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Natural botanical extractive as Biopotential Antifeedant against Diamondback moth, *Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae)

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Abstract
A laboratory trial was conducted to assess the insect-pest rejected compatibility of six ecofrindly botanical alcoholic extracts *viz*., *Azadirachta indica* A. Juss., *Adhatoda vasica* Nees. *Curcuma domestica* Val. *Lantana camara* Linn., *Ricinus communis* Linn. and *Vitex nugendo* Linn. With control (untreated) were tested to find out their comparative antifeedant effects were worked against third instars larvae of Diamondback moth, *Plutella xylostella* Linn. It is evident that *V. nugendo* was more effective and *Lantana camara*, the least. On the basis of their order of merit and EC_{50} values result is summarized as under *viz*., *V. negundo* (0.1155) > *Azadirachta indica* (0.1380) > *Adhatoda vasica* (0.2055) > *Ricinus communis* (0.2399) > *Curcuma domestica* (0.2974) >*Lantana camara* (0.4432) and the order of merit being : 1.000 > 1.194 > 1.779 > 2.077 > 2.574 > 3.837, times less protective, respectively as *V. negendo* taken as unit. It is evident that *Vitex nugendo* Linn. was more effective and *Lantana camara*, the least.

Keywords: Feeding deterrent, *Azadirachta indica, Vitex negundo*

1. Introduction
The Diamondback moth, *Plutella xylostella* Linn. (Lepidoptera: Plutellidae) is sporadic in nature and has been in regular occurrence in northern India, causing considerable damage to brassicacius crops and vegetables in our country [1, 2]. The pest is distractive in its larval stages. The larvae feed and caused enormous destruction by making holes in the leaves [3, 4]. The damaged plant stunt shows poor growth and results in deterioration of the yield of cabbage [5-7]. Diamondback moth, *Plutella xylostella* Linn is a most damaging pest of cruciferous plants, *viz*. mustard, cabbage, cauliflower, turnip, radish, rapeseed etc [8-10]. Diamondback moth, *Plutella xylostella* Linn, and other cole crops cause enormous damage [11, 12]. Brassicaceous crop and vegetables are attacked by a dozen of insect pests [13-15], off which, *P. xylostella* (Linn.) is the most serious and destructive insect pest [16-18]. Its larvae feed leaves, by cutting and in severely attacked crops and vegetables, the plant's growth is arrested and consequently, the yield is considerably reduced [19, 20].

Biological resources and the potential for sustainable exploitation of crops of medicinal and aromatic plants in our country are huge and represent an important component of sustainable agricultural development in India [21, 22]. From existing data, our country has a flora of over 3,700 different plant species, cultivated or spontaneous, with therapeutic and insecticidal action, of which 800 species have properties defined and 370 species have been recognized as having the qualities of insecticidal effects, but which have not yet been fully studied from the scientific point of view [23-26]. Botanicals derived from plants are currently recognized as biodegradable, systemic, eco-friendly and non-toxic to mammals and are thus considered as safe alternatives [27-29].

Efforts have been made to develop a control schedule by using plant origin insecticides in the vegetative phase and less persistent in the middle phase to save the crop from pest's ravages [30-32]. In contrast, naturally occurring indigenous plant products traditionally used against insect pests of crops and vegetables as antifeedants appear to be quite safe and promising [33, 34]. Several authors have reported the antifeeding and insecticidal action of man naturally occurring indigenous plant extractives and derivative and phagodeterrent effects of botanicals [40, 41]. The uses of the plant extracts are being sought with the following favorable properties [35-37]. The target insects do not even touch the selected plant species to feed. Therefore they have been utilized for insect management.

The products of selected plants are less deleterious to a human being in manufacturing, handling and in the application and are very effective against insect pests [38, 39]. They are nonphytotoxic and have no residual hazards for beneficial organisms *viz*., parasites, predators and pollinators. These are comparatively cheaper than synthetic insecticides [40, 41].

However, no appropriate attempts have been made to compare the relative bio-efficacy of indigenous herbal products on cruciferous crops. Therefore, the present investigations were undertaken to explore and evaluate the relative anti-insect efficacy of six herbal extracts for the effective control of diamondback moth, *P. xylostella*, a serious pest of brassicaceous crops and vegetables in most of the states of India [43, 44].

Visualizing the present situation of using synthetic insecticides, it has been proved that these insecticides are very effective in minimizing the population of harmful insect pests. Still, at the same time, their harmful effects are more dominant in respect of causing environmental pollution and deadly hazardous to human beings and domestic animals.

2. Materials and Methods

The present study was conducted in the post-graduate Department of Zoology, Entomology, Biopesticides and Toxicological Laboratory, D.B.S. College, affiliated with CSJM University, Kanpur, India.

2.1. Mass culturing of Diamondback moth, *Plutella xylostella*

The diamondback moth, *Plutella xylostella* required for the study, was mass-reared on mustard in the laboratory. The moisture content of the grains was adjusted to 11.0 percent by sun-drying to have uniform moisture content. The mass culturing was initiated by confining 10-20 freshly emerged beetles in the plastic containers of 59 x 21 x 18 cm having mustard leaves which were then covered with cloth and secured tightly with a rubber band. Such containers were stacked on iron shelves. Mass culturing of larvae of *Plutella xylostella* was done at room temperature in the plastic container and observed daily. Larvae were collected for the study.

2.2. Procurement of raw plant materials: In the present investigation ten indigenous botanicals were collected *viz Azadirachta indica* A.Juss., *Adhatoda vasica* Nees. *Curcuma domestica* Val. *Lantana camara* Linn., *Ricinus communis* Linn. and *Vitex nugendo* Linn. and their parts as per Table 1, were used for their antifeedant biopotency against larvae of *Plutella xylostella* in laboratory trials.

Table 1: List of naturally occurring indigenous floral materials for their extractions

Sr. No.	Botanical Name	Common Names	Family	Part Used
1.	*Azadirachta indica* A.Juss	neem	Meliacea	Seed kernel
2.	*Adhatoda vasica* Nees.	Pavettia	Acanthaceae	Leaves
5.	*Curcuma domesticus* Val.	Turmeric	Zingeberaceae	Rhizome
6.	*Lantana camara* Linn.	Aripple	Verbenaceae	Aerial Part
7.	*Momordica charantia* Linn.	Bittar Guard	Cucurbitaceae	Unripe fruit
10.	*Vitex nugendo* Linn.	Nirgundi	Verbenaceae	Leaves

2.3. Preparation of powder: Fresh collected green plant parts (leaves, Flowers and seeds, rhizomes etc) were washed with distilled water and kept in the laboratory for 7 days for air drying followed by one-day sun drying before making powder. Electric grinder was used to have coarse powder, then these were passed through a 60-mesh sieve to get a fine powder. Powders were kept in polythene bags at room temperature and properly sealed to prevent quality loss.

2.4. Extraction of Selected Plant Materials

For the extraction, Soxhlet Apparatus was used; about 20g of powder of each category of powder were extracted with 300 ml of different solvents (n-hexane, acetone, methanol, petroleum ether and distilled water). Extraction of each type of powder was done in about 12 hrs. After soxhlet extraction, the material was run on a rotary evaporator. The extracts were concentrated on a rotary evaporator by removing the excess solvent under a vacuum. After evaporation of solvent with a rotary evaporator the remaining extracted material was kept in a water bath to remove the remaining solvent from the extracts. The extracts were stored at 4°C before application.

2.5. Preparation of 50 Percent Stock Solution from Pure Extract

50ml. Extract in each case was taken into reagent bottle and 50ml. Benzene was added to it to dissolve the constituents of the materials. This was the 50 percent stock solution, the mouth of the bottles was stopped with airtight corks and kept in the refrigerator.

2.6. The Insecticidal Formulations

The different concentrations of the herbal botanicals were prepared from the stock solution using benzene as solvent and Triton X-100 as an emulsifier. The level of solvents and emulsifier were kept constant at the rate of 5 percent and 0.5 percent, respectively, in the final spray.

2.7. Preparation of 0.5 Percent Emulsifiable Water

0.5 ml. of Triton X-100 was accurately measured into a large bottle with the help of a measuring cylinder, then 99.5 ml of distilled water was added and the bottle was shaken well to dissolve the emulsifier. Thus emulsifiable water of 0.5 percent strength was obtained and used to prepare different concentrations of the extracted materials (Schmidt and El, 1997).

2.8. Preparation of Concentrations

To make the various concentration of extract the required quantity of the 50 percent stock solution was calculated with the help of the following formula:

$$\text{Amount of Stock} = \frac{\text{Amount required x Concentration required}}{\text{---}}$$

Solution

The calculated amount of various ingredients required to make different concentrations from the 50 percent stock solution and the amount of ingredients taken are presented in the following table:

Table 2: Preparation of different formulations of the selected plant materials: Concentration (%) Concentration of Stock Solution

Concentration (%)	Amount of Stock Solution (ml)	Amount of Benzene (ml)	Amount of Emulsifiable Water (ml)	Total Amount
0.25	2.50	22.50	475.00	500.00
0.50	5.00	20.00	475.00	500.00
1.00	10.00	15.00	475.00	500.00
1.50	15.00	10.00	475.00	500.00
2.00	20.00	5.00	475.00	500.00

2.8. Field Collection and culture of Pulse Beetle: The larvae of *Plutella xylostella* was drawn from laboratory mass cultures reared in glass jars at ambient laboratory temperature. The larvae of *Plutella xylostella* used for the experiment were 3rd instar larvae were used for the experiment and fed on mustard.

3. Experimental Protocol

For testing the repellent effect of plant extracts were used as food for larvae of *Plutella xylostella* treated with different concentrations. The treated foods were kept on moist filter paper in a jar (23cm x 10 cm). Thirty 24 hours, starved larvae of *Plutella xylostella* were released in each jar along with control. The treated seeds were dipped in Benzene + emulsified water only. After four hours of the release larvae of *Plutella xylostella.* Treated larvae either rejected feeding and forced them to move from treated jars A to an empty untrated jar B through the plastic pipe The ones found in the plastic pipe were considered feeding rejected individuals. The antifeedant data (in treated and untreated jars) were recorded for 14 days at an interval of 24 hours for each observation. The data was collected on the number of larvae of *Plutella xylostella,* which reached the treated food and feeding rejection over control was recorded. The data was collected on the number of larvae of *Plutella xylostella,* which reached the treated food and feeding rejection over control was recorded. The data on a number of larvae used and a number of test insects that reached to the food in each replication were was calculated the data was subjected to Probit analysis (Finney, 1952) [42] and the result were compared based on respective EC $_{50}$ values (Godine, 1959) [43].

Table 3: Summary of Log Conc./Probit Protection Regression Column of extract as Protectants against larvae of *Plutella xylostella*

Plant Extracts	Het.	X2	Regression Equation	EC50	Relative EC50	Fiducial Limit
Azadirachta indica	3	0.94	Y=1.8X+2.65	0.1380	1.194	M1=0.0102 M2=0.0301
Adhatoda vasica	3	0.71	Y=0.53X+1.41	0.2055	1.779	M1=1.0234 M2=0.0202
C. domestica	3	0.77	Y=0.89X+2.40	0.3776	3.269	M1=1.5865 M2=0.1390
L. camara	3	1.17	Y=2.8X+0.60	0.4432	3.837	M1=0.1608 M2=0.0344
R. communis	3	0.79	Y=0.84X+3.84	0.2399	2.077	M1=1.7533 M2=1.0066
Vitex nugendo	3	0.28	Y=0.61X+4.13	0.1155	1.000	M1=1.6295 M2=1.0877

All cases x was found non-significant hetrogenous at P = 5. Y = Probit KILL. x = Log conc. X 10^2

D.F. = Degree of freedom, EC_{50} = Conc. calculated to give 50 % antifeeding activity. Het.= HeterogenecityIn

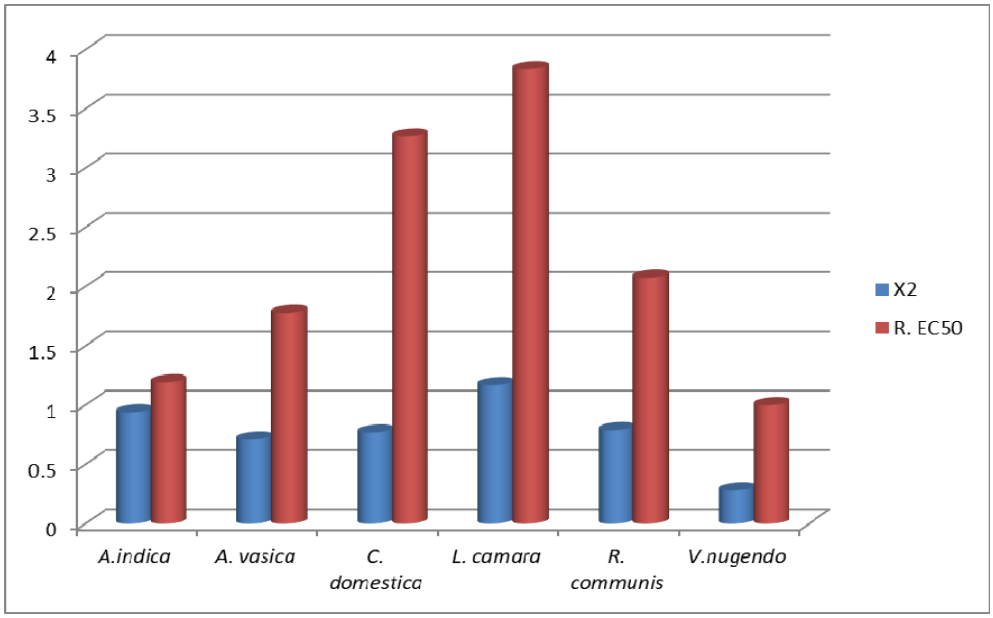

Fig 1: Summary of Log Conc./Probit Protection Regression column

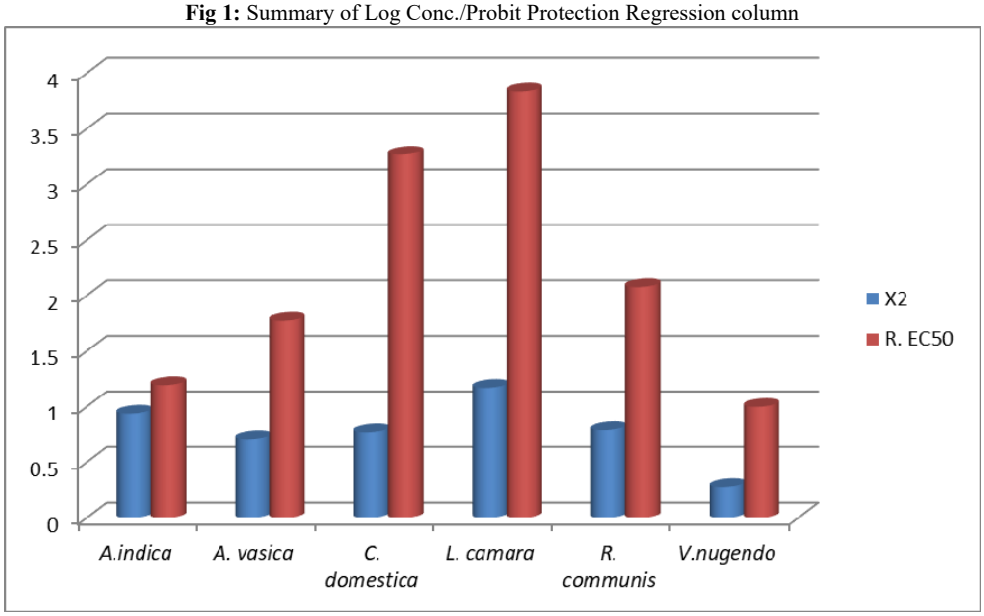

Fig 2: Summary of Log Conc./Probit Protection Regression column

4. Result and discussion

It is seen from the table 2 and Fig.1 and 2 that all the plant extracts have proved to have more or less antifeedant activity against the larvae of *Plutella xylostella*. All the plant extracts have proved to more or less feeding rejection against the larvae of *Plutella xylostella*. Among all selected plant extracts, only three plant extracts gave promising antifeedant activity with a minimum EC50 value (less than 0.50%) and rest seven showed the less antifeedant effect on larvae of *Plutella xylostella*. The leaves extract of *V. Nugendo* gave the highest and most significant antifeedant bio-potency to larvae of *Plutella xylostella.* On the basis of their order of merit and EC_{50} values result is summarized as under *viz*., *V. Nugendo* (0.1155) > *Azadirachta indica* (0.1380) > *Adhatoda vasica* (0.2055) >*Vitex nugendo* (0.1155) > *Ricinus communis* (0.2399) > *Curcuma domestica* (0.2974) >*Lantana camara* (0.4432) and the order of merit being : 1.000 > 1.194 > 1.779 > 2.077 > 2.574 > 3.837, times less protective, respectively as *V. nugendo* taken as unit. It is evident that *Vitex nugendo* Linn. was more effective and *Lantana camara*, the least.

In the support of the present findings several workers also reported antifeedant activity of various plant extracts. Sudhaker *et al.* (1978) tested the antifeeding and insecticidal properties of ether extract and *Crimum defixum* showed antifeeding property in the laboratory conditions [46]. Misra and Singh, (1992) found the antifeeding properties of *Azedirachta indica* against desert locust, *Schistocerca gragaria*. Out of them 5.0 % neem, *A. indica* leaf extract showed highest antifeeding activity [47].

Many workers has been also reported the antifeedant activity of plant extracts as antifeedant responses *viz*; [Muralikrishna *et al.* (1990) [48], Rao *et al.* (1999) [49], Maredia, *et al.* (1992) [50], Tewari and Moorthy (1985) [51],Tripathi *et al.* (1999) [52], Govindachari *et al.* (2000) [53], Suindararajan and Kumuthakalaralli (2001) [54], Omer *et al.* (2004) [55], and Omer (2006) [56].

Murugan *et al.* (1988) reported that neem limonoids was found to be significant antifeedant against the cotton ballworm, *Helicoverpa armigera* Hubner [57]. Abudulai *et al.* (2001) tested nemm,*Azadirachta indica* reported that neem extract possesses strong feeding rejectant aginst *Nezara viridula.* [58]. Chandel *et al.* (2005) tested leaves extract of nirgundi, *Vitex negundo* Linn. And neem, *Azadirachta indica* extract showed strong antifeedant biopotency to *Dysdercus koenigii* Fabr [59]. Chauhan *et al.* (2011) reported significant antifeedant activity of extracts of *Azadirachta indica* and *Toona ciliata* against larvae of *Spilarctia obliqua* Walker.[60].

5. Conclusion

The findings of the present investigations indicate that botanical derivatives might be useful as insect control agents for commercial use. Among six plant extract, only *Vitex nugendo* leaves extract showethe d highest protectivity, followed by *Azadirachta indica* against the larvae of *Plutella xylostella*. All the extracts tested were effective to some degree of antifeedancy rejected feeding. More studies on major biochemical constituents responsible for feeding rejectant activity to the larvae of *Plutella xylostella* on mustard.

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