetic resistance, increasing cost of application, environmental health hazards and pollution etc. (Ahmed et al., 1981). This has created an interest in the development of altenative strategies for pest management for which botanical insecticides are well suited. The plant based insecticides (botanicals) are well known for their insecticidal and insect repellent characters and lower toxicity to the environment (Zahid et al., 2016). The use of simple formulations of plants such as leaves, flower or seed, fruit extracts need to be popularized which are also safe to non-target organism, including humans

The common weed *Lantana camara* L. was used for its biological activities against the pest *Pieris brassicae* in the present study. Antifeedant activity, toxicity and Gonado somatic indices i.e. Female Fertility Index (FFI) and Male Fertility (MFI) for this pest have been studied.

II. MATERIALS AND METHODS:

2.1 Rearing of test insecct

The stock culture of *Pieris brassicae* was maintained under laboratory conditions. Egg clusters were collected from Cole crops in the field and kept in petri dishes (10 cm) diameter kept over the moist filter

papers. Newly hatched larvae were transferred to cabbage/cauliflower leaves. The larvae were provided with fresh food daily till pupation.The adults were provided with 50 % honey solution. Male and female adults were reared separately for the study of Gonado Somatic Index (GSI).

2.2 Collection of plant material and extraction

Fresh leaves of *Lantana camara* were collected from the bushes, washed thoroughly to remove dirt and other garbage material. The leaves were then shade dried till the moisture completely evaporated and leaves turned out to be crispy. After drying, leaves were powdered finely. Extraction was done by Soxhlet method (Sharma, 1988).

50 g of leaf powder was used for extraction. The soxhlet apparatus was run for 28 h. The solvent having extract was then subjected to rotator evaporator. A total of 250 mg viscous, dark green coloured extract was obtained. This extract was then diluted in 1000 ml of Methanol to make 250 ppm and stored properly in dark bottles for antifeedant and larvicidal studies.

2.3 Antifeedant effect

Antifeedant effect of the plant extracts was studied against the third and fourth instar of test insect. The testing was done by leaf-dip method at the desired concentrations of 0.5%, 1.0%, 2.5% and 5.0%. The third and fourth instars of the test insects were pre-starved for three hours. Three replicates, of single starved larvae were released in the five beakers covered with muslin cloth, each for experimental and control, to prevent carnivorous behaviour. Fresh cabbage leaves were kept in a separate beaker to calculate the moisture loss. Observations were recorded after 24 hours for 3 days. After three days uneaten leaves were collected and the antifeedant effect was calculated as Waldbauer's (1968) equation.

$$\text{Percent (\%) reduction in food consumption} = \frac{Cc - Ct}{Cc} \times 100$$

Where:

Cc = Consumed amount in untreated leaf
Ct = Consumed amount in treated leaf

2.4 Toxicity effect

Twenty larvae each of third, fourth and fifth instars of *P.brassicae* were collected from the rearing stock for the solvent i.e. Methanol and were kept singly in a glass beaker (9.5 x 7 cm). The mouth of each beaker was covered with muslin cloth, tightened with rubber band to prevent escape of the larva.

Equal number of larvae were kept under control for both the species. Larvae were treated with pure solvent, i.e. Methanol; devoid of leaf extract.

For the toxicity study, Knock Down Effect (KD), the beaker and food were sprayed with different concentrations of leaf extracts. Ten larvae were then released in the beaker, timing and percent of the mortality was noted. This process was repeated till complete mortality of all 20 larvae. The larvae which did not show any movement on touching with camel's brush were considered as dead.

The larvae under treated and control group were fed with fresh cabbage leaves. The beakers were washed thoroughly for prevention of fungal attack and other infections.

2.5 Gonado Somatic Index

The Gonado Somatic Index is the calculation of gonad mass as a proportion of the total body mass. It is a tool for measuring the sexual maturity of animals in relation to ovary and testes development. Newly

emerged adults of test insect were kept in separate groups of five male and females each for their body and gonads weight on third, fifth and seventh day. The adults were provided with 50% honey solution.

Under experimental groups, the adults were given honey solution mixed with Methanol extracts, whereas, control group were provided with honey solution only devoid of extract.

2.6 Female Fertility Index (FFI) IN Adult females:

Mean weight of each (control and experimental) groups of five adults females and the ovaries were weighed and FFI was calculated as:

$$\text{FFI (adults)} = \frac{\text{Wet weight of ovaries}}{\text{Wet weight of females}} \text{ x } 100$$

Similarly, mean weight of each (control and experiment) groups of five adult males and testes were weighed and MFI was calculated as:

$$\text{MFI (adults)} = \frac{\text{Wet weight of testes}}{\text{Wet weight of males}} \text{x } 100$$

III. RESULTS AND DISCUSSION

3.1 Antifeedant effect

Data on antifeedant effect of *L. camara* on 3rd and 4th stage larvae of *P. brasicae* are shown in Table 1.

Food consumption in the control group showed an increase from 3rd to 4th instar larvae. However, in the treatment group, consumption declined.

At 0.5% concentration, Antifeedant Index was 16.42% in 3rd instar which decreased to 10.2% in 4th instar larva. At 1.0% concentration, AFI was 28.57% in 3rd instar which decreased to 20.01% in 4th instar larvae. At 2.5% concentration, AFI was 40.0% in 3rdinstar which decreased to 36.5% in 4th instar larvae.

Thus, Antifeedant Index increased with increased concentration of Methanol extract fed larvae (Table 1).

Any substance reducing the food ingestion by insects is categorized as an antifeedant and generally has adverse effects on insect feeding behaviour (Hummelbrunner and Isman, 2001) which observed in our study also. Sharma and Gupta (2009) reported that 2 percent leaf water extract extract of *Azadiracha indica* resulted in maximum protection to foliage over control at all concentrations used followed by *Eucalyptus* sp. against *P. brassicae.* The antifeedant activity of methanol extracts of *Melia azedarach* was reported by Zhu (1989) against *P. rapae*. Muralikrishana et al. (1990) reported 100% protection to cabbage leaves with petroleum ether extract (1%) against *P. brassicae.* Complete feeding inhibition of cabbage leaves treated with *Lantana camara* at 1 percent against first instar larvae of *P. brassicae* was reported by Mehta et al. (1996). Leaf extracts of *Justica vasica* L. and *Capparis* spinosa L. also caused strong feeding inhibitor in *Spodoptera litura* (Sadek, 2003; Ladhari et al., 2013).

Table 1: Antifeedant effect of the *Lantana camara* leaf extract on different instars of *Pieris brassicae*

Treatment	% Conc. (g/L)	Instars	Consumed food (mg) (Control)	Consumed food (mg) (Treated)	Antifeedant (%)

METHANOL	0.5	3rd	700	582	16.42
		4th	980	880	10.20
	1.0	3rd	700	500	28.57
		4th	980	775	20.91
	2.5	3rd	700	420	40.0
		4th	980	622	36.53
	5.0	3rd	700	280	60.0
		4th	980	480	51.02

3.2 Toxicity effect

Promising results were obtained for Knock Down (KD) toxicity with methanol solvent extract using four different concentrations (0.5%, 1.0%, 2.5% and 5.0%). Plant extract proved to be feeding deterrent and larvicidal agent (Table 2).

It was observed that percent mortality increased with increase in dose and its application time in all larval instars. Various morphological deformities such as larvae failed to shed cuticle, stunted growth, unable to hold the wall of the beaker due to lack of control and co-ordination of muscles, rotten intestine, unable to form pupae and some larvae died due to incomplete pupation were observed in the treated larvae. Pre treated adults an emergence had crippled wings and deformed body that also effects their fertility. Narendran et al. (1999) also observed several deformities in head size, body length, remains of old cuticle, darkened colour of wings of *S. litura* when treated with bark extracts of several *Cassia fistula* and leaf extracts of *Murraya koenigii* at 1000ppm. Several studies have also reported that mortality was dose dependent. Many botanicals offer antifeedant as well as toxic effects against *Spodoptera litura* (ulrichs et al., 2008; Arivoli and Samuel, 2012) and *Pieris brassicae* (Ali et al., 2017). Satpathi and Ghatak (1990) observed 90% mortality of *Henosepilachna vigintiopunctata* (F.) larvae with *Nerium indicum* petroleum ether extract. Larval mortality of 22.8 percent with methanol extract of *Melia azedarach* against *Plutella xytostella* was reported by Dilawari et al. (1994). Leatemia and Isman (2004) also also reported that higher concentrations of plants can cause high larval mortality even with only a small amount of treated food being consumed. Similarly, 100% mortality of larval steps was observed with increase in concentration of dose and application time in the present study also.

Table 2: Knock Down (KD) toxicity of methanol extract on larval instars of *P. brassicae* (±SD)

% Conc. (g/L)	KD toxicity values	3rd instar larvae		4th instar larvae		5th instar larvae	
		Duration (days)	Mortality (%)	Duration (days)	Mortality (%)	Duration (days)	Mortality (%)
0.5	KD_{1oo}	4.2±0.83	100	4.8±0.44	100	7.4±0.54	100
	KD_{50}	3.0±0.9	50	3.6±0.54	50	6.8±0.44	50
	KD_0	2.0±0.0	0	3.4±0.5	0	6.4±0.5	0
1.0	KD_{100}	2.0±0.7	100	4.6±0.5	100	6.0±1.0	100
	KD_{50}	3.2±0.83	50	3.4±0.54	50	6.4±0.5	50
	KD_0	4.6±0.54	0	3.2±0.83	0	4.6±0.54	0
2.5	KD_{100}	1.6±0.54	100	3.4±0.54	100	5.2±0.44	100
	KD_{50}	2.6±0	50	3.2±0.44	50	5.4±0.54	50
	KD_0	3.4±0.54	0	2.8±0.83	0	4.2±0.4	0
5.0	KD_{100}	1.2±0.44	100	2.4±0.5	100	4.2±0.83	100
	KD_{50}	2.2±0.44	50	2.8±0.44	50	3.0±1.0	50

	KD_0	3.2±0.44	0	1.2±0.4	0	1.2±0.4	0

3.3 Gonado Somatic Index

A comparative account of control and extract of different concentration on FFI and MFI is given in Tables 3 and 4 respectively.

In comparison to control group where overall body weight, weight of ovaries and percent FFI increased with age, a decrease in all these values were observed in methanol treated extract (Table 3).

Similarly, a decrease in overall body weight, weight of testes and percent MFI was also observed in methanol treated extract in comparison to control (Table 4). Table 5 presents correlation coefficients (r) between body weight, weight of ovaries/testes and percent FFI and MFI of adults of control group and methanol treated extract. A positive correlation was observed in all the parameters.

In the present study, methanol extract not only showed antagonistic effects on the survival of different larval instars but also hampered the reproductive potential of the male and female adults of *P. brassicae* by decreasing the proper size, weight ad morphological makeup of gonads and thus causing a decrement in their Gonado Somatic Index values in comparison to the adults of the adults of control group, which showed an increase in all above mentioned aspects. The reduction clearly depicts decrease in their reproductive ability which will negatively affect the size of progeny and in turn, will help in controlling the pest population. The impact on gonads may lead to complete mating, non-production of eggs as sperms and consequently complete sterility.

Sombatsiri and Tigvattanont (1983) reported reduction in fecundity in *Dacus dorsalis* after being treated with neem products. Oocyte development in *Locusta migratoria* was completly suppressed when nymphs were fed on *Ageratum houstonianum* extract. Linton et al. (1997) reported reduced reproductive potential of testes of *Schistocerca gregaria* showing the showing the effects of *Azadirachtin.* Anani et al. (2004) have also reported that neem reduced *Sesamia calamistis* and *Eldana saccharina* fecundity by 30 and 46%, respectively.

Insecticidal and repellent properties of leaf extract of *L. camara* observed in the present study indicates that it can be used to control the larvae of cabbage butterfly in cole crops.

Table 3: Comparative account of Control and Methanol solvent extracts (0.5%, 1.0%, 2.5% and 5.0% concentration) on FFI in *Pieris brassicae*

Experimental group	**Average body weight (mg)**	**Average weight of ovaries (mg)**	**FFI= Weight of ovaries × 100 / Weight of female insect (%)**
Control			
2 days	115.87	25.92	22.36
4 days	119.95	32.16	26.81
6 days	128.54	36.15	28.12
0.5 % concentration			
2 days treated	112.25	23.64	21.06

4 days treated	111.28	22.90	20.57
6 days treated	110.81	20.22	18.24
1.0 % concentration			
2 days treated	110.52	22.07	19.96
4 days treated	108.03	20.13	18.63
6 days treated	106.75	17.02	15.94
2.5 % concentration			
2 days treated	108.88	21.02	19.30
4 days treated	105.93	19.59	18.49
6 days treated	104.30	18.64	17.87
5.0 % concentration			
2 days treated	105.23	18.87	17.93
4 days treated	103.60	17.28	16.67
6 days treated	101.57	15.73	15.48

Table 4: Comparative account of Control and Methanol solvent extracts (0.5%, 1.0%, 2.5% and 5.0% concentration) on MFI in *Pieris brassicae*

Experimental group	**Average body weight (mg)**	**Average weight of testes (mg)**	**FFI= Weight of testes× 100 / Weight of male insect (%)**
Control			
2 days	107.29	16.33	15.22
4 days	108.05	17.10	15.82
6 days	110.72	19.21	17.34
0.5 % concentration			
2 days treated	105.25	13.03	12.38
4 days treated	105.82	11.17	10.55
6 days treated	103.29	9.53	9.22
1.0 % concentration			

2 days treated	101.31	10.08	9.94
4 days treated	100.16	9.0	8.98
6 days treated	98.30	7.23	7.35
2.5 % concentration			
2 days treated	98.67	8.70	8.81
4 days treated	98.10	7.54	7.68
6 days treated	97.17	5.05	5.19
5.0 % concentration			
2 days treated	95.19	6.14	6.45
4 days treated	94.35	5.18	5.49
6 days treated	93.88	4.27	4.54

Table 5: Correlation coefficient (r) of control and methanol treated extract of adults of *P. brassicae* (all values significant at P<0.01)

Control Parameters	Percent treatments							
	0.5%		1.0%		2.5%		5.0%	
	Female	Male	Female	Male	Female	Male	Female	Male
Body weight	0.961	0.545	0.967	0.981	0.973	0.981	0.996	0.974
Weight of ovaries/testes	0.903	0.998	0.967	0.980	0.999	0.881	0.993	0.999
FFI/MFI	0.787	0.991	0.879	0.978	0.974	0.955	0.959	0.999

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