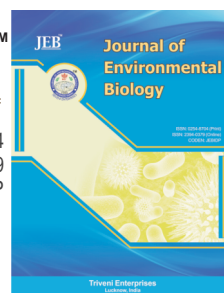
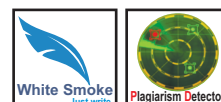


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Antifeedant and ovipositional deterrent activity of medicinal plants of Western Himalaya on *Plutella xylostella*



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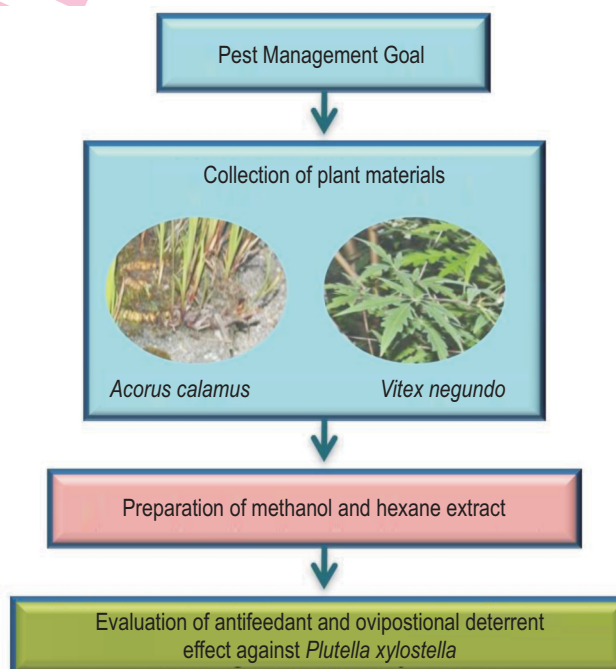
Abstract

Aim: Medicinal plants of Himalayan region is inadequately known for their role in pest management. The present study was conducted to assess the response of different extracts of *Acorus calamus* (L.) and *Vitex negundo* (L.) on antifeedant and ovipositional deterrent activity of *Plutella xylostella*.

Methodology: *Acorus calamus* (Rhizome) and *Vitex negundo* (leaves) were taken as an experimental material. Methanol (polar solvent) and hexane (non-polar solvent) were used for extraction. Antifeedant and ovipositional deterrent effect of two plants were worked out against *Plutella xylostella*. The data were statistically analysed by t-tests for paired comparisons.

Results: Stronger ovipositional deterrent effects was observed in *A. calamus* (methanol extract) with higher oviposition deterrent indices (ODI) (38.7), followed by hexane extract of *V. negundo*. The leaves treated with plant extracts of *A. calamus* and *V. negundo* deterred the female of *P. xylostella* to some extent to lays eggs. After three days of treatment, methanol extract of *A. calamus* showed a residual deterrent effect to female of *P. xylostella* due to its low volatile nature.

Interpretation: Application of methanol and hexane extracts of *A. calamus* and *V. negundo* on host plants render them less attractive and show ovipositional deterrent to females of *P. xylostella*.



Introduction

Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is an important insect-pest that damages the cabbage plants (Sarfraz *et al.*, 2006). It is adapted to wide range of agro-climatic conditions and cause serious economic losses to cabbage, cauliflower, broccoli, canola and brussels sprouts in 128 countries (Martínez-Castillo *et al.*, 2002; Ahmad and Ansari, 2010). In last half century, this pest has developed resistance to chemical insecticide which have been used for their management, so safe and alternative to synthetic insecticide is needed (Talekar and Shelton, 1993). Plants are nature's chemical factories, providing the nature's richest source of chemicals (Singh, 2000). Plant families like Rutaceae, Annonaceae, Malvaceae, Labiateae, Canellaceae, Asteraceae and Meliaceae are a vast source of secondary plant substances (Jacobson, 1989), and approximately 2500 plants have been reported to possess insecticidal properties (Dhaliwal and Koul, 2011).

Plants like *Acorus calamus* and *Vitex negundo* manufacture different kinds of allelochemicals like glycosides, saponins, polyphenol which change the behavior of the insects and therefore, it is difficult for insects to develop resistance to these pesticides (Dhaliwal and Koul, 2011).

Antifeedant effect is essentially a pre-injective activity, based on chemosensory response, where insect rejects food treated with the compound and ingestion is reduced. This results in reduced growth and fecundity and prolonged developmental time. Antifeedant act as allomone substances which inhibit feeding and do not kill the insect-pests directly, but rather limit its developmental potential considerably and act as phagorepellent. The plant products applied in any form *i.e.*, either as extracts or oils affects egg laying and egg hatching, influencing the reproduction potential of a pest is known as oviposition deterrents. Plant extract produces pungent smell which causes malfunctioning of the ovariole in female lepidopteran pest (Elumalai *et al.*, 2005).

Mid hills of western Himalaya are endowed with a wide range of promising flora known for their medicinal values and history of usages to management of insect-pests (Tewary *et al.*, 2005). For successful exploitation of natural compounds, screening of different plant extracts against polyphagous insects is required. The bioactivity of phytochemical extracts varies significantly with solvents used for the extraction and test insect (Ramya *et al.*, 2008). Bioactive properties of *A. calamus* L. and *V. negundo* L. are well known. However, studies on the antifeedant and ovipositional deterrent activity of these plants are scanty and no quantitative reports are available on their activity against *P. xylostella*. Therefore, the present study was planned to evaluate the antifeedant and ovipositional deterrent activity of two medicinal plants against Diamondback moth, *P. xylostella*.

Materials and Methods

Plant extracts preparation: Plants *viz.*, rhizome of *A. calamus* and leaves of *V. negundo*, were collected from the Kangra district (H.P). Different plant parts were cleaned three to four times with ordinary water and shade dried, then grounded in a grinder and these fine powder *viz.*, brown (*A. calamus*) and greenish (*V. negundo*) were subjected to extraction. Dried powder forms were mixed with non-polar solvent (hexane) and polar solvent (methanol) for 48 hrs and thereafter, passed through Whatman filter paper. Under reduced pressure and temperature (38°C), extracts were evaporated to obtain crude extract by using rotary evaporator. Desired concentrations were prepared by adding emulsifier (Triton X-100) to crude extract.

Experimental plants: For antifeedant and ovipositional deterrent experiments, cabbage plants, were grown in a tray (58×32×11.5cm) placed in green house conditions at 23±2°C temperature, 60±5% RH and 14:10 (L: D) photoperiod. Each tray contained approximately 50 plants. These trays were put into rearing cages to avoid pre-egg laying and feeding on the seedling. After 20 days, seedling were separated and grown individually in single pots (7×10cm). For both experiments, forty five-day-old single potted cabbage plants were used.

Test insects: A culture of *P. xylostella* was established from a field collected population of Diamondback moth from cabbage grown in vegetable farm and maintained on cabbage in a growth chamber room at 25±2°C, 70±5% RH and 16:8 (L:D) photoperiod during larval to adult stage. For culture maintenance, second instar larvae were transferred to cabbage leaves kept in plastic jars (25×20 cm) covered with a muslin cloth (4 mm). The larvae were regularly provided with fresh leaves without removing the infested one so as to enable them to shift to fresh leaves on their own, to improve their survival rate and reduce the handling time considerably. The pupae were collected and placed in clean cages for adult emergence. For mating and oviposition, adult moths were collected with an aspirator and transferred to oviposition cages (27×21×21 cm). For oviposition, the bottom of the boxes were covered with tissue paper and on which cabbage leaves were provided by placing their petiole in Erlenmeyer flask filled with tap water. Adult moths were provided with 5% honey solution as food in cotton wool in Petri dishes (35 mm).

Oviposition experiments: Oviposition experiments were designed to evaluate the oviposition deterrence activity of adult females of *P. xylostella* to methanol and hexane extract of *A. calamus* and *V. negundo* (treatments) comparison to the crucifer host (control). Two cabbage potted plants were placed into each cage on opposite sides of oviposition cages (27×21×21 cm). Leaves of one potted plant was treated with plant extracts and other plant was treated with only solvent. For preparing formulation of plant extract, Triton X-100 (0.1% (w/v) and solvent (10%) was added. The control formulation was prepared without

adding the plant extract. The treatments were applied using a small hand-held sprayer (Shivam Agrotech, Rajkot, Gujarat, India). The plants were sprayed until run-off (approximately 100 ml per plant) and then left to dry for one hour before being placed in a cage. The cages were arranged randomly on the laboratory bench. Five male and female moths were collected after 10-12 hrs of emergence, adult moths were sexed on the basis of their wing colour, male moth possess dark brown wings, however female moth possess tan coloured wings and released into oviposition cages. Placement of adult moths in the cages was done at around 6 p.m. Once placed in the cages, moths were allowed to freely mate and oviposit on the offered host plants. Cotton wools saturated with 5% honey solution were placed in cages to serve as feeding sites. After exposing the plants to adult female for 24 hr number of eggs laid on treated and un-treated plant was counted. Eggs laid at other sites in the cage were ignored. The experiment was three replicates for each concentration.

Ovipositional response to concentrations: Different concentrations (0.625, 1.25, 2.5 and 5.0%) of crude extract was formulated by serial dilution method. The oviposition deterrence activity of different plant extracts at different concentrations was measured by this experiment. For each concentration, there were three replicates.

Residual activity: The residual activity of different plant extracts applied to treated surface of potted plant to female of *P. xylostella* were evaluated from 0 to 3 days. There were three replicates per treatment.

Antifeedant activity: The third instar larvae of *P. xylostella* were used for measuring the antifeedant activity of different plant extracts. The leaf discs of cabbage (30 cm²) were dipped in four concentrations ranging from 0.625 to 5.0 % and then placed over bed of agar gel in Petri-plates. One set was also maintained without any treatment in Petri-plate to serve as control. Third instar larvae (n=10) of diamondback moth, pre-starved for 6 hrs were released in the centre of the leaf disc in Petri plates and were kept in the growth chamber for 24 hrs and experiment was replicated five times. In each treatment, leaf area was determined

using digital leaf area analyzer (Winfolia; Regent Inc, Canada) prior to initiating the experimentation and after 24 hrs of contact.

Statistical analyses: The data on number of eggs laid by adult female on treated potted plant and un-treated plant were analyzed by t-test for paired comparisons by using statistical package for the social sciences (SPSS) software version 16. The significance of differences was checked at the level of 5%. Before performing analysis the data were log-transformed.

$$\text{Oviposition deterrent indices} = \frac{100(C-T)}{C+T}$$

Where, T and C are the mean number of eggs laid on treated potted plant and un-treated plant. For the calculation of index, data were not log-transformed. Oviposition deterrent indices (ODI) were calculated as per Huang *et al.* (1995).

The per cent antifeedance for each treatment was calculated as per Govindachari *et al.* (1994).

Results and Discussion

Cabbage leaves treated with plant extract of *A. calamus* (methanol) and *V. negundo* (hexane), significantly deterred the oviposition of diamondback moth under dual choice test ($t=4.09$, $df=4$, $P=0.016$, and $t=4.98$, $df=4$, $P=0.008$, respectively). At a concentration of 0.625% (w/v) methanol extract of *A. calamus* showed highest deterrent effect (ODI=11.7) as compared to hexane extract of *V. negundo* (ODI=6.6). However non-significant deterrent effect on female moths was observed when leaves treated 0.625% (w/v) concentration of *A. calamus* (hexane) and *V. negundo* (methanol) extract ($t=3.69$, $df=4$, $P=0.302$ and $t=1.33$, $df=4$, $P=0.253$, respectively). Lowest ODI values of the test materials indicated less deterrent effect (Table 1). Host-plant finding by adult insects in crucifers may depend on the emanation of plant volatiles and plant acceptance may depend on glucosinolates that are perceived upon contact with the plant (Renwick, 2002; Reed *et al.*, 1989). Previous study showed that ethanolic fruit extracts of syringe plant have been shown oviposition deterrence to *P. xylostella* (Chen *et al.*, 1996). In the present, cabbage potted plants treated with methanol and hexane extract of *A. calamus* and *V. negundo* inhibits the egg

Table 1 : Oviposition by Diamondback moth, *Plutella xylostella* on cabbage leaves treated with *Acorus calamus* and *Vitex negundo* plant extracts at a concentration of 0.625% (w/v)

Plants	Plant parts	Solvent	Mean number of eggs laid (\pm SE) ^a			ODI ^c
			Treated	Control	P value ^b	
<i>Acorus calamus</i>	Rhizomes	M	238 \pm 16	301 \pm 30	0.016	11.7
<i>Acorus calamus</i>	Rhizomes	H	225 \pm 25	246 \pm 21	0.302	4.5
<i>Vitex negundo</i>	Leaves	M	174 \pm 15	190 \pm 15	0.253	4.4
<i>Vitex negundo</i>	Leaves	H	219 \pm 18	250 \pm 19	0.008	6.6

M: Methanol; H: Hexane; ^aaverage of five replication, SE=standard error; ^bstatistical test refers to t-test for pair comparison between treated and control leaves; ^cODI=oviposition deterrent index

Table 2 : Oviposition by Diamondback moth, *Plutella xylostella* on cabbage leaves treated with *Acorus calamus* and *Vitex negundo* plant extracts according to concentration

Concentration (%w/v)	Mean number of eggs laid (\pm SE) ^a			ODI ^c
	Treated	Control	P value ^b	
<i>Acorus calamus</i> (M)				
0.625	238 \pm 16	301 \pm 30	0.016	11.7
1.25	194 \pm 12	285 \pm 14	0.000	19.0
2.5	103 \pm 22	185 \pm 25	0.002	28.5
5.0	106 \pm 10	240 \pm 21	0.001	38.7
<i>Acorus calamus</i> (H)				
0.625	225 \pm 25	246 \pm 21	0.302	4.5
1.25	168 \pm 15	189 \pm 07	0.091	5.9
2.5	179 \pm 08	228 \pm 22	0.002	12.0
5.0	150 \pm 25	226 \pm 28	0.021	20.2
<i>Vitex negundo</i> (M)				
0.625	174 \pm 15	190 \pm 15	0.253	4.4
1.25	163 \pm 08	200 \pm 27	0.064	10.2
2.5	110 \pm 18	145 \pm 16	0.040	13.7
5.0	120 \pm 18	175 \pm 14	0.017	18.6
<i>Vitex negundo</i> (H)				
0.625	219 \pm 18	250 \pm 19	0.008	6.6
1.25	168 \pm 23	215 \pm 32	0.026	12.3
2.5	152 \pm 38	231 \pm 20	0.013	20.6
5.0	95 \pm 30	160 \pm 18	0.037	25.5

M: Methanol; H: Hexane; ^aaverage of five replication, SE=standard error; ^bstatistical test refers to t-test for pair comparison between treated and control leaves; ^cODI=oviposition deterrent index

Table 3: Oviposition by Diamondback moth, *Plutella xylostella* on cabbage leaves treated with *Acorus calamus* and *Vitex negundo* plant extracts (1.25 % w/v) at different days after treatment

DAT ^a	Mean number of eggs laid (\pm SE) ^a			ODI ^c
	Treated	Control	P value ^b	
<i>Acorus calamus</i> (M)				
0	194 \pm 12	285 \pm 14	0.000	19.0
1	248 \pm 18	369 \pm 06	0.000	19.6
2	206 \pm 18	265 \pm 32	0.039	12.5
3	99 \pm 15	114 \pm 11	0.088	7.0
<i>Acorus calamus</i> (H)				
0	168 \pm 15	189 \pm 07	0.091	5.9
1	188 \pm 12	207 \pm 13	0.027	4.8
2	136 \pm 16	153 \pm 11	0.055	5.9
3	91 \pm 23	98 \pm 13	0.672	3.7
<i>Vitex negundo</i> (M)				
0	163 \pm 08	200 \pm 27	0.064	10.2
1	145 \pm 10	177 \pm 15	0.029	9.9
2	159 \pm 14	138 \pm 19	0.137	-7.1
3	108 \pm 22	100 \pm 16	0.306	-3.8
<i>Vitex negundo</i> (H)				
0	168 \pm 23	215 \pm 32	0.026	12.3
1	326 \pm 14	379 \pm 12	0.005	7.5
2	173 \pm 16	197 \pm 14	0.025	6.5
3	83 \pm 06	90 \pm 11	0.219	4.0

^aDAT= days after treatment; M: Methanol; H: Hexane; ^aaverage of five replication, SE=standard error; ^bstatistical test refers to t-test for pair comparison between treated and control leaves; ^cODI=oviposition deterrent index

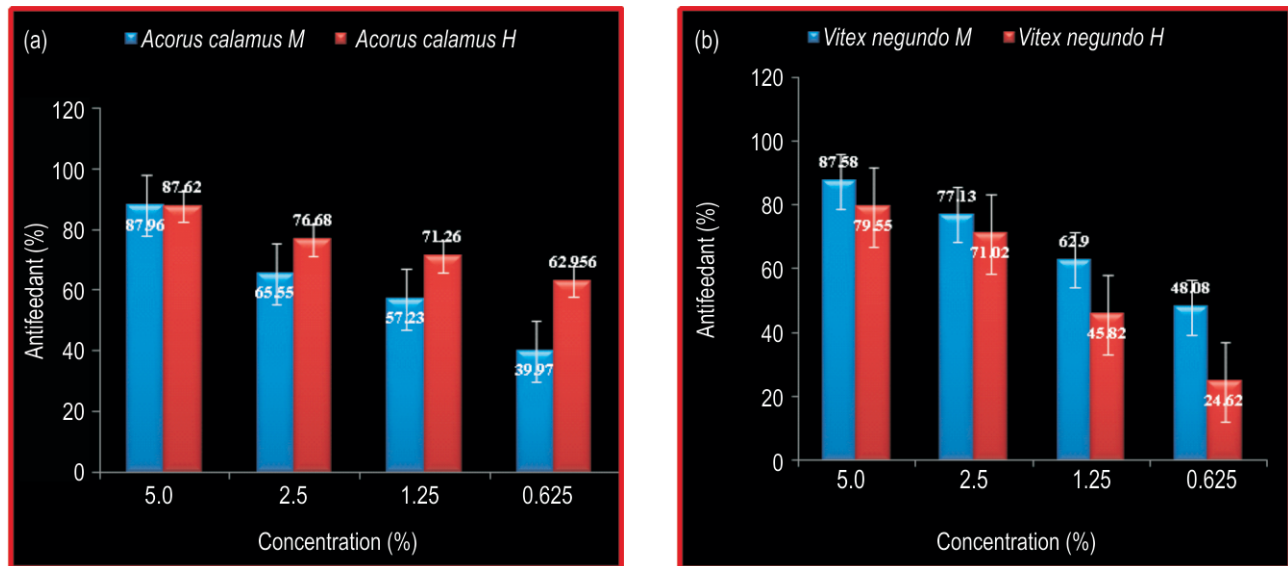


Fig. 1 : Effect on antifeedant activity against third instar larvae of *Plutella xylostella* (a) *Acorus calamus* and (b) *Vitex negundo*.

laying of the females of *P. xylostella* (Table 2). Earlier, oviposition deterrent activity of *A. calamus* and *V. negundo* to females of *P. xylostella* due to presence of active compound β -asarone (Murthy *et al.*, 2005; Liu *et al.*, 2013 and Raja *et al.*, 2009). However, *V. negundo* leaves contained an alkaloid nishidine, flavonoids like flavones, luteolin-7-glucoside, casticin, iridoid glycoside, an essential oil and other constituents like vitamin-C, carotene, benzoic acid, δ -gualene and C-glycoside (Khokra *et al.*, 2008). Feeding cabbage leaves treated with extract of *A. indica* at 0.5 per cent concentration to *P. xylostella* resulted in 50.33 per cent reduction in egg laying by this pest (Patil and Goud 2003), confirming the present study. Methanol and dichloromethane extract of *A. calamus* showed oviposition deterrent activity to *Callosobruchus maculatus* (Jayakumar *et al.*, 2005). Oviposition deterrence activity of limonoid allelochemicals and *Chrysanthemum morifolium* to *P. xylostella* were also reported by Akhtar and Isman (2003) and Liu *et al.* (2006).

The results of the present study indicated that untreated and treated leaves with lowest concentration (0.625 and 1.25 % w/v) of hexane extract of *A. calamus* showed a non-significant difference for egg laying by diamondback moth ($P=0.302$, $t=1.183$, $df=4$ and $P=0.091$, $t=2.212$, $df=4$, respectively) and *V. negundo* methanol extract ($P=0.253$, $t=1.334$, $df=4$ and $P=0.064$, $t=2.546$, $df=4$). The ODI values at 0.625 % concentration were 4.5 and 4.4 for *A. calamus* hexane and *V. negundo* methanol extract, respectively. Female of diamondback moth laid significantly lower number of eggs at higher concentration on cabbage leaves treated with hexane and methanol extract of *A. calamus* and *V. negundo*, respectively.

As the concentration of *A. calamus* and *V. negundo* extract increased, an increase in ODI value was also observed

(Table 2). At the highest concentration none of the plant extract completely deterred the oviposition by diamondback moth. Methanol and hexane extract of *A. calamus* and *V. negundo* at a concentration of 5.0% (w/v) showed 38.7 and 25.5 ODI, respectively. Results from the present study showed that *P. xylostella* larvae were able to detect the *A. calamus* and *V. negundo* extract. At high doses these botanical insecticides also had a significant impact on the behaviour of larvae. Our results collaborate with the findings of Rana *et al.* (2013) who also observed that increase in concentration of extract of *Artemisia annua* resulted in decrease in egg laying of *Callosobruchus chinensis*. Moreover, Prathibha *et al.* (2014) also observed that as the concentration of *Eugenia jambolana* increased from 20-100 % the mean oviposition deterrent activity increased to *Culex quinquefasciatus*. The results of this study collaborate with the findings of Adesina and Ofuya (2015) who observed increase in concentration of methanol leaf extract of *Secamone afzelii* resulted in reduction in number of egg laying from 51.61 to 30.62. The present results revealed that cabbage plant treated with methanol extract of *A. calamus* and hexane extract of *V. negundo* deterred the females of *P. xylostella* to lay eggs in a concentration-dependent manner.

The leaves treated with hexane extract of *V. negundo* showed a decline oviposition deterrent effect as ODI value decreased from 12.3 at 0 day to 4.0 at 3 day, however hexane and methanol extract of *A. calamus* and *V. negundo* showed relatively less change in ODI values. However, oviposition deterrence of *A. calamus* methanol extract increased after first day then decreased with time. A significant differences were evident in the number of eggs laid by diamondback moth on leaves treated with methanol extract of *A. calamus* and control leaves (first day, $t=18.92$, $df=4$, $P=0.00$; second day, $t=3.424$,

df=4, $P=0.039$), while on third day no significant differences were found ($P=0.088$, df=4, $t=2.247$). Adult females laid significantly different number of eggs on leaves treated with *V. negundo* hexane extract and control leaves (first day, $t=5.455$, df=4, $P=0.005$; second day, $t=4.92$, df=4, $P=0.025$) (Table 3). A non significant difference in egg laying in both treatments and control were observed with *A. calamus* hexane and *V. negundo* methanol extract at 0, 2 and 3 DAT, however on the first day there was significant difference in egg laying. Leaves treated with *A. calamus* and *V. negundo* showed different ODI values that tended to decline with increasing time (0 to 3 DAT). Highest residual activity of methanol extract of *A. calamus* was observed at 3 DAT, which maybe due to its low volatility, but varied residual activity given by hexane extract of *A. calamus*. In contrast, the effect of unstable compound of *V. negundo* decreased with increasing time (0 to 3 DAT) (Table 3). Patil *et al.* (2003) reported the mean egg laying by *P. xylostella* decreased from 56.7 to 32.66 on plant surface treated with *A. calamus* (aqueous extract) as the time increased from 0 to 3 days. Similarly, Basukriadi and Wilkins (2014) also reported the residual effect of yam bean seed extract (1%) as egg laying decreased from 1 to 3 days from 77 to 59 of *P. xylostella*. Plant extracts having nonvolatile properties are more useful compounds for oviposition deterrent (Renwick, 1988).

In the present study, the antifeedant activity varied significantly based on the solvents used for extraction. Antifeedant effects of different plant extracts were evaluated based on leaf area consumed by larvae of *P. xylostella*. Among the two plant extracts, methanol and hexane extract the *A. calamus* (5%) concentration showed 87.96 and 87.68 % antifeedant activity to *P. xylostella*, respectively. However, among the plant extract of *V. negundo* the maximum antifeedant activity was with methanol extract (87.58%) as compared to hexane extract (79.55%) at 5% concentration. Antifeedant activity of both plants extracts decreased with the decrease in concentration (Fig. 1). During the present investigation, it was observed that larvae highly preferred to feed on control cabbage leaf and less leaf area was consumed on the treated cabbage leaf. The present investigation is in agreement with Murthy *et al.* (2005) who reported that mustard leaves treated with aqueous extract (2.5%) of *A. calamus*, *V. negundo* and *Strychnos nux-vomica* L., when fed to larvae reduced feeding of *P. xylostella*. Leaf disc of *Brassica oleracea* L. when treated with aqueous extract of *Vitex trifolia* L., *A. calamus* and *Lantana camara* L gave protection against third instar larvae of *P. xylostella* (Hemchandra and Singh, 2006; Sharma *et al.*, 2001). Similarly, antifeedant activities of essential oils of *A. calamus* were also reported against lepidopteran pest by Melani *et al.* (2016).

The results of the present study reveals that cabbage plants treated with methanol and hexane extracts of *A. calamus* and *V. negundo* showed oviposition deterrent and antifeedant

activity to *P. xylostella*. So, both the plant extracts may be useful for the management of this pest under real field conditions.

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