



Insecticidal activity of ethanolic extracts from seed and leaf of bakain (*Melia azedarach*) and its effect on oviposition and egg hatching of *Pieris brassicae* in mid hills of Uttarakhand

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ABSTRACT

The effect of ethanolic extracts from the leaves and seeds of bakain (*Melia azedarach* L.) on insecticidal activity, oviposition deterrent and egg hatchability of *Pieris brassicae* were studied under laboratory conditions at V C S G College of Horticulture, Bharsar, Pauri Garhwal, Uttarakhand during 2010. Six concentrations, viz. 1, 2, 4, 6, 8 and 10% of ethanolic extracts from both leaves and seeds of bakain were prepared and compare with 0.5% nimbecidine and control (water). As the doses increased, mortality of 2nd instar larvae of *P. brassicae* was also increased at each concentration from 1 to 10% after 24 hr of treatment. Highest mean mortality (66.67 %) was recorded at 10% concentration of bakain seeds followed by 10% bakain leaves (56.67% mean mortality). LC₅₀ values from seeds and leaves extracts were 8.30% and 9.59%, respectively against 2nd instar larvae of *P. brassicae*. This LC₅₀ indicates the toxicity of *M. azedarach* to the insects at higher concentrations. Egg laying by female adults at treated surfaces (muslin) proportionately decreased as the concentration of extracts increased. Egg laying at 10% bakain seed treated surface (8.00 eggs), 8% (14.67 eggs) and 6% (19.67 eggs) and bakain leaves at 10% (11.33 eggs) and 8% (17.33 eggs) were significantly better than nimbecidine 0.05% (23.00 eggs) and control (65.33 eggs). All the substrates treated with different concentrations had significantly adverse effect on the egg hatching as compared with the control. Distinct adverse effects on hatching were reported when the eggs were directly treated in different concentrations of extracts. Maximum 100% egg hatching reduction was recorded at 10% concentrations from both leaves and seed extracts. Finally it was concluded that *M. azedarach* was toxic to *P. brassicae* larvae with high LC₅₀ values and it had oviposition deterrent effect and adverse effect on egg hatchability.

Key words: Egg hatching, Insecticidal activity, Leaves and seed extracts, *Melia azedarach*, Oviposition, *Pieris brassicae*

Botanical insecticides are one of the best alternatives for commercially available synthetic pesticides in organic farming as these synthetic chemicals have great hazardous effects on the nature. They are plant-derived insecticides, either naturally occurring plant materials or the products simply derived from such plants (Gupta *et al.* 2005). There is a great variety of families of plants that possess potent anti-insect compounds. Numerous plant species have been reported to possess pest control properties but only a few seem to be ideally suited to practical utilization. Among these, neem (*Azadirachta indica* A. Juss) and bakain (*Melia azedarach* L) are the most promising plants from the entomological perspective (Schmutterer 1990). 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). *Melia azedarach* (Family: Meliaceae) is a deciduous tree that is native to northeastern India. It has several common names such as, white cedar, Persian lilac, tulip cedar and Chinaberry. Extracts of fruits, seeds, leaves of *M. azedarach* have shown many characteristics of medicinal and pesticidal activities against several pathogenic and pest organisms respectively (Senthil Nathan and Saehoon 2005). In the mid hills of Uttarakhand, *M. azedarach* is common tree and farmers in this area use its leaves in their storage bins to protect their commodities from insect pests.

Off season vegetable cultivation is very common in mid hills hence; these vegetable crops are attacked by number of insect pests. Among these insect pests, cabbage butterfly (*Pieris brassicae* L.) (Lepidoptera: Pieridae) is a serious pest

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of cabbage, cauliflower, broccoli, brussels sprout. In India, this insect is distributed along the Himalayan region throughout the plain except the southern parts (Raqib 2004). It also attacks turnip, radish, sarson and toria (Bhalla and Pawar 1977). 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 and causes serious damage economically (Siraj 1999). As Uttarakhand Government is giving emphasis on the organic farming hence, scientists have great pressure to manage the insect pests organically by use of botanicals and biocontrol agents. Therefore, keeping above facts in view present investigation was carried out to study the effect of drupes and leaves of *M. azedarach* on the cabbage butterfly, *P. brassicae*.

MATERIALS AND METHODS

Plant samples containing drupes and leaves of bakain were collected from the different places of Pauri Garhwal and were air-dried for 6-7 days and then dried in an oven at 30°C for 24 hours. Then dried plant samples were crushed into pieces using a mallet. The plant material was extracted by Soxhlet extraction method.

Soxhlet extraction was used to extract more active principle from the plant materials as the ordinary method of extraction was not efficient to yield good amount of active principle of the plant material. Known amount (500 g) of plant material of each sample 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°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).

Bakain extract (10 g) was weighed in a 100 ml flask, to which ethanol was added and the volume was made up to 100 ml. This constituted 10% ethanol-diluted extract which was designated as the stock solution of bakain (w/v). Different concentrations, i.e. 1, 2, 4, 6, 8 and 10% as required for different experiments were obtained by diluting the standard stock solutions serially with distilled water. The nimbecidine (0.05%) was used as a standard check for the different experiments excluding bioassay.

The mass culture of *Pieris brassicae* was established in the laboratory from the eggs collected from insecticide free cabbage field of Vegetable Research Block of the

College. Field collected eggs were kept in Petri plates (10 cm diameter) over the filter papers for hatching. After hatching, neonate larvae were transferred to cabbage/cauliflower leaves with their petioles dipped in water in glass vials (14 cm × 4 cm) inside the wooden rearing cages (36 cm × 34 cm × 26 cm) and 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 separately in glass jars (10 cm × 14.5 cm) over a piece of filter paper. In each jar, resting place was provided to the newly hatched adults for normal expansion of wings. The emerging adults were offered a 15% sucrose solution absorbed in cotton swabs and some shoots of cabbage/cauliflower. Muslin cloth was provided as an oviposition substrate.

Bioassay technique was used to test the toxicity of *M. azedarach* against *Pieris brassicae*. 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 considered as control. Each treatment was replicated thrice. Ten pre-starved laboratory reared 2nd instar larvae were released on the treated leaves in Petri plates. The second instar larvae were pre-starved for eight hours. Data on mortality of larvae were recorded after 24 hours and corrected for control mortality through Abbott's formula (Abbott, 1925). The whole set of experiments was maintained at temperature of 28±2°C with 67±2 % RH. Moribund insect counted as dead. The data obtained on mortality of insect was subjected to the probit analysis (Finney 1971) and LC₅₀ values of extracts were obtained.

Percentage of Mortality:

$[\text{Number of dead larvae}/\text{Number of larvae introduced}] \times 100$

Corrected percentage of mortality:

$[1 - (n \text{ in T after treatment}/n \text{ in C after treatment})] \times 100$

where n, number of larvae, T, treated, C, control.

The muslin pieces (20 cm × 10 cm) to be used as oviposition substrate were dipped for 30 s to impregnate in different concentrations together with nimbecidine and control and left to air dry. Four pairs of well fed 1 day old adults (1: 1) were released in glass jars (15 cm × 10 cm) and the treated substrates were introduced into the oviposition glass jars. Each replication consisted of eight treatments, i.e. 1, 2, 4, 6, 8 and 10% bakain extract together with the standard nimbecidine and another control with water. Each treatment was replicated thrice. All test units were kept in an incubator maintained at 30 ± 1°C. The number of eggs deposited on the muslin was counted after 48 hr.

To impregnate the muslin pieces (20 cm × 10 cm), as oviposition substrates, they were dipped for 30 s in different concentrations of the water diluted ethanolic bakain extracts and in nimbecidine and left to air dry. Four pairs of well fed

1 day old adults (1 : 1) were released in glass jars (15 cm × 10 cm) and the treated oviposition substrates were introduced into the jars. Treated substrates containing one day old eggs were included in the experiment. Muslin with 25 eggs were kept inside separate glass jars and then placed inside the BOD incubator at $30 \pm 1^\circ\text{C}$. Thereafter, the eggs were observed for hatching. Three replications were maintained for all the treatments.

Four pairs of adults were released into glass jars (15 cm × 10 cm) containing 15% sucrose solution soaked in cotton swabs. Muslin was provided as an oviposition substrate. Muslin pieces containing a minimum of 25 eggs in different glass jars were included in the bioassay. The 1 day old egg laden muslin containing counted number of eggs (25) were dipped in different experimental concentrations of the water-diluted extracts and nimbecidine for 5 s (Matthews 1966). The egg dipping in water alone served as control. The eggs on muslin were then retained in glass jars and placed inside the BOD incubator to record hatching.

The analysis program Probit (Finney 1971), the lethal concentrations for 50% of the mortality, LC_{50} was at 24 hr. The fiducial limits and degrees of freedom of the χ^2 goodness of fit tests, and regression equations, were recorded. Data from effect of extracts on fecundity, egg hatching and ovicidal action were subjected to analysis of variance (ANOVA) using a completely randomized design to determine critical difference (CD) among treatments/concentrations. The difference of two means between treatments/concentrations exceeding CD value was significant at 5% level.

RESULTS AND DISCUSSION

Insecticidal activity

Perusal to Table 1 all the concentrations of bakain seeds were superior than concentrations of bakain leaves and caused higher mortality of *Pieris brassicae* larvae after 24 hr. The

highest mean mortality 66.67% of larvae was recorded at 10% concentration of bakain seeds followed by 56.67% mean mortality at 10% concentrations of bakain leaves, whereas, 46.67% and 43.33% mortality was observed at 8% concentration of seeds and leaves, respectively. At 1% concentration of both seed and leaves, minimum percent mortality of larvae, i.e. 3.33% was recorded. Similarly, Atwal and Pajni (1964) reported 33.3 and 40% mortality against *P. brassicae* with alcohol extracts (10 and 5%, respectively) from drupes of bakain. Larval mortality of 22.8% with methanol extract of *M. azedarach* against *P. xylostella* has been reported by Dilwari *et al.* (1994). Whereas, Hernandez and Vendramin (1997) reported larval mortality above 80 per cent with the aqueous extract of *Melia azedarach* and *Azadirachta indica* against *S. frugiperda*. On the basis of probit analysis, LC_{50} was calculated as 9.59% concentration of bakain leaves and LC_{50} by drupes of bakain was 8.30% against the 2nd instar larvae of *Pieris brassicae* (Fig. 1&2). Lower concentrations of extracts from leaves and drupes were very less toxic to the larvae of *P. brassicae*. Coria *et al.* (2008) proved that ethanolic leaf extract of *M. azedarach* is a strong larvicidal action against the insects. Results clearly showed that extracts from drupes are more toxic as compared to extracts from leaves of bakain. Based on the results obtained and discussed as above, it can be said that extracts of *M. azedarach* had larvicidal activity against *P. brassicae* with high LC_{50} values.

Effect of extracts treated substrates on oviposition and hatching of eggs

Results revealed that as the concentration of extracts increased, egg-laying proportionately decreased (Table 2). Adult females laid significantly less number of eggs on the treated substrates in all treatments in comparison with the control. This discrimination was more prominent in 10% concentration of bakain seeds where only 8.00 eggs were

Table 1 Larvicidal activity of seed and leaf extracts of bakain at various concentrations applied for 24 hr to 2nd instar larvae of *P. brassicae*

Treatment	Concentration (%)	Larval mortality (%)	LC_{50} (%)	Fiducial limits	Regression equation	χ^2
Bakain leaf	1	3.33	9.59	7.68-14.31	Y= 2.48+2.57X	1.40
	2	10.00				
	4	16.67				
	6	26.67				
	8	43.33				
	10	56.67				
Bakain seed	1	3.33	8.30	6.75-11.52	Y= 2.66+2.54X	3.52
	2	13.33				
	4	20.00				
	6	33.33				
	8	46.67				
	10	66.67				

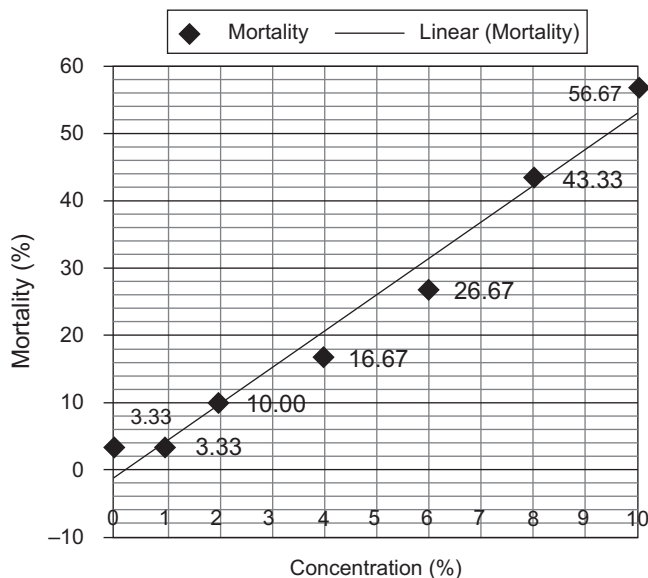


Fig 1 Linear mortality of 2nd instar larvae of *Pieris brassicae* at different concentrations of bakain leaf extracts.

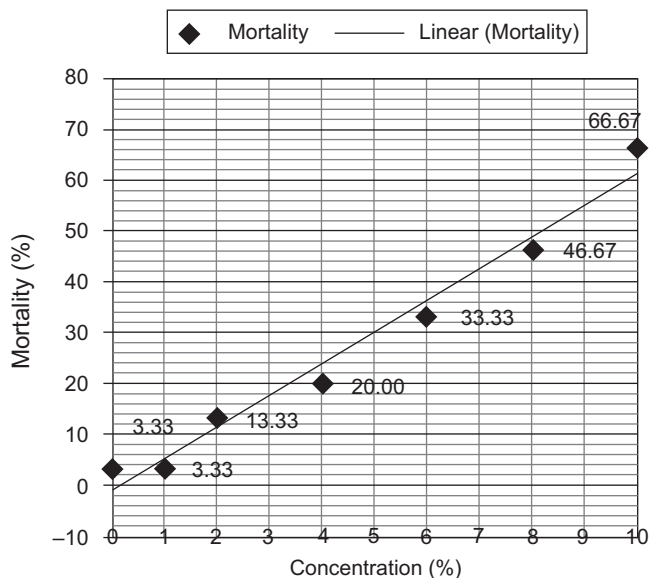


Fig 2 Linear mortality of 2nd instar larvae of *Pieris brassicae* at different concentrations of bakain seed extracts.

laid followed by 11.33 eggs on 10% bakain leaves as compared with 60.33 eggs on the control. Both treatments of bakain at 10% and 8% were significantly better than the nimbecidine 0.05% in suppression of egg laying by the adults. Bakain seed at 10% (8.00 eggs), 8% (14.67 eggs) and

6% (19.67 eggs) and bakain leaves at 10% (11.33 eggs) and 8% (17.33 eggs) were significantly better than nimbecidine 0.05% (23.00 eggs). Highest number of egg laying were recorded at 1% (52.67 eggs) of bakain leaves and 1% (42.67 eggs) of bakain seed as compared to 65.33 eggs were recorded at control (Table 2). Female *P. brassicae* laid very less number of eggs on the substrates which were treated with higher concentrations as compared to the low concentrations and control. Therefore, extracts from *M. azedarach* had oviposition deterrent effects. Gajmer *et al.* 2002 also reported that extracts of *M. azedarach* manifested repellent activity by reducing the number of eggs laid by females of *Earias vitella* on the treated substrates. Such oviposition repellency was also noticed in *Cnaphalocrocis medinalis* by neem extracts (Saxena *et al.* 1981). Result clearly showed that besides inhibitory action of extracts treated substrates for oviposition it also help in reduction in hatching of eggs at the substrates. Minimum egg hatching was recorded at 10% concentrations of both leaves and seeds extracts, i.e. 24.00% and 12.00% egg, respectively followed by 29.33 eggs hatching at 8% bakain seed as compared to 45.33% egg hatching in 0.5% nimbecidine and 94.67% in control. All the treatments had significantly adverse effect on the egg hatching as compared with the control (Table 2).

Table 2 Effect of ethanolic extracts of seed and leaves of *M. azedarach* on oviposition and egg hatching of *Pieris brassicae*

Treatment	Concentration (%)	Mean number of eggs laid	Mean egg hatching (%)
Bakain leaf	1	52.67 (7.29)*	85.34 (67.81)**
	2	48.00 (6.96)	73.34 (59.01)
	4	40.33 (6.39)	58.67 (50.08)
	6	35.67 (6.01)	49.33 (44.62)
	8	17.33 (4.22)	45.33 (42.31)
	10	11.33 (3.42)	24.00 (29.12)
	Bakain seed	1	42.67 (6.56)
2		37.33 (6.15)	74.67 (59.84)
4		30.00 (5.52)	58.67 (50.01)
6		19.67 (4.49)	45.33 (42.31)
8		14.67 (3.89)	29.33 (32.74)
10		8.00 (2.90)	12.00 (20.09)
Nimbecidine	0.5	23.00 (4.84)	45.33 (42.27)
Control	Water	65.33 (8.10)	94.67 (76.83)
SEm±		(0.16)	(2.83)
CD (P=0.05)		(0.45)	(8.20)

*The figures presented in parentheses are square root transformed values ($\sqrt{x+0.5}$), **The figures presented in parentheses are angular transformed values.

Ovicidal effect of extracts

All the concentrations used to treat the eggs of *P. brassicae* had significantly good effect on inhibition of hatching (Table 3). All the eggs were not hatched at 10% concentration of Bakain leaves and seeds, hence 100% ovicidal activity of extracts was observed as compared to control where 22.67 eggs were hatched out of 25 exposed

Table 3 Ovicidal effect of ethanolic extracts of leaves and seed of *M. azedarach* on *Pieris brassicae*

Treatment	Concentration (%)	Total no. of eggs treated	Mean no. of eggs hatched	Mean percent mortality
Bakain leaf	1	25	19.33 (4.45)*	22.67 (28.36)**
	2	25	17.33 (4.22)	30.67 (33.42)
	4	25	14.00 (3.80)	44.00 (41.52)
	6	25	9.00 (3.08)	64.00 (53.15)
	8	25	5.67 (2.46)	77.34 (61.80)
	10	25	0.00 (0.71)	100.00 (90.00)
Bakain seed	1	25	17.00 (4.18)	32.00 (34.42)
	2	25	14.00 (3.80)	44.00 (41.52)
	4	25	11.33 (3.44)	54.67 (47.69)
	6	25	6.67 (2.67)	73.34 (59.01)
	8	25	1.67 (1.39)	93.34 (77.76)
	10	25	0.00 (0.71)	100.00 (90.00)
Nimbecidine	0.5	25	12.33 (3.58)	50.67 (45.38)
Control	Water	25	22.67 (4.81)	12.00 (19.81)
	SEm±		(0.15)	(2.79)
	CD (P=0.05)		(0.45)	(8.10)

*The figures presented in parentheses are square root transformed values ($\sqrt{x+0.5}$), **The figures presented in parentheses are angular transformed values.

eggs with 12.00% egg mortality, whereas 12.33 eggs hatched and 50.67% egg mortality was recorded at 0.5% nimbecidine. Highest egg hatching 19.33 eggs and 17.00 eggs were recorded at 1% concentration of both bakain leaves and seeds, respectively, whereas egg mortalities were 22.67% and 32.00% in both the treatments at 1% concentration, respectively. Highest concentration (10%) was superior to the lower concentrations (1 and 2%) of both bakain leaves and seed but on a par with moderate (4, 6 and 8%) concentrations and nimbecidine. Similar results were also reported by Gajmer *et al.* (2002) where, 10 % methanolic extracts of *M. azedarach* reduced the egg hatching of *E. vitella* and only 36% egg hatching was recorded. Similarly, Jeyakumar and Gupta (1999) reported the ovicidal action of neem extracts against *Helicoverpa armigera*.

Finally it may be concluded that ethanolic extracts of leaves and seeds of bakain had little toxicity to the larvae but extracts reduced the egg laying by the adults, so the oviposition deterrent effect and it also reduced the egg hatchability, hence extracts revealed the ovicidal action against *P. brassicae*. It manifested that Meliaceae plant family has growth regulatory effect against insect pests (Gajmer *et al.* 2002, Banchio *et al.* 2003). The growth regulatory effect is the most important physiological effect of *M. azedarach* on insects. The results of this study indicate the plant-based compounds may be an effective alternative to conventional insecticides for the control of *P. brassicae*. Plant

allelochemicals may be quite useful in increasing the efficacy of biological control agents because plants produce a large variety of compounds that increase their resistance to insect attack (Senthil Nathan *et al.* 2005). Hence, by the use of extracts of bakain in their fields farmers may reduce the incidence of cabbage butterfly in cole crops.

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