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Anti-Feedant, Repellent and Growth Regulatory Effects of Four Plants Extracts on *Pieris Brassiceae* Larvae (Lepidoptera: Pieridae)

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Abstract

Background: Over 30 decades a number of natural plant products have been considered for use as insect anti-feedants and repellents in the agriculture. Plants are able to synthesize a broad range of different chemical compounds called secondary metabolites, with many of them as promising new sources of natural pesticides.

Results: Aqueous extracts of four locally available plants namely, *Artemisia vulgaris, Datura arborea, Nico-tiana tobacum* and *Zanthoxylum alatum* were prepared, and their feeding deterrence, repellent action, and mor-tality affects on the *Pieris brassiceae* larvae was studied. Discernable effects were recorded at 20 mg kg^{-1}, and at higher concentrations of 50 mg kg^{-1}, 100 mg kg^{-1} and 150 mg kg^{-1}, the intensity of the effects gradually increased. Among the four extracts, *Z. alatum* was the most potent as a feeding deterrent and repellent, and also caused the highest mortality of *Pieris brassiceae* larvae. *Datura aborea* was seen to exhibit Insect growth regulating (IGR) activity in causing late pupation of the larvae.

Conclusion: All the results exhibited significant differences ($P \leq 0.005$) in feeding deterrence, repellent action, mortality, and late pupation when compared with the non-treated controls. The importance of the four botanicals as eco-friendly and easily accessible local pest control alternatives are discussed.

Keywords: *Artemisia vulgaris; Zanthoxylum alatum; Nicotiana tobaccum; Datura aborea Pieris brassiceae* larvae; Feeding deterrence; Anti-feeding activity

Introduction

Insect-pests management has a long history of the use of chemical insecticides/ pesticides. These chemical com-pounds can leave potentially toxic residues in the soil, crops and vegetables, which can be hazardous to the non-target organisms, the environment, and even man. Over the past 3 decades, plant-derived extracts and phyto-chemicals have been intensely investigated in an effort to develop environment friendly alternatives to the conventional chemical insecticides [1-15]. Meliaceae, Rutaceae, Asteraceae, Labiateae, Piperaceae and Annonaceae are the plant families which show the most promising effects as natural botanical insecticides/ pesticides [12,16]. Plants are able to synthesize a broad range of different chemical compounds called secondary metabolites [17] and many of them are promising new sources of natural pesticides [4,18-20]. Secondary plant compounds found in botanical insecticides have been the recent focus of many investigations [21-28]. Neem (*Azadirachta indica*, Juss.) has emerged as an excellent alternative to chemical insecticides for the management of insect-pests and was studied by many researchers [25,29-32]. The extracts of *Artemisia vulgaris* (Linn.), *Datura aborea* (Linn.), *Nicotiana tobaccum* (Linn.) and *Zanthoxylum alatum* (Roxb.) showed repellent effect and anti-feedant effect on different insect larvae and their lifecycles [33,34]. A number of plant substances have been considered for use as insect anti-feedants and repellents, but apart from some natural mosquito repellents, little commercial success has ensued for plant substances that modify arthropod behavior [4].

Cabbage and cauliflower (*Brassica oleracea* Linn.) are economically important crops, and are often produced under small holder conditions [35]. Over the years, these crops have been cultivated more intensively, resulting in high pest infestation rates [36-40]. About 38 insect-pests of cole crops have been reported till date and among them the cabbage white butterfly, *Pieris brassiceae* (Linn.) is one of the most destructive causing damage at all the growth stages including seedling, vegetative growth and flowerering [40-45]. It is a pest with a wide host range and is known to infest 83 species of food plants belonging to the family Cruciferae [39]. The present investigation was undertaken is to find effective control for larval stages of *P. brassiceae* by using plant extracts easily available locally, and with user friendly protocols for the farmers.

Materials and Methods

Preparation of plant extracts

The leaves of *Z. alatum* were collected from Mawtneng village near Umroi, and the leaves of *A. vulgaris* and *D. aborea* were collected from around the campus and brought to the laboratory. They were washed in distilled water and then air dried at room temperature (± 27°C) for about 72 hours. The leaves of *N. tobaccum* were bought from the local market and dried at 50°C in the oven for 24 hours. The dried leaves of the different plants were separately powdered using a mortar and pestle. For the preparation of the different plant extracts, the powder (250 gms) of the respective plant was taken, and hexane+ethyl ether (250 ml+250 ml) was added to it. The mixture was vigorously agitated using a mechanical shaker and left undisturbed for 48 hours. The mixture was then filtered out using Whatman filter paper No.1. The filtrate was evaporated to dryness at 60°C in a rotatory-evaporator (BUCHI Rotavapour R II). The extract thus obtained was stored in air-tight containers and kept under refrigeration till further use.

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Rearing of the larvae

The first instar larvae of *P. brassiceae*, were manually collected from the cropping fields near the University cam-pus in Mawlai and brought to the laboratory. They were reared at room temperature (± 27°C) at 12 L:12 D, in 1 liter glass jars and fed on fresh cabbage leaves for about a week [25,46,47].

Experimental Set-up

The experiments were conducted at room temperature in the laboratory. For each experiment, 10 larvae of *P. brassiceae* (third instar) were exposed to four concentrations (20 mg kg^{-1}, 50 mg kg^{-1}, 100 mg kg^{-1} and 150 mg kg^{-1}) of the plant extract. A control having the same set up but without the extract was also set up. Each experiment was replicated thrice. Thus for the whole study a total of 192 experiments were conducted.

Feeding deterrence of the prepared aqueous plant extracts were assayed by using a Leaf Disc Choice test [48]. A Feeding Deterrence Index (FDI) was calculated using the formula **FDI=100[(C-T)/(C+T)]** where, C and T are the control and treated leaf respectively consumed by the insect [2,12].

Leaf discs were cut and measured for the Leaf Area Index by using the LI-COR 3100C. Leaves were dipped in a surfactant (SDS 0.1% w/v) solution [12] and then dipped in the plant extract (test) solution for 30 seconds. Control leaves were thoroughly washed in running water and then dipped in a surfactant (SDS 0.1% w/v) solution. 2nd instar larvae were placed in trays containing the extract-treated / control leaves and activity was observed for 5 hours. For each of the plant extract, *n* number (*n*=10) of larvae were used. At the end of 5 hours the larvae were washed with distilled water and then fed on untreated leaves. After 5 hours, all the leaves from the control and from the experimental trays were measured again for the leaf area index.

The repellent action of the plant extracts against the *P. brassiceae* larvae was assessed by the method detailed above. Increasing order of the plant extract concentrations (20 mg kg^{-1}, 50 mg kg^{-1}, 100 mg kg^{-1} and 150 mg kg^{-1}) were used for the leaf dips. For the mortality and IGR activity studies, the experiments were set up as detailed above. However, the applications of the plant extracts were done by spraying directly on the larvae.

Data were analyzed by using Microsoft excel and t-test was applied, at a significance level of P<0.05.

Results

Feeding deterrence

The results of feeding deterrence experiments are depicted in table 1. On calculating the Feeding Deterrence In-dex, *Z. alatum* has the best deterrent action against *P.brassiceae* larvae with the highest FDI value. *A. vulgaris* has the least value when compared to the other three plant extracts (Figure 1).

Repellent action

Repellent action experiments revealed some activity at 50 mg kg^{-1} (Table 2). All the larvae were strongly repelled at 150 mg kg^{-1} concentration of the plant extract. Within 4 hours of the experiment larvae were totally repelled from the treated leaves. Among the four plant extracts, *Z. alatum* gave the best result as a repellent, repelling 9 larvae, followed by *A. vulgaris* repelling 8 larvae. Statistically, the results were found to be significant at a significance level of P ≤ 0.005 (Figure 2).

Concentration→	50 mg kg^{-1}	100 mg kg^{-1}	150 mg kg^{-1}	Mean
Name of plant extract ↓				
Artemisia vulgaris	26.923	11.475	20	19.466
Datura arborea	23.809	15.254	22.448	20.503
Nicotiana tobaccum	37.037	34.615	28	33.217
Zanthoxylum alatum	28	41.463	43.478	37.647

Table 1: FDI value when sprayed with different concentrations of the four plant extracts.

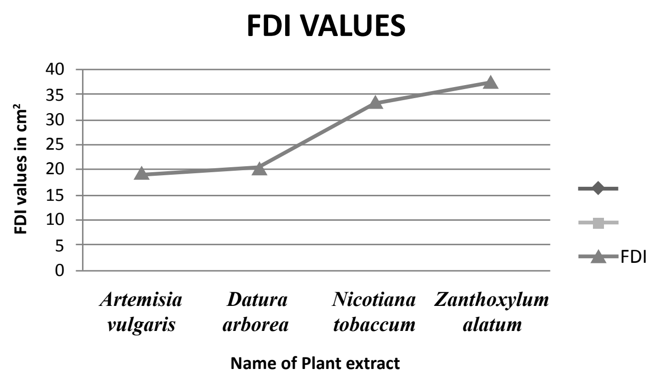

Figure 1: Graphical presentation of FDI value when sprayed with different concentrations of the four plant extracts.

Concentrations→	20 mg kg^{-1}	50 mg kg^{-1}		100 mg kg^{-1}			
Name of Plant Extracts↓		Mean	SD	Mean	SD	Mean	SD
Artemisia vulgaris	0	2.333	±0.577	6	±1	8.666	±0.577
Datura arborea	0	2.333	±0.577	5.333	±1.527	6.666667	1.527
Nicotiana tobaccum	0	4	±1	6.333	±2.081	8	±1.732
Zanthoxylum alatum	0	3.666	±1.527	6.333	±1.527	8.666667	±0.577

Table 2: Repellent activity of the four extracts Values are means of three readings, SD in parentheses.

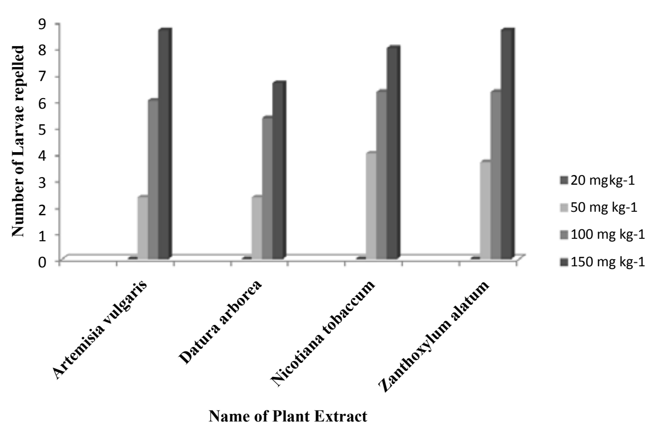

Figure 2: Graphical presentation of the repellent activity of the four plant extracts.

Mortality

During the experiment, a small number of larvae (≤ 30%) died in the control trays (Table 3). However, mortality in the experimental trays was considerably higher, particularly with *Z. alatum* extract (150 mg kg^{-1} concentration) where 60% mortality (Figure 3) of the larvae was observed ($P \leq 0.005$).

IGR effects

IGR results revealed a significant difference on the duration of pupation of the larvae (Table 4). In the controls, pupation occurred on the 14th day (± 2 days), whereas, in the experimental trays, pupation occurred on the 18th (± 2 days). Data were analyzed using two way sample t-test with t= 0.000175 df =6 significant at $P \leq 0.005$.

Emergence in the control occurred after 12 days whereas in the experimental set-up it was delayed to 18 to 20 days. Late emergence was predominantly induced at 150 mg kg^{-1} concentration of *D. aborea*. (Table 4 and Figure 4).

Discussion

Aqueous extracts of the four locally available plants prepared were used against P. brassiceae larvae and pupae. From the results obtained, all the four plant extracts viz. *N. tobaccum, A. vulgaris, D.atura sp.* and *Z. alatum* exhib-ited viable anti-feedant/deterrent and repellent activity. Late pupation and high mortality rate was also observed in both the larval and in the pupal stages. *Z. alatum* has the highest FDI value of 37.647 cm^2 while *A. vulgaris* had the least, with an FDI value of 19.466 cm^2. All the four extracts induced reduced feeding rates when compared with the controls. The highest concentration (150 mg kg^{-1}) of all the extracts had the strongest repellent action. Among the four plant

Name of plant extracts	*Artemisia vulgaris*	*Datura arborea.*	*Nicotiana tobaccum*	*Zanthoxylum alatum*
Experimental				
Mean	4.33333333	3.33333333	4.66666667	4.66666667
SD	± 0.577	± 0.577	± 0.577	± 0.577
Control				
Mean	0.666667	1	1	1.66666667
SD	± 0.577	± 1	± 1	± 0.577

Table 3: Shows the larval mortality of the four plant extract values are means of three readings, SD in parentheses.

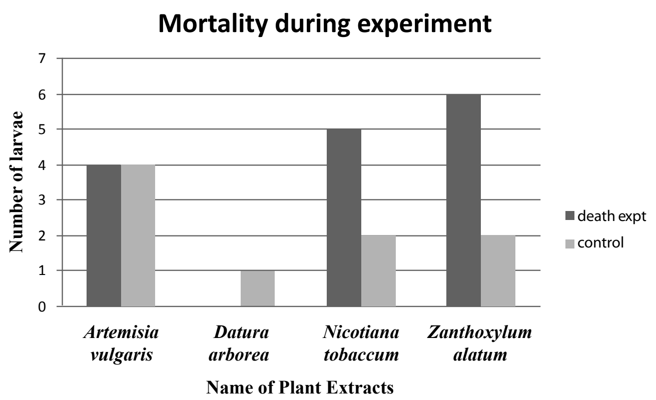

Figure 3: Graphical presentation of the larval mortality of the four plant extract.

Name of plant extracts ↓	Mortality (%)	Late Pupation (%)
Artemisia vulgaris	40	60
Datura arborea	10	100
Zanthoxylum alatum	60	40
Nicotiana tobaccum	50	50

Table 4: Occurrence of mortality and late pupation with 150 mg kg^{-1} concentration.

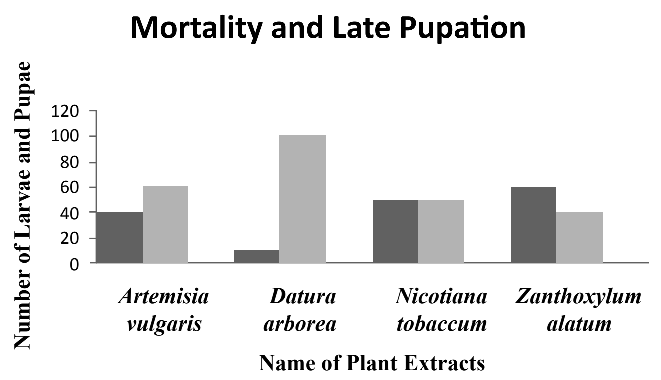

Figure 4: Occurrence of mortality and late pupation with 150 mg kg^{-1} concentration.

extracts, *Z. alatum* and *A. vulgaris* were the most effective repellents. Further, it was observed that *Z. alatum* at 150 mg kg^{-1} concentration caused high mortality when sprayed directly on the 4th instar larvae of *P. brassiceae*. Larvae exhibited reduced feeding, and 100% larval mortality was attained within five days. Late pupation was induced on the pupae of *P. brassiceae* when 150 mg kg^{-1} of *D. arborea* plant extract was directly sprayed on the pupae. Extracts are known to contain certain chemicals which have the potential to interfere with the physiol-ogy of the larvae by reducing their growth and survival [40,41]. Metspalu (2003) working with *P. brassiceae,* showed that even if other factors were favorable, if food was limited, the larval period as well as the pupation period increases [50]. In the present experiments too, the induction of reduced feeding may have been the initial effect, which led to the elongation of the pupa stage.

It is evident from the above that all the four extracts have the potential of being used at different times of the crop-ping cycle for the management of *P. brassiceae* infestations. Further, considering the fact that two of the species used are wild herbaceous plants abundant in the region (*A. vulgaris, D. arborea*), a third (*Z. alatum*) is used for traditional medicinal purposes and is common in home gardens and also abundant in the wild, and the fourth (*N. tobaccum*), a commercial tobacco species grown in the state, it is evident that they are easily available for wider use. The simple aqueous extraction and cost effectiveness are user friendly traits which make these botanicals excellent candidates for promotion. Natural insecticides such as the present extracts are obtained from renewable natural resources, and they quickly degrade without leaving any persistent harmful residues in the environment, thus making them eco friendly alternatives to chemical pesticides against cruciferous crops which are abundantly grown by the farmers of the region.

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