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EFFECT OF CHLOROFORM EXTRACTS OF *VINCA ROSEA* AND *CALLISTEMON LANCEOLATUS* ON FEEDING BEHAVIOUR OF *HELICOVERPA ARMIGERA* HUB.

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Summary

The chloroform extracts of *Callistemon lanceolatus* and *Vinca rosea* alone and their different mixtures (*viz*., 1:1, 1:3 and 3:1) were tested on feeding against third instar larvae of *Helicoverpa armigera* Hub. by leaf dip method under laboratory conditions. The results revealed that, out of these two plants tested highest antifeedant activity was observed after 24 hr in chloroform extract of *V. rosea* (5270 ppm) followed by *C. lanceolatus* (5605 ppm). Among the various mixtures tested 3:1 ratio of both the plants (*C. lanceolatus* and *V. rosea,* AI_{50} = 1216 ppm) showed highest antifeedant activity followed by 1:1 (3044 ppm) and 1:3 (5354 ppm).

सारांश

कैलिस्टोमान लैन्सियोंलेटस तथा विन्का रोजिया का अकेले तथा उनके विभिन्न मिश्रण (1:1, 1:3 व 3:1 अनुपात) का परिक्षण हेलियोथिस अर्मीजेरा के सूंड़ी का तृतीय सूंड़ी अवस्था का खानें के व्यवहार पर पत्ती डुबोकर प्रयोगशाला में किया गया। परिक्षण में पाया गया कि इन दोनों के अलावा दो पौधों का खाद्य प्रतिकार व्यवहार के प्रति 24 घण्टों तक क्लोरोफार्म जो विन्का रोजिया (5270 पी.पी.एम.) से अच्छा रहा तथा इसके बाद कैलिस्टोमान लैंसियोलैटस (5605 पी.पी.एम.) का प्रभाव रहा। विभिन्न मिश्रण के परिक्षण में 3:1 अनुपात शोधित दोनों पौधों पर (कैलिस्टोमान लैसियोलैटस व विन्का रोजिया ए.आई.$_{50}$=1216 पी.पी.एम.) में सबसे ज्यादा खाद्य प्रतिकार व्यवहार रहा जबकि इसके बाद 1:1 के अनुपात (3044 पी.पी.एम.) तथा 1:3 के अनुपात (5354 पी.पी.एम.) के प्रयोग में पाया गया।

Introduction

The gram pod borer, *Helicoverpa armigera* (Hub.) is an International pest because of its polyphagous and multigenerational nature as well as high damage potential covering more than 182 species of plants belonging to 47 botanical families (Russell *et al*., 1998). To control this pest presently many synthetic insecticides are being widely used which can leave potentially toxic residues in food products and can be deleterious to non-target organisms in the environment (Isman, 2006). In search of alternative methods, plant-derived extracts and phytochemicals have been intensively investigated for the past 30 years in an effort to develop alternatives to conventional insecticides with reduced health and environmental impacts. Such phytochemicals are secondary plant metabolites and include phenolics, alkaloids, terpenoids, flavonoids and acteogenins (Parmar and Singh., 1993). Jointly or independently they may contribute to the protection of plants against herbivores. It has been well recognized that plant-derived antifeedants could be developed into products suitable for IPM. Much concern has been focused, therefore, on the distribution, nature and practical use of chemical substances having the antifeeding activity for insects in plants. Over 2,000 plant species including neem have been reported to possess biological activity against different type of insects. Amongst these, two indigenous plants *viz*., sadabahar or Periwinkle (*Vinca rosea* L., Family: - Apocynaceae) and bottle brush (*Callistemon lanceolatus* DC., family:- Myrtaceae) that are widely grown in many parts of the country are well known for its medicinal properties. However, very little efforts were made to explore the pesticidal properties of these duo plants.

Materials and Methods

Leaf collection, processing and extraction**:** Green fresh leaves of *V.rosea* and *C.lanceolatus* were collected from Diamond Harbour, District- South 24 Parganas, West Bengal and Indian Agricultural Research Institute (I.A.R.I.) New Delhi, India, respectively shade dried for one week and then ground to powder. Finely ground plant material (500 gm) of respective species were dipped separately into 2.5 lit chloroform in round bottom flasks and kept 24 hours. The supernatant was filtered through a Whatman No.1 filter paper and the process was repeated twice. The pooled extracts was subjected to vacuum distillation at 54^0 C to obtain blackish chloroform extract which was kept under room temperature to get the solid chloroform extracts. Maintenance of Insect culture**:** A stock culture of *H. armigera* was maintained on artificial diet (Singh, 1983) under laboratory condition having controlled environment, *i.e.* 27 ± 1° C temperature and 70 ± 5 % relative humidity, a photo phase of 14 hr and scoto phase of 10 hr. The rearing lab was exposed once every month to germicidal lamp (UV lamp) for 12 hr to prevent microbial infection. Feeding bioassay**:** The third instar larvae of *H. armigera* weighing 30-40 mg obtained from the culture were exposed to botanical extracts on cabbage leaves (Kathuria and Kaushik, 2005). Leaf discs of 4 cm diameter were cut from cabbage leaves. After washing the leaf discs they were dipped in the different concentrations of respective extracts for twenty seconds and then air-dried. The treated discs were then transferred individually to clean petri plate (8 cm x 1.5 cm) and one 7 ± 1day old larvae were placed in each petri plate. Each treatment and control was replicated twenty times. The treated larvae were observed daily and area of leaves fed by larvae on 1 day after treatment (DAT) was measured with leaf area meter for per cent antifeedance calculation. The per cent antifeedance was calculated using the following Abbott's modified formula (1925) as under:

$$\text{\% leaves protection} = \frac{\text{Leaf area given (cm}^2\text{)} - \text{Leaf area consumed (cm}^2\text{)}}{\text{Leaf area given (cm}^2\text{)}} \times 100$$

Table 1. Antifeedant effect of chloroform extract of *C. lanceolatus* and *V. rosea* and their various ratios against *H. armigera* by leaf dip method

Conc. (ppm)	Per cent antifeedance				
	C. lanceolatus	V. rosea	1:1	1:3	3:1
5000	44.88 ± 2.59 g	45.52 ± 4.11 h	53.14 ± 4.59 f	42.84 ± 4.26 e	61.62 ± 4.59 g
3000	40.72 ± 2.57 f	38.54 ± 2.59 g	46.34 ± 3.65 e	36.74 ± 3.67 d	52.50 ± 4.16 f
1000	36.97 ± 1.57 e	35.63 ± 2.57 f	40.99 ± 4.15 d	33.33 ± 2.69 d	46.02 ± 3.65 e
700	28.78 ± 1.50 d	28.57 ± 1.26 e	38.79 ± 2.47 d	21.30 ± 2.58 c	39.91 ± 2.57 d
500	21.21 ± 0.88 c	21.57 ± 1.58 d	30.18 ± 3.49 c	15.07 ± 1.15 b	37.08 ± 3.15 d
300	12.14 ± 1.25 b	12.96 ± 0.69 c	23.56 ± 2.15 b	13.26 ± 0.84 b	29.68 ± 2.48 c
100	10.44 ± 0.89 b	8.56 ± 0.49 b	13.28 ± 1.89 a	10.31 ± 1.16 b	22.96 ± 1.67 b
70	5.49 ± 0.45 a	4.26 ± 0.33 a	10.56 ± 0.98 a	3.06 ± 0.11 a	13.26 ± 0.68 a
Cont.	–	–	–	–	–
CD (0.5%)	2.75	2.38	2.11	2.89	3.28

$$\text{\%Antifeedance} = \frac{\text{\% leaves protection in treatment - \% leaves protection in control}}{100 - \text{\%leaves protection in control}} \times 100$$

The data recorded for various parameters was subjected to probit analysis for the calculation of AI_{50} by using GWBASIC and AGRES software program.

Results and Discussion

The chloroform extracts of both the plants as well as their mixture at various ratios (1:1, 1:3 and 3:1), significantly deterred feeding by larvae. The per cent reduction of feeding was dose dependent and with various extracts and their mixtures. The per cent antifeedance was maximum of 45.52 at 5000 ppm was significantly different from others (Table 1) in case of chloroform extract of *V. rosea*. At other doses the per cent antifeedance ranged between 38.56 to 4.26. The data presented in Table 1 also showed that chloroform extract of *C. lanceolatus* was least effective among these two plants and per cent antifeedancy was 44.88, 40.72, 36.92, 28.78, 21.21, 12.14, 10.44 and 5.49 at 5000, 3000, 1000, 700, 500, 300, 100 and 70 ppm concentration respectively. Out of the three mixtures tested 3:1 ratio (*C. lanceolatus*: *V. rosea*) documented highest antifeedancy and mean leaf area consumed decreased with increased concentration and ranged between 61.62 - 13.26 per cent followed by 1:1 (53.14 - 10.56 %) and 1:3 (42.84 - 3.06 %). It is also evident that antifeedancy of 3:1 mixture of *C. lanceolatus* and *V. rosea* (AI_{50} = 1216 ppm) almost at par with the Neem oil (1050 ppm) but 5.26 times higher than the NSKE (5520 ppm) and in contrast all the other extracts and their mixtures were lower than the neem oil. How ever, chloroform extract of *V.rosea* showed higher antifeedancy than the NSKE even after 24 hours of the experiments. Interestingly, all the mixtures tested against 7±1 day old larvae of *H. armigera* were proved more effective then NSKE. Based on the AI_{50} values presented in the table 2 the ascending order of antifeedance of various chloroform extracts and their mixture at various ratios was *C. lanceolatus* (5605 ppm) > *V. rosea* (5270 ppm) > 1:3 (5258 ppm) > 1:1 (3044 ppm) > 3:1 (1216 ppm).

In East Asia, *V. rosea* has long been considered to have natural medicinal properties, because it's rich in alkaloids such as vincristine, vincaleukoblastine, ajmalicine, raubacine and reserpine (Kumar *et al.*, 2004). Very little work has been done to manage insect pests, although it has been reported that *Vinca* leaf-derived materials mainly alkaloids have antibacterial

(Nyein *et al*., 1999), antifungal (Kshirsagar *et al.,* 2004) and nematicidal activity (Alagumalai *et al*., 1991). Recently, Patil and Goud (2003) reported that chloroform extracts of *V. rosea* serve as strong ovipositional repellent and egg hatch inhibitor against diamond back moth, *Plutella xylostella*. In our study, chloroform leaf extract of *V. rosea* has potent antifeedant activity against third instar larvae of *H. armigera*. Earlier, Rajendran and Gopalan (1980) reported that leaf extracts from *Catharanthus (Vinca) roseus* affected the normal growth, development and moulting of *Dysdercus cingulatus* resulting the insects had different combinations of nymphal characters. The leaf extracts of *C. roseus* produced a maximum of 47.5 % adultoids at 120/ìg/nymph. Sharma *et al*. 2001 observed the insecticidal, antifeedant and growth inhibitory activities of the leaf essential oils of *Callistemon lanceolatus* (*Callistemon citrinus*) against third instar larvae of *Spodoptera litura*. At 10% concentration *Callistemon lanceolatus* caused 100% deterrence of feeding, and after 4 days of exposure of the larvae *C. lanceolatus* treated food, larval weight gain was lower than in the control. Kaushik (1999) reported that diet having the crude leaf powder of *C. lanceolatus* leads to slow growth and development in *H. armigera* larvae. The larvae could not survive beyond L_3 stage and per cent survival was only 20% at pre-pupal stage. Mixtures of plant extracts were proven effective in controlling many insect pests. Chowdhury *et al*., 2001 reported that the activity of azadirachtin was considerably enhanced by incorporation of different concentrations of turmeric oil in the mixtures which were attributed to the possible additive, synergistic and/or stabilizing effect of turmeric oil. In our present study also 3:1 ratio was found to be most effective than 1:3 and 1:1. Many botanical insecticides including azadirachtin is having diverse mode of action. At higher concentration (75 %) of chloroform extract of *Callistemon* in mixture might be blocking the other side of action or inhibiting the ion channels where probably *Vinca* acts upon or crude chloroform extract containing number of pure active compounds. Higher concentration of one compound might be showing antagonistic effect to other compounds present in the mixture that might be the reason for lower activity of 1:3 and 1:1 mixtures.

Table 2. Comparative efficacy of chloroform extract of *C. lanceolatus* and *V. rosea* alone and their various combianations on feeding activity against *H. armigera* by leaf dip method

Extracts	$\chi2$ (df=6)	Regression Equation Y=	AI_{50} (ppm)	Fiducial limits
C. lanceolatus	8.481	5.198+0.791X	5605	3961-7931
V. rosea	2.012	5.219+0.787X	5270	3183-8726
1:1	3.508	5.377+0.730X	3044	1967-4706
1:3	5.758	5.212+0.760X	5258	3701-7470
3:1	6.530	5.701+0.765X	1216	898-1647
Neem oil	2.927	6.359 + 1.331 X	1050	620 - 1770
NSKE	3.545	6.158 + 0.665 X	5520	3900 - 7810

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