Biological activity of some plant extracts against *Pieris brassicae* (Linn.)

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ABSTRACT

In the present study, aqueous extract of eight plants, namely *Azadirachta indica* A. Juss, *Melia azedarach* Linn., *Lantana camara* L. Moldenke., *Cannabis sativa* Linn., *Nerium indicum* Mill., *Eucalyptus* sp., *Ricinus communis* Linn. and *Solanum nigrum* Linn. were tested for antifeedant and toxic effects against *Pieris brassicae* (Linn.). The ethanol extract of four potential plants were further tested for their biological activity against the test insects. Aqueous extract of *A. indica* and *M. azedarach* repelled maximum number of larvae protected 94.0 per cent and 89.2 per cent cabbage foliage, respectively. Aqueous extract of *M. azedarach, N. indicum* and *A. indica* showed higher mortality of larvae (19.6, 19.6 and 18.5%, respectively) while *R. communis* was the least toxic resulting in 8.9 per cent larval mortality. In case of ethanol extract, seed extract of *M. azedarach* protected 58.3 per cent cabbage foliage while *Eucalyptus* sp. protected minimum cabbage foliage. The maximum protection to the cabbage foliage was provided at 5 per cent of *M. azedarach* (88.3%) and *A. indica* (82.5%). Ethanol extract of *A. indica* exhibited statistically higher larval mortality of 50.0 per cent and *N. indicum* the lowest mortality of 3.2 per cent. In general, antifeedant and larval mortality was dose dependent.

Key words: *Pieris brassicae*, plant extracts, antifeedant effect, toxic effects

INTRODUCTION

Eversince man started cultivating the crop plants, many control measures were adopted to prevent them from diseases and insect pests. Use of synthetic chemicals is one among them. In recent years, the increasing information on hazardous effects of synthetic insecticides on plant and animal health has alarmed scientists to seek some alternative ways, which are ecofriendly. About 450 pest species of insects and mites have now developed resistance to one or more major synthetic pesticides (Georghiou, 1986). Botanical insecticides are one of the best alternatives for these hazardous chemicals. They are plant-derived insecticides, either naturally occurring plant materials or the products simply derived from such plants (Gupta *et al.*, 2005).

A number of plant species like *Azadirachta indica* A. Juss, *Melia azedarach* Linn., *Lantana camara* L. Moldenke., *Cannabis sativa* Linn., *Nerium indicum* Mill., *Eucalyptus* sp., *Ricinus communis* Linn., *Solanum nigrum* Linn. etc are known to possess insecticidal properties, although only a few of these have been exploited commercially. The compounds from these plants have a number of useful activities like toxicity, repellence, feeding and oviposition deterrence and insect growth regulator activity etc. (Mordue, 2004).

The cabbage white butterfly, *Pieris brassicae* (Linnaeus) (Lepidoptera: Pieridae), is a serious pest of cauliflower and cabbage (Bhalla and Pawar, 1977). In India, *P. brassicae* is distributed along the Himalayan region throughout the plain except the southern plain (Raqib, 2004). It was recorded as a serious pest of cabbage, cauliflower, broccoli, brussels sprout in different parts of the world. It also attacks turnip, radish, sarson and toria. A single larva consumes 74-80 sq.cm. leaf area. The larvae eat all the parts of the plant like leaves, branches, pods and the seeds of cabbage and cauliflower (Siraj, 1999) and causes serious damage economically. Moreover, this pest has developed resistance against few insecticides.

MATERIAL AND METHODS

Rearing of test insect

The stock culture of *P. brassicae* was maintained under laboratory conditions. For this purpose, egg clusters were collected from the cole crops in the field and kept in petri plates (10cm diameter) over the filter papers. Newly hatched larvae were transferred to cabbage/cauliflower leaves with their petioles dipped in water in glass vials (14cm x 4cm) inside the wooden rearing cages (36cm x 34cm x 26cm) with glass panes on three sides and the top and wire mesh on the front door. Fresh leaves were provided daily to the caterpillars till pupation.

One day old pupae were collected from the walls of the rearing cages and were sexed as suggested by Chandra and Lal (1975). The pupae of both the sexes were kept

Table 1. Description of selected plant species

Local name/ Common names	Botanical name	Family	Distribution	Period of flowering/ fruiting	Parts used
Neem, Nimba, Margosa tree	*Azadirachta indica* A.Juss.	Meliaceae	Throughout India in tropical and subtropical regions	March-April/ July-August	Whole plant
Dharek, Darek, Persian lilac	*Melia azedarach* Linn.	Meliaceae	Sub-Himalayan tracts	March-April	Leaves, bark, drupes
Wild sage, Caturangi	*Lantana camara* L. Moldenke	Verbenaceae	Throughout the country	Through out the year	Whole plant
Kaner, Karveer, Kuruvira, Oleander	*Nerium indicum* Mill.	Apocynaceae	Found in Himalayan regions, in Gangetic plains, Punjab, H.P., Haryana, Kashmir	April–June/ Fruiting in winter season	All plant part
Bhang, Hemp, Charas, Ganja	*Cannabis sativa* Linn.	Cannabinaceae	Grown in temperate and tropical regions of India up to elevation of 2500m	June – October	Flower, fruits and leaves
Castor, Arandi, Eranda, Arand	*Ricinus communis* Linn.	Euphorbiaceae	Cultivated throughout India in moist tropical and warm temperate regions up to an elevation of 2000m	May- August	Seed and leaves
Makai, Bandakh, Kakamachi.	*Solanum nigrum* Linn.	Solanaceae	Found throughout India in dry parts, up to an elevation of 2100m	June – October	Entire plant
Saffeda, Blue gum.	*Eucalyptus* spp.	Myrtaceae	Found throughout India as a landscape tree	Through out the year	Leaves and flowers

Source: Chauhan (1999)

separately in glass jars (10cm x 14.5cm) over a piece of filter paper. In each jar, resting place was provided to the newly hatched adults for normal expansion of wings. The adults were provided with sugar solution (10%) soaked in cotton swabs and some shoots of cabbage/ cauliflower.

Collection of plant materials

Samples of test plants viz. *Azadirachta indica* A. Juss, *Melia azedarach* Linn., *Lantana camara* L. Moldenke., *Cannabis sativa* Linn., *Nerium indicum* Mill., *Eucalyptus* sp., *Ricinus communis* Linn. and *Solanum nigrum* Linn., were collected from different places of Punjab namely Ropar, Rupnagar, Ludhiana and Sisuan, as detailed in Table 1. The samples containing leaves, stems, seeds or flowers as the case may be, of the selected plant materials were air-dried for 6-7 days and then dried in an oven at 30^0C for 24 hours. The plant material was extracted by two methods such as Simple extraction method and Soxhlet extraction. Following methods were used for simple extraction.

Aqueous and Ethanol extraction

The aqueous extract of plant material was prepared under laboratory conditions on per cent basis as per the method of Gahukar (1996) and Sharma *et al.* (1997). A stock solution of 10 per cent was made by mixing 10g plant material of each plant in 90ml distilled water. It was kept in a beaker for 24 hours (stirred in between thrice). The solution was passed through the muslin cloth and filtered again through Whatman filter paper No-1. This filterate was considered as stock solution. Further dilutions were made from this stock solution with distilled water by using single dilution method. For ethanol extraction, 10 gram powder of the plant material was mixed in a beaker in 90ml ethanol and kept for 24 hours (stirred in between thrice). The mixture was filtered with the help of Whatman filter paper No-1. Further dilutions required were made from this stock solution with distilled water by using single dilution method.

Soxhlet extraction

The ordinary method of extraction was not efficient to yield good amount of active principle of the plant material. To extract more active principle from all the plant materials, Soxhlet extraction was used. Known amount (500g) of plant material of each species was filled into the Soxhlet apparatus. A cotton plug was used at the place of thimble to stop the entry of the crude material into the siphoning tube. The required solvent (ethanol) was filled up five times more than total amount of the sample material into the flask of the apparatus. The apparatus was then

connected with the water supply to the condenser. The temperature of the heating mantle was maintained at 60-65^{0}C (boiling point of ethanol). The process was carried out for 5 to 6 hours for each sample. The extract was transferred to Petri plates and solvent was allowed to evaporate. The evaporated material was weighed and stored in the refrigerator for further use. The desired stock solution of each extract was made by adding more solvent until the plant material was dissolved completely. Further dilutions were made by adding distilled water with emulsifier (0.5-1.0 % of Triton X- 100).

Antifeedant effect

Antifeedant effect of all the plant extracts was studied against the second instar larvae of test insects. Fresh castor leaves were washed with distilled water and dried in shade. The testing was done by leaf-dip method at the desired concentrations, ranging from 1-10 per cent, and kept in Petri plates, after drying in shade. The leaves were dipped in the extract for 5-10 seconds and then shade dried. The leaves treated with distilled water were considered as control. The second instar larvae of the test insect were pre-starved for eight hours, released on the leaves in Petri plates and were kept at room temperature for 24 hours. Observations were recorded to study the antifeedant effect of the plant extracts and it was calculated by using the formula:

Percent area consumed = 100 -

$$\left[\frac{\text{Area of leaf fed in treatment}}{\text{Initial leaf area given for feeding}}\right]\times 100$$

The data were corrected with respect to control using the Abbott's formula (Abbott, 1925) as under:

Corrected per cent antifeedant effect $\frac{T-C}{100-C}\times 100$

Where, T = per cent area consumed in treatment
C = per cent area consumed in control

Toxic bioassay

Fresh cabbage leaves were washed with distilled water and dried in shade. The leaves were treated by leaf-dip method (for 5-10 seconds) in the desired concentrations (1-10%), and kept in Petri plates after drying in shade. Leaves treated with distilled water were consideres as control. The second instar larvae were pre-starved for eight hours, released on the treated leaves in Petri plates, at room temperature. Data on mortality of larvae were recorded after 24 hours and corrected for control mortality through Abbott's formula (Abbott, 1925).

RESULTS

The data in Table 2 reveal that up to 54.2 per cent protection to cabbage foliage was recorded in case of *A. indica* treated leaves. Also, aqueous extract of *A. indica* (81.8%) and *M. azedarach* (81.7%) at 10 per cent gave statistically equal protection to the foliage. In general, antifeedant effect was found to increase in a dose dependent manner. Irrespective of plant extract, high concentration resulted in maximum mean protection to foliage (68.1%), while the lowest concentration resulted in least protection (9.5%).

Figure 1 reveals that 4.6 to 88.3 per cent protection to cabbage foliage was obtained as a result of feeding by the second instar caterpillars of *P. brassicae* on four test plant extracts. The protection to cabbage foliage at all the concentrations of *M. azedarach* was higher as compared to other plant extracts. The maximum protection was provided at 5 per cent of *M. azedarach* (88.3%) and *A. indica* (82.5%). The minimum (4.6%) protection to the cabbage foliage was observed at 1 per cent of *A. indica*. In general, the antifeedant effect of different concentrations, irrespective of extracts, decreased with decrease in concentration from 5 to 1 per cent.

Toxic effects

The data presented in Table 3 reveal that upto 28.6 per cent mortality was observed against larvae of *P. brassicae* as a result of feeding on cabbage leaves, dip treated in aqueous extracts of eight plant species. Aqueous extract of *M. azedarach, N. indicum* and *A. Indica* showed higher mortality of larvae (19.6, 19.6 and 18.5%, respectively) while *R. communis* was the least toxic resulting in 8.9 per cent larval mortality. Larval mortality at 10 per cent was low, compared to mortality response at 2.5 and 5 per cent. The mortality of *P. brassicae* caterpillars ranged from 3.2 to 50 per cent (Fig. 2) when second instar caterpillars of *P. brassicae* were fed for 24 hours on cabbage leaves

Table 2. Antifeedant effects of aqueous extracts of some plant extracts on *P. brassicae* second instar larvae

Plants	Concentrations (in %)				Mean
	10	5	2.5	1.0	
A. indica	81.8	69.9	50.0	15.1	54.2
M. azedarach	81.7	66.7	41.8	5.7	48.7
L. camara	67.7	52.8	44.4	1.9	41.7
N. indicum	69.2	68.6	29.9	19.6	46.8
Eucalyptus sp.	75.9	61.7	43.2	22.0	50.7
S. nigrum	61.3	38.2	16.9	3.2	29.9
R. communis	58.5	38.1	16.9	3.2	29.2
C. sativa	48.9	40.6	8.7	5.0	25.8
Mean	68.1	54.4	31.5	9.5	-

CD (p = 0.05) Extract: 2.9 Concentration : 4.1
Extract X Concentration : 8.0

Table 3. Mortality response of some plant extracts on *P. brassicae*

Plants	Concentrations (in %)				Mean
	10	5	2.5	1	
A. indica	14.3	26.2	26.2	7.1	18.5
M. azedarach	11.9	26.2	28.6	11.9	19.6
N. indicum	19.0	26.2	21.4	11.9	19.6
S. nigrum	5.2	14.3	11.9	10.9	10.6
R. communis	5.2	9.5	18.1	2.9	8.9
C. sativa	9.5	23.8	23.8	6.2	15.8
L. camara	14.3	11.9	16.7	2.9	11.4
Eucalyptus sp.	16.7	16.7	16.7	13.3	15.8
Mean	12.0	19.3	20.4	8.4	-

CD (p=0.05Extract : 3.5 Concentration : 5.1
Extract X Concentration :NS

treated with ethanol extract. *A. indica* exhibited statistically higher mortality of 50 per cent at 2.5 per cent extract and *N. indicum* the lowest mortality of 3.2 per cent. Ethanol extract of *A. indica* and *M. azedarach* exhibited higher mortality of 35.5 and 30.9 per cent,

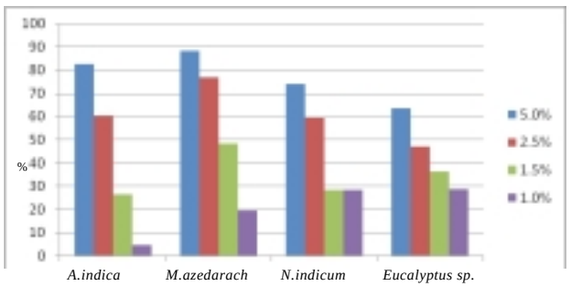

Fig. 1. Antifeedant effect of ethanol extract some plants against *P. brassicae*

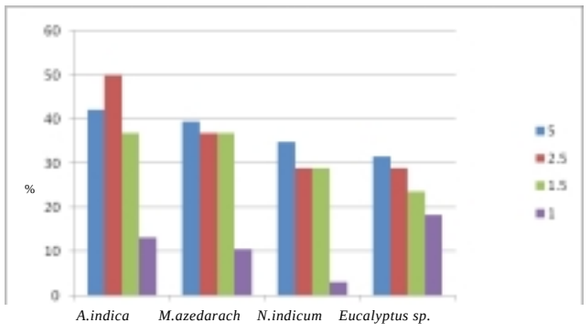

Fig. 2. Mortality response of ethanol extract of some plants against *P. brassicae*

respectively, than that caused by *N. indicum* and *Eucalyptus* sp. (23.8 and 25.7%, respectively) extract. Mortality was dose dependent one.

DISCUSSION

Antifeedant effect

Aqueous plant extract against *Pieris brassicae* (Linn.), at different concentrations caused 3.2-81.8 per cent protection to the foliage over control (Table 1). Extract of *A. indica* resulted in maximum protection to foliage over control at all the concentrations used followed by *Eucalyptus* sp. against *P. brassicae*. Singh *et al*. (1987) reported that 2 per cent leaf water extract of *A. indica* had a significant antifeedant effect, thereby reducing mean cabbage leaf area consumption of *P. brassicae*. Similarly, Streets (1976/1977) reported feeding inhibition up to 90 per cent against the third instar larvae of *Leptinotarsa decemlineata* (Say) with crude leaf extract of *A. indica*. Charleston *et al*. (2005) also reported feeding deterrent activity of aqueous extract of *M. azedarach* and *A. indica* against the larvae of *Plutella xylostella* (Linn.).The antifeedant activity of *Lantana* extract has been shown by Mehta *et al*. (1996), who reported complete feeding inhibition of cabbage leaves treated with *L. camara* at 1 per cent against first instar larvae of *P. brassicae*. Similarly, Deka *et al.* (2001) reported that aqueous extract of *L. camara* reduced the infestation of tea leaves from 27.63 to 38.90 per cent against the tea mosquito bug, *Helopeltis theivora* Waterhouse. Zhou *et al*. (2004) also found that aqueous extract of *N. indicum* produced strong antifeedant effect against *Aphis gossypii* Glover.

In the present study, it was found that extract of *M. azedarach*, *A. indica*, *N. indicum* and *Eucalyptus* sp. protect the cabbage leaves over control against second instar larvae of *P. brassicae* (Table 6). The antifeedant activity of methanol extract of *M. azedarach* was reported by Zhu (1989) against *P. brassicae*, *P. rapae* (Linn.) and *P. xylostella.* Similarly, Muralikrishana *et al.* (1990) reported 100 per cent protection to cabbage leaves with petroleum ether extract (1%) of *L. camara*, *A. indica*, *E. globulus* and *R. communis* against larvae of *Henosepilachna vigintioctopunctata* (Fab.). Irrespective of plant extract (alcohol or aqueous) protection to the foliage obtained at different concentration was dose dependent i.e. higher protection to the foliage was obtained at higher concentrations and vice-versa.

Toxic Effects

Results reveal that aqueous extract of *M. azedarach*, *N. indicum*, *A. indica* and *Eucalyptus* sp. resulted in statistically equal mean larval mortality over control against larvae of *P. brassicae*. Hernandez and Vendramin

(1997) reported larval mortality above 80 per cent with the aqueous extract of *Melia azedarach* and *Azadirachta indica* against *S. frugiperda*. In the present study, statistically equal larval mortality of *P. brassicae* larvae was observed with *L. camara*, *S. nigrum* and *R. communis*. Earlier, Verma and Srivastava (1988) reported that alcoholic extract of *S. indicum* (4%) reduced the population of *Macrosiphum rosae* L. Similarly, Oudhia (2000) obtained mortality of 23-35 per cent against orange-banded blister beetle, *Zonabris postulate* Thunb. with the aqueous leaf extract of *L. camara*.

M. azedarach and *A. indica* caused more mortality than *Eucalyptus* sp. and *N. indicum*. Atwal and Pajni (1964) reported 33.3 and 40 per cent mortality against *P. brassicae* with alcohol extract (10 and 5%, respectively) of *M. azedarach*. Satpathi and Ghatak (1990) observed 90 per cent mortality of *H. vigintioctopunctata* larvae with *N. indicum* petroleum ether extract. Larval mortality of 22.8 per cent with methanol extract of *M. azedarach* against *P. xylostella* has been reported by Dilwari *at al*. (1994).

Based on the results obtained and discussed as above, it can be said that aqueous extracts of *A. indica* and *M. azedarach* were highly effective against *P. brassicae*. Based on these data, the effects of aqueous extracts of test plants could be arranged in the order: *A. indica = M. azedarach > N. indicum > Eucalyptus* sp. *= R. communis = C. sativa > S. nigrum > L. camara*. As observed in case of aqueous extract, ethanol extract of *A. indica* and *M. azedarach* were also effective against *P. brassicae*. Based on this data, the effect of ethanol extract can be arranged in the order: *A. indica = M. azedarach > N. indicum = Eucalyptus* sp.

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