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Effect of plant extracts on feeding activity of cabbage butterfly, *Pieris brassicae*

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Abstract

The antifeedant activity of various plant extracts were evaluated against cabbage butterfly under laboratory conditions (range of temperature 27-30°C and RH 75-80%). An experiment was conducted to find out antifeedant activity of leaf extracts prepared in cow urine at 2% and 5% concentrations of various plant species viz., *Azadiracta indica*, *Eupatorium triplinerve*, *Cannabis sativa*, *Nerium indicum*, *Parthenium hysterophorus*, *Melia azedarach*, *Lantana camara*, *Annona squamosa*, *Datura stramonium*, against 3rd instar larvae of *P. brassicae*. These were tested by using 'no-choice feeding' bioassay method. The observation on leaf area consumed was recorded on graph sheets and was used for calculations of other parameters viz., Mean leaf area consumed (cm²), Per cent feeding, Per cent Feeding inhibition, Antifeedant activity (%) and Preference index (C-value).

Keywords: Antifeedant, instar, bioassay, *P. brassicae*, preference index (C-value)

Introduction

Cabbage, *Brassica oleracea* var. *capitata* Linn. is an essential cole crop and commercially produce as a leafy vegetable in India. The leafy vegetables, especially Cole crops, make up a major portion of the diet of humans and are sources of phyto-nutriceuticals: vitamins (C, A, B1, B6, B9, and E), minerals, dietary fiber and phytochemicals (Dias, 2012) [4]. The cabbage butterflies, *Pieris brassicae*, *P. canidia* and *P. rapae* have also been found to be major pests of cabbage and cauliflower in India (Bhatia & verma, 1993; Bhutani & Jotwani, 1984; Firake *et al.*, 2012 and Lal, 1975) [6, 7, 12, 13]. In one of the studies, it has been documented *P. brassicae* and Cabbage leaf webber, *Crociodolomia binotalis* underlay 69% & 28-51% damages respectively to the Cole crops in India (Rai *et al.*, 2014) [20]. In India, it passes winter in the plain and migrates to hilly regions during summer (Gupta, 1984) [6]. This pest damages all plant stages in cabbage crops, i.e., seedlings, vegetative and flowering stages (Ullah *et al.*, 2016) [27]. Young caterpillars of *P. brassicae* are gregarious leaf feeders (Hasan & Ansari, 2011) [8]. The larvae of *P. brassicae* feed on all plant parts including leaves, twigs, fruits and seeds of cabbage and cauliflower (Siraj, 1999) [22]. A single larva can consume up to 74 to 80 cm² of leaf and causes serious damage to the host plant (Younas, *et al.*, 2004) [30]. Extreme infestations of *P. brassicae* completely destroy the plant foliage and ultimately kill the plant (Hasan & Ansari, 2010) [7].

Plants are rich sources of natural substances that can be utilized in the development of environmentally safe methods for insect control and are able to synthesize a broad range of different chemical compounds called secondary metabolites and many of them are promising new sources of natural pesticides. Secondary plant compounds found in botanical insecticides have been the recent focus of many investigations. Neem (*Azadiracta indica*, Juss.) has emerged as an excellent alternative to chemical insecticides for the management of insect-pests and was studied by many researchers. The extracts of *Datura aborea* (Linn.), *Nicotiana tobaccum* (Linn.) and *Zanthoxylum alatum* (Roxb.) showed repellent effect and anti-feedant effect on different insect larvae and their lifecycles (Paul and Sohkhlet, 2012) [17]. Numbers of workers uses the plant materials and cow urine for the control of insect pests of field crops (Geetanjal and Tiwari, 2014).

Botanicals are comparatively safer to the parasitoids like *Cotesia glomerata* which voraciously parasitizes the *P. brassicae* caterpillar (Ullah *et al.*, 2016) [27] when compared to synthetic

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insecticides (Yi *et al.*, 2016)^[29]. Botanicals are even less toxic to the developing larvae of parasitoids in the host insects exposed to them. In addition, the majority of botanicals contains a diverse mix of active compounds and thus, do not result in pest resistance (Pavela, 2011)^[19]. The present investigation was undertaken to find effective control for larval stages of *P. brassicae* by using plant extracts easily available locally, and with user friendly protocols for the farmers.

Materials and Methods

Maintenance of Culture

Culture of cabbage butterfly, *P. brassicae* L.

The culture of *P. brassicae* (Linn.) was raised in glass jars on fresh leaves of cabbage, *Brassica oleracea* L. var. *capitata*. The nucleus culture of the test insect larvae was collected from the Vegetable Research and Demonstration block, Department of Vegetable Science, College of Horticulture, VCSG UHF, Bharsar and brought to the laboratory, were reared on fresh cabbage leaves till pupation and healthy pupae were procured for the next generation. The culture was

maintained at 27°C and 70 ± 5% RH. The adults obtained from above culture were released in separate glass jars (18x12 cm²) the walls of which were lined with white paper for egg laying. White paper strips were also kept in the jars for egg laying and muslin cloth and strips were checked daily for egg laying and eggs were removed and placed in separate jars on fresh succulent cabbage leaves for hatching. The neonate larvae were reared on fresh cabbage leaves to maintain the test culture of *P. brassicae* (Linn.). In adulthood, the culture had been covered with muslin cloth and continuously supplemented with 10% honey solution to adults as a food. To get homogenous population and one generation passed larvae were used for the experiment. Panwar and Chibber (2006).

Selection and collection of plant species for the preparation of extracts

The criteria of selection of plant species, having a bioactive compound was based on literature. Desired plants at the peak of its vegetative growth stage were collected from various locations.

Table 1: Details of the leaf extracts of different plant species used in the experiment

S. No	Common Name	Scientific name	Family	Plant part used
1.	Neem	<i>Azadirachta indica</i>	Meliaceae	Leaves
2.	Wild sage	<i>Lantana camara</i>	Verbenaceae	Leaves and stem
3.	Kaner	<i>Nerium indicum</i>	Apocynaceae	Leaves
4.	Bakain	<i>Melia azedarach</i>	Meliaceae	Leaves
5.	Bhang	<i>Cannabis sativa</i>	Cannabinaceae	Leaves
6.	Datura	<i>Datura stramonium</i>	Solanaceae	Leaves
7.	Gajar Ghass	<i>Parthenium hysterophorus</i>	Asteraceae	Leaves and flowers
8.	Crofton weed	<i>Eupatorium adenophorum</i>	Asteraceae	Leaves
9	Sharifa	<i>Annona</i> spp.	Annoneace	Leaves

Preparation of plant extracts

The test plants parts were collected from different location, whereas, Cow urine was collected from desi breed cow. The plants collected from various families were brought to the laboratory, washed with dechlorinated water, shade dried under room temperature for 7-9 days and then the plant materials were powdered individually using an electric blender. 10gm of each powdered plant material weight separately by using a top separately balance and dissolve in 90 ml of cow urine to get 1:9 w/v Rani *et al.* (2009)^[26] were sieved using a kitchen strainer. The prepared solution were kept for fermentation for 15 days then the extracts filtered by using muslin cloth after that it was kept in refrigerator at 40 C and working solutions of the desired concentrations were prepared afresh prior to application. To prepare 2 and 5 per cent concentrations of plant extracts in cow urine separately, For 2 per cent dissolve 2 ml of stock solution (Plant extract +Cow urine 1:9w/v) in 98 ml of water. For 5 per cent dissolve 5 ml of stock solution (Plant extract +Cow urine 1:9w/v) in 95 ml of water.

Screening of plant extracts for their antifeedant activity against cabbage butterfly, *Pieris brassicae* L.

The antifeedant activity of various plant extracts were

evaluated against cabbage butterfly under laboratory conditions (range of temperature 27-30°C and RH 75-80%). 'No-choice' feeding bioassay following Singh *et al.* (1995)^[23] technique was used for the determination of antifeedant activity.

Cabbage leaves were utilized to treat and feed the larvae during the experimentation. Required concentrations were prepared from the stock solution.

The fresh and soft leaves were plucked, thoroughly washed and dried with the help of filter paper and the leaf discs (area= 5×5 cm²) were cut from them. These leaf discs were later dipped in the extracts for approx. 2 minutes and air dried for a while.

These leaf discs were kept in the centre of pre sterilized corning glass Petri-dishes (dia. 9cm) containing an inner lining of moist filter paper.

All the treatments were replicated three times along with one control. Pre-starved (3 hours) larvae of uniform size were released in each Petri-dish and were allowed to feed until more than 75 per cent leaf discs were eaten away in control. The data on the mean leaf area consumed was plotted and recorded on graph paper in different concentrations.

$$\text{Per cent feeding given by Pande and Shrivastav (2003)} = \frac{\text{Initial area given for feeding} - \text{leaf area left after feeding}}{\text{Initial area given for feeding}} \times 100$$

$$\text{Per cent feeding inhibition given by Pande and Shrivastav (2003)} = \frac{C-T}{C+T} \times 100$$

Where

C = Consumption of control leaves,

T= Consumption of treated leaves

$$\text{Antifeedant activity given by Singh and Pant (1980)} = \frac{\text{Area eaten in untreated leaf} - \text{Area eaten in treated leaves}}{\text{Area eaten in untreated leaf}} \times 100$$

$$\text{The preference index (C- value) given by Kogan and Goeden (1970)} C = \frac{2A}{M+A} \times 100$$

Where

A= eaten area of the treated leaf,

M= eaten area of the control leaf

The antifeedant activity of each plant extracts was worked out on the basis of preference indices (C-values) according to the following scale as given by Sharma and Bisht (2008)

C-value of 1 = feedant on test plant extracts equal to standard

C-value >1 = preference to test plant extracts, and

C-value <1 = lesser acceptance of the test plant extract

On the basis of C-values the experimental categories as under:

C – Values	Antifeedant categories
0.1-0.25	Extremely antifeedant
0.26-0.50	Strongly antifeedant
0.51-0.75	Moderately antifeedant
0.76-0.99	Slightly antifeedant
>1	Preferred plant extract

Statistical analysis

The data obtained from the different treatments were computed to determine the mean values. The mean values after suitable transformation were subjected to statistical analysis to test significance as per (Gomez and Gomez. 1984)^[8] for interpretation of the results.

Observations were recorded as given below

1. Mean leaf area consumed (cm²)
2. Antifeedant activity (%)
3. Feeding Inhibition (%)
4. Feeding Percentage
5. C- Value (Preference index)

Results and Discussion**Antifeedant of plant extracts against cabbage butterfly**

An experiment was conducted to find out antifeedant activity of leaf extracts prepared in cow urine at 2% and 5%

concentrations of various plant species viz., *Azadiracta indica*, *Eupatorium triplinerve*, *Cannabis sativa*, *Nerium indicum*, *Parthenium hysterophorus*, *Melia azedarach*, *Lantana camara*, *Annona squamosa*, *Datura stramonium*, against 3rd instar larvae of *P.brassicae*. These were tested by using 'no-choice feeding' bioassay method. The observation on leaf area consumed was recorded on graph sheets and was used for calculations of other parameters viz., Mean leaf area consumed (cm²), Per cent feeding, Per cent Feeding inhibition, Antifeedant activity (%) and Preference index (C-value). The results of antifeedant activity of various plant extracts have been presented below mentioned sub headings:

Mean leaf area consumed (cm²) at 2 per cent concentration

Data presented in Table 4.1 and Fig. 4.1 revealed that from the provided leaf area of 25 cm² of treated leaves for upto 75% consumption of larval feeding, the minimum consumption of leaf discs was found in T3 (Cow urine+*Azadiracta indica*) 6.72 cm² @ 2% and next effective treatments were T7 (Cow urine+ *Melia azedarach*) 9.27 cm², T4 (Cow-urine + *Parthenium hysterophorus*) 10.68 cm², T8 (Cow urine+ *Annona* spp.) 11.18 cm² while maximum leaf consumption in T1 (Control) 14.35 cm². All treatments were statistically significant over T1 (Control) except T5 (Cow urine + *Eupatorium adenophorum*) 13.62 cm².

Mean leaf area consumed (cm²) at 5 per cent concentration
Data presented in Table 4.1 clearly showed that the consumption of leaf discs was minimum in T3 (Cow urine+*Azadiracta indica*) 4.04 cm² at 5% and next effective treatments were T7 (Cow urine+ *Melia azedarach*) 7.84 cm², T8 (Cow urine + *Annona* spp.) 10.61 cm², T4 (Cow urine+ *Parthenium hysterophorus*) 10.82 cm² while maximum leaf consumption in T1 (Control) 14.35 cm². All treatments were statistically significant over T1 (Control) except T5 (Cow urine + *Eupatorium adenophorum*) 12.30 cm².

Table 2: Effect of Plants extracts on feeding behaviour of *P. brassicae* larvae.

T. No.	Treatments	Leaf area consumed (cm ²)	
		2% ± SE(m)	5% ± SE(m)
T1	Control	14.35 ±0.58	14.35 ±0.58
T2	Cow urine + <i>Lantana camara</i>	12.67*±0.62	11.01*±0.38
T3	Cow urine + <i>Azadiracta indica</i>	06.72*±0.58	04.04*±0.60
T4	Cow urine + <i>Parthenium hysterophorus</i>	10.68*±0.36	10.82*±0.57
T5	Cow urine + <i>Eupatorium adenophorum</i>	13.62 ±0.36	12.30 ±1.44
T6	Cow urine + <i>Datura stramonium</i>	11.73*±0.57	12.20*±0.64
T7	Cow urine+ <i>Melia azedarach</i>	09.27*±0.51	07.84*±0.60
T8	Cow urine+ <i>Annona</i> spp.	11.18*±0.58	10.61*±0.65
T9	Cow urine+ <i>Cannabis sativa</i>	11.46*±0.67	11.42*±0.67
T10	Cow urine+ <i>Nerium indicum</i>	11.42*±0.57	12.12*±0.56
	SE(d)	0.78	1.02
	C.D. (0.05)	1.63	2.15

*Significant at 5% level of significance compared with control

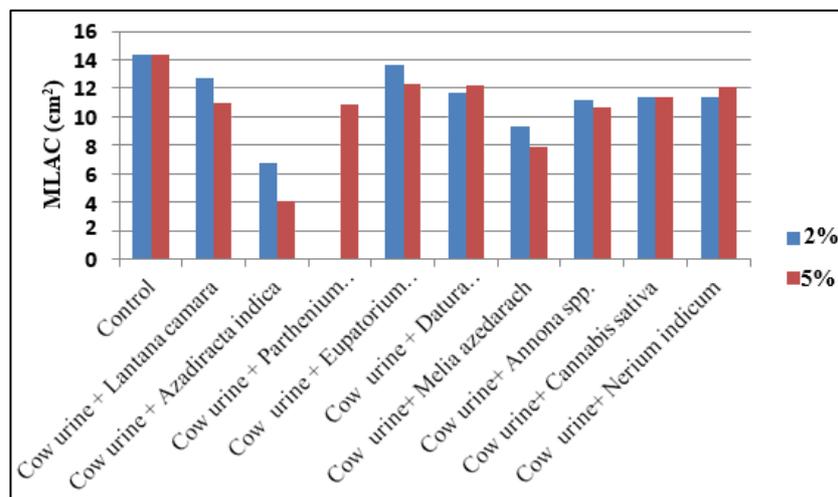


Fig 1: MLAC (Cm2) at 2% and 5% concentrations

Feeding per cent at 2 per cent concentration

The data contained in Table 4.2 and Fig.4.2 clearly demonstrate that minimum per cent feeding was recorded in T3 (Cow urine+*Azadiracta indica*) 26.88% and next effective treatments were T7 (Cow urine+ *Melia azedarach*) 37.08%, T4 (Cow urine + *Parthenium hysterophorus*) 42.72%, T8 (Cow urine+ *Annona* spp.) 44.72%, T10 (Cow -urine+ *N. indicum*) 45.67% at 2 per cent and maximum feeding percent found in T1 (Control) 57.39%.

Feeding per cent at 5 per cent concentration

The lowest per cent feeding was recorded in T3 (Cow urine + *Azadiracta indica*) 16.16% and next effective treatments were T7 (Cow urine+*Melia azedarach*) 31.36%, T8 (Cow urine+ *Annona* spp.) 42.43%, T4 (Cow urine + *Parthenium hysterophorus*) 43.28%, T9 (Cow urine+ *C. sativa*) 45.67% while maximum feeding per cent found in T1 (Control) 57.39%.

Antifeedant activity (%) at 2 per cent concentration

In Table 4.2 and Fig.4.3 the maximum antifeedant activity was recorded in T3 (Cow urine+*Azadiracta indica*) 73.12% followed by T7 (Cow urine+ *Melia azedarach*) 62.92%, T4 (Cow urine + *Parthenium hysterophorus*) 57.28%, T8 (Cow urine+ *Annona* spp.) 55.28%, T10 (Cow urine+ *N. indicum*) 54.32% while minimum antifeedant activity was found in case of T1 (Control) 42.60%.

Antifeedant activity (%) at 5 per cent concentration

The maximum antifeedant activity were recorded in T3 (Cow urine+*Azadiracta indica*) 83.84% followed by T7 (Cow urine+ *Melia azedarach*) 68.64%, T8 (Cow urine+ *Annona* spp) 57.56%, T4 (Cow urine + *Parthenium hysterophorus*) 56.72%, T2 (Cow urine +*Lantana camara*) 55.96%, while minimum antifeedant activity was found in case of T1 (Control) 42.60%.

Feeding inhibitions (%) at 2 per cent concentration

In Table 4.2 and Fig.4.4 the maximum feeding inhibitions was observed in T3 (Cow urine+*Azadiracta indica*) 36.21% followed by T7 (Cow urine+ *Melia azedarach*) 21.51%, T4 (Cow urine + *Parthenium hysterophorus*) 14.66%, T8 (Cow urine+ *Annona* spp.) 12.42%, T10 (Cow urine+ *N. indicum*) 11.38% and T5 (Cow urine+ *Eupatorium adenophorum*) 2.61% showed minimum feeding inhibition.

Feeding inhibitions (%) at 5 per cent concentration

The maximum feeding inhibitions recorded in T3 (Cow urine+*Azadiracta indica*) 56.06% followed by T7 (Cow urine+ *Melia azedarach*) 29.34%, T8 (Cow urine+ *Annona* spp.) 14.98%, T4 (Cow urine + *Parthenium hysterophorus*) 14.02%, T2 (Cow urine +*Lantana camara*) 13.17% and T5 (Cow urine+ *Eupatorium adenophorum*) 7.69% showed minimum feeding inhibition.

Preference index at 2 per cent concentration

Overall mean preference index in Table 4.2 indicated that none of the plant extract formulations were found to belong extremely antifeedant category. But the preference indices on treated cabbage leaves discs were significantly less. The minimum preference index was found in T3 (Cow urine+*Azadiracta indica*) 0.58 and next effective treatments with less preference index found in T7 (Cow urine+ *Melia azedarach*) 0.75, T4 (Cow urine + *Parthenium hysterophorus*) 0.85, T8 (Cow urine+ *Annona* spp.) 0.88.

Preference index at 5 per cent concentration

The minimum preference index was found in T3 (Cow urine+*Azadiracta indica*) 0.44 and it was found strongly antifeedant and next effective treatments with less preference index found in T7 (Cow urine+ *Melia azedarach*) 0.71, T8 (Cow urine+ *Annona* spp.) 0.85, T4 (Cow urine + *Parthenium hysterophorus*) 0.86, T2 (Cow urine+ *Lantana camara*) 0.87.

Discussion

Anti-feedant activity of plant extracts against *P. brassicae* L.

Leaf area consumed (cm²)

Plants extracts prepared in cow urine have a significant effect on feeding behavior of the *P. brassicae*. In the present investigations Cow urine+*Azadiracta indica* and Cow urine+ *Melia azedarach* was found effective in reducing feeding of the *P. brassicae*. when treated in cabbage leaves discs. Among the plants extracts evaluated, minimum leaf consumption (6.72 cm²) and (4.04 cm²) was recorded with Cow urine + *Azadiracta indica* at 2% and 5% concentrations, respectively, as compared to the control (14.35 cm²). Similar results were observed by Sharma *et al.* (2009) who studied the antifeedant and toxic effect of an aqueous extract of *Azadiracta indica*, *Melia azedarach*, *Lantana camara* against *P. brassicae* L. The

maximum protection i.e. 88.3 and 82.5% to the cabbage foliage was provided @ 5% of *M. azedarach* and *A. indica*, respectively.

Feeding per cent

The lowest per cent feeding (26.88%) and (16.16%) was recorded with the use of Cow urine+*Azadiracta indica* at 2% and 5%, respectively, followed by Cow urine+ *Melia azedarach* (37.08%) and (31.36%) while the highest per cent feeding was recorded in control (57.39%).

Antifeedant activity

Antifeedant property of each of the plant extract was accessed by comparing the average of the leaf area consumed in the treated leaves with that of control. The observations indicated that maximum antifeedant activity i.e. 73.12% and 83.84% was found when the leaves were treated with Cow urine+*Azadiracta indica* at 2% and 5%, respectively, followed by Cow urine+ *Melia azedarach* (62.92%) and (68.64%) against 3rd instar larvae of *P. brassicae* while the lowest antifeedant activity was found in control (42.60%). The above findings are in strong conformation with Vattikonda and Sangam (2016) who reported the highest antifeedant activity of azadirachtin (a secondary metabolite of *Azadiracta indica*) against fourth instar larvae of *P. demoleus*. Nathan (2006) reported that majority of limnoid compounds present in the leaves of *Azadiracta indica* showed more antifeedant activity. Bakavathiappan *et al.* (2012) observed the antifeedant activity was directly proportional to the concentration of the extract.

Meshram *et al.* (1996) also advocated the antifeedant property of *Azadiracta indica* against the third instar larvae of *P. demoleus*.

Feeding inhibitions

The maximum feeding inhibitions (36.21%) and (56.06%) was show by Cow urine+*Azadiracta indica* at 2% and 5%, respectively, followed by Cow urine+ *Melia azedarach* (21.51%) and (29.34%), Cow urine+*Parthinium hystrophorus* (14.66%). The present finding was in close association with the research conducted by Streets (1977) who reported the feeding inhibition up to 90 per cent against the third instar larvae of *Leptinotarsa decemlineata* with crude leaf extract of *A. indica*. Summarwar and Pandey (2013) observed that leaf extract of *A. indica* showed complete inhibition of feeding at 5% extract (antifeedant index 100 per cent) compared to untreated leaf disc (antifeedant index 19.62 percent).

Preference index (C-Value)

The Preference index of cow urine+*Azadiracta indica* fall under strong antifeedant category (range 0.44) at 5% concentration whereas at 2% concentration showed moderately antifeedant activity (range 0.58). Similar studies were conducted by Geetanjali and Tiwari (2014) who studied the growth regulatory effects of Cow urine, Neem leaf extracts prepared in water and Cow urine (5% and 10%) where they calculated that the preference index showed the strong antifeedant activity of NLCUE @10% (0.49), whereas, NLCUE @5% (0.61) showed moderately antifeedant activity.

Table 3: Effect of Plants extracts on feeding behaviour of *P. brassicae* larva.

T. No	Treatment	Feeding percent		Antifeedant activity		Feeding inhibition		Preference index		Antifeedant category
		2%	5%	2%	5%	2%	5%	2%	5%	
T1	Control	57.39	57.39	42.60	42.60	0.00	0.00	1.00	1.00	
T2	Cow urine + <i>L. camara</i>	50.68	44.05	49.32	55.96	06.22	13.17	00.94	00.87	Slightly antifeedant
T3	Cow urine+ <i>A. indica</i>	26.88	16.16	73.12	83.84	36.21	56.06	00.58	00.44	Moderately Antifeedant at 2%, Strongly antifeedant at 5%
T4	Cow urine+ <i>P. hystrophorus</i>	42.72	43.28	57.28	56.72	14.66	14.02	00.85	00.86	Slightly antifeedant
T5	Cow urine+ <i>E. adenophorum</i>	54.48	48.81	45.52	50.80	2.61	7.69	00.97	00.92	Slightly antifeedant
T6	Cow urine + <i>D. stramonium</i>	46.93	49.20	53.08	51.20	10.03	8.10	00.90	00.92	Slightly antifeedant
T7	Cow urine+ <i>M. azedarach</i>	37.08	31.36	62.92	68.64	21.51	29.34	00.75	00.71	Moderately antifeedant
T8	Cow urine+ <i>Annona</i> spp.	44.72	42.43	55.28	57.56	12.42	14.98	00.88	00.85	Moderately antifeedant
T9	Cow urine+ <i>C. sativa</i>	45.85	45.67	54.16	54.32	11.18	11.37	00.89	00.89	Slightly antifeedant
T10	Cow urine+ <i>N. indicum</i>	45.67	48.47	54.32	51.52	11.38	8.42	00.89	00.92	Slightly antifeedant

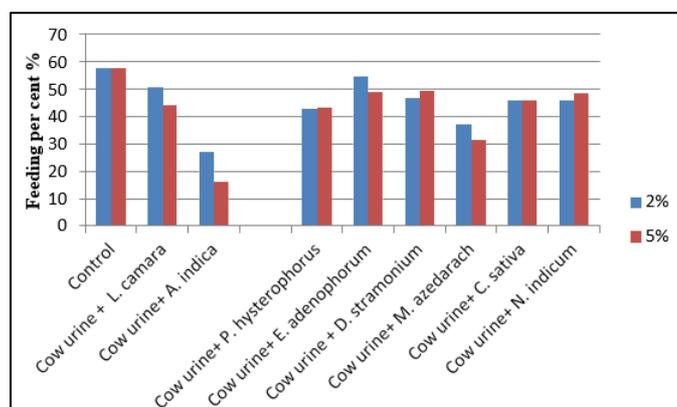


Fig 2: Feeding per cent at 2% and 5% concentrations

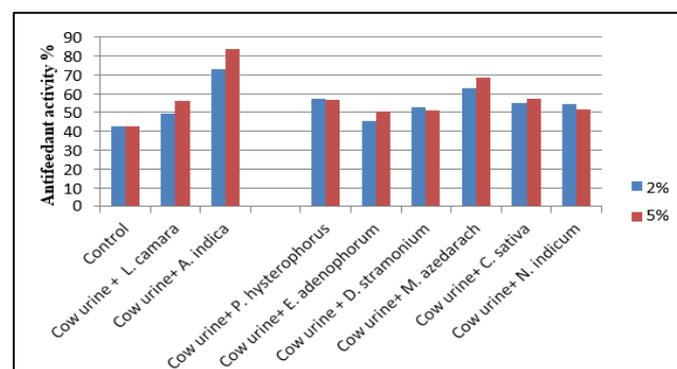


Fig 3: Antifeedant activity (%) at 2% and 5% concentrations

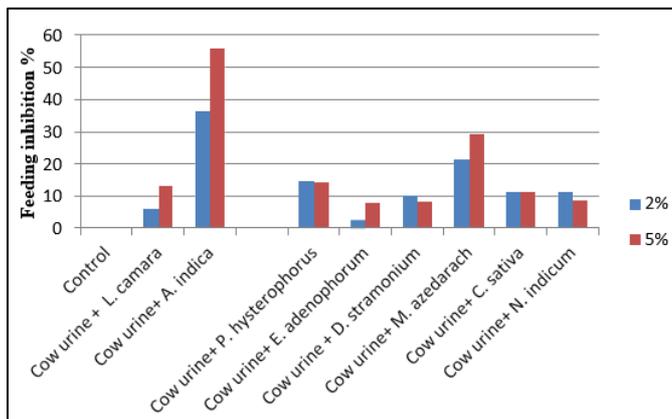


Fig 4: feeding inhibition (%) at 2% and 5% concentrations



Fig 5: Larvae of *Pieris brassicae* attack cabbage crop



Fig 6: Plastic jars containing botanicals

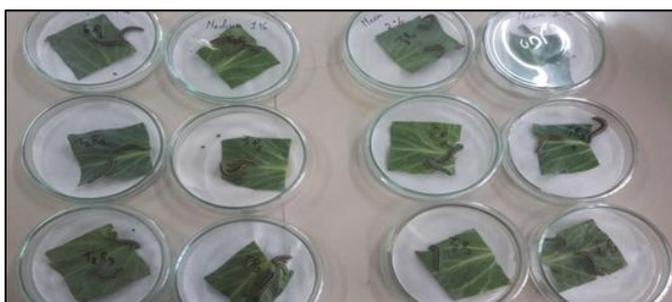


Fig 7: Antifeedant activity of *P. brassicae* on cabbage leaf

Conclusion

From the analysis of the present findings it was concluded that plants extracts prepared in cow urine showed promising results in terms of lowering the feeding per cent of *P. brassicae*. The studies clearly demonstrated that it is possible to reduce the total load of chemical insecticides on the environment. Hence, isolation of the active ingredients responsible for such antifeedant activity could possibly facilitate in new formulations for effective activity at lower concentrations.

Among the different plants extracts, Cow urine+*Azadiracta indica* and Cow urine+ *Melia azedarach* were found to be most effective in feeding behavior, growth and development against cabbage butterfly.

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