

## Effect of methanolic extracts of periwinkle (*Vinca rosea*) and bottlebrush (*Callistemon lanceolatus*) alone and their mixtures against neonate larvae of gram pod borer (*Helicoverpa armigera*)

JAYDEEP HALDER<sup>1</sup>, CHITRA SRIVASTAVA<sup>2</sup> and PREM DUREJA<sup>3</sup>

Indian Agricultural Research Institute, New Delhi 110 012

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### ABSTRACT

The insect growth regulatory, cumulative larval mortality and adult inhibitory activity of methanolic extracts of 'Sadabahar' (*Vinca rosea* L.) and bottle brush (*Callistemon lanceolatus* L.) alone and their mixtures at different ratios were evaluated against the neonate larvae of *Helicoverpa armigera* (Hubner) by artificial diet incorporation method. It has been recorded that highest growth regulatory activity (GI<sub>50</sub>) was noticed at 1 : 3 mixture both in 7 and 9 days of treatment (62, 69 ppm), followed by 1 : 1 (70, 77 ppm) and 3:1 (81, 83 ppm). Same trend was also observed for cumulative larval mortality (LC<sub>50</sub>) and adult inhibitory activity (I<sub>50</sub>) and the values were 80, 101, 127 and 36, 40, 47 ppm, respectively. Incorporation of both extracts and their mixtures at different ratios into the artificial diet significantly reduced the larval growth as compared to control. Among the two plants tested, *V. rosea* was showed higher growth regulatory activity and larval toxicity than *C.lanceolatus*. However, the adult inhibitory activity was more in *C.lanceolatus* (72 ppm) than *V. rosea* (76 ppm).

**Key words:** *Callistemon lanceolatus*, Methanolic extracts, Mixtures, *H. armigera*, *Vinca rosea*

The gram pod borer [*Helicoverpa armigera* (Hubner)] being highly polyphagous, collectively attacks a wide range of food, fibre, oil and fodder crops as well as many horticultural and ornamental crops. In fact, polyphagy, high mobility, high fecundity and facultative diapause are key physiological, behavioural and ecological characteristics facilitate survival of *Helicoverpa* species even in unstable habitats. Outside the range of its normal distribution, its outbreak/damage has been reported from Hungary, Sicily, Romania, Slovakia, Spain, Sweden, Switzerland and United Kingdom. Globally, this pest causes yield loss worth about US\$ 2 billion annually (ICRISAT 2003). Gram pod borer (*H. armigera*) is the most dreaded and polyphagous pest and causes estimated US\$ 927 million loss in pigeonpea (*Cajanus cajan* L. Mill sp.) and chickpea (*Cicer arietinum* L.) (Sharma 2001). Recently to control this polyphagous, polymorphous pest of many neurotoxic insecticides are

widely in use, which are damaging to the environment and/or pose a threat to public health via food residues, ground water contamination or accidental exposure. The adverse effects of synthetic pesticides have amplified the need for effective, biodegradable pesticides with greater selectivity. Alternative strategies have included the investigation for new type of insecticides and use of traditional botanical pest control agents.

Pesticides derived from plants have the potential to play a major role in pest management in sustainable agricultural production. They are renewable, non-persistent in the environment and relatively safe to natural enemies, non-target organisms and human beings. Plants produce a range of chemical substances to protect themselves from insect pests. Such chemicals are secondary plant metabolites and include alkaloids, terpenoids, flavonoids and acetogenins. Approximately >350 compounds are insecticidal and more than 900 isolates are feeding deterrents alone (Koul 2005) and a quite a good number of insect growth inhibitors and growth regulators have been isolated from various plant species (Dhaliwal and Koul 2007). Over 2500 plant species including neem have been reported to possess biological activity against different type of insects. Amongst these, sadabahar or Periwinkle (*Vinca rosea* L.) and bottle brush (*Callistemon lanceolatus* DC, Myrtaceae) have been the

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<sup>1</sup>Scientist, (e mail: jaydeep.halder@gmail.com); IIVR Varanasi

<sup>2</sup>Principal scientist, (e mail: chitrasriv@gmail.com); Division of Entomology

<sup>3</sup>Emiratus Scientist (e mail: pd\_dureja@yahoo.com); Division of Agricultural Chemicals

focus of a large number of studies over the decades. The methanolic crude leaf extract of *V.rosea* produces maximum 47.5% adultoids at 120 µg/nymph. The longevity of malformed adults was 7.28 days at 180 µg compared to 20.14 days in the control. Sharma *et al.* (2001) reported that when larvae of *S. litura* were exposed to *C. lanceolatus* treated food for 4 days, larval weight was lower than that of control. Keeping all these in mind the present study was undertaken to evaluate the methanolic extracts of these two plants alone and their different mixtures, viz 1 : 1, 1 : 3 and 3 : 1 against neonate larvae of *H. armigera* by artificial diet incorporation method.

## MATERIALS AND METHODS

### Leaf collection and processing

To conduct the experiments in 2007 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, New Delhi respectively and washed thoroughly and then shade-dried for one week. Dried leaves were powdered and used for extraction with methanol. Finely grounded plant material (500 g) dipped in 2.5 litre methanol was kept for 24 hr in a round bottom flask. The supernatant was filtered and solute was extracted thrice

Table 1 Relative effect of methanol extracts of *Vinca rosea* and *Callistemon. lanceolatus* alone and their mixtures on larval toxicity, growth and development of neonate larvae of *Helicoverpa armigera* by artificial diet incorporation method

Extract	Bioactivity	Heterogeneity $\chi^2$ (df=6)	Regression equation Y=	Lethal concentration (fiducial limits) (ppm)
<i>V. rosea</i>	IGR *(7 DAT)	2.733	3.498+0.765X	92 (67–124)
	IGR*(9 DAT)	3.179	3.432+0.776x	105 (78–140)
	CLM (LC <sub>50</sub> )	2.273	3.153+0.816x	18 (142–237)
	NAI (I <sub>50</sub> )	3.568	2.774+0.182x	76 (61–94)
<i>C. lanceolatus</i>	IGR*(7 DAT)	2.290	3.441+0.751x	119 (89–159)
	IGR*(9 DAT)	3.379	3.237+0.701x	129 (98–169)
	CLM (LC <sub>50</sub> )	1.895	3.253+0.824x	189 (101–170)
	NAI (I <sub>50</sub> )	4.227	3.054+1.046x	72 (56–92)
1 : 1 mixture	IGR *(7 DAT)	3.012	3.512–0.806x	70 (51–96)
	IGR *(9 DAT)	3.709	3.439+.828x	77 (57–103)
	CLM (LC <sub>50</sub> )	2.416	3.420+0.787x	101 (76–134)
	NAI (I <sub>50</sub> )	9.601	3.176+1.134x	40 (30–54)
1 : 3 mixture	IGR*(7 DAT)	1.535	3.581+0.792x	62 (44–86)
	IGR *(9 DAT)	2.810	3.486+0.822x	69 (50–95)
	CLM (LC <sub>50</sub> )	2.795	3.488+0.795x	80 (58–108)
	NAI (I <sub>50</sub> )	5.992	3.338+1.061x	36 (26–50)
3 : 1 mixture	IGR*(7 DAT)	2.670	3.539+0.765x	81 (59–111)
	IGR*(9 DAT)	2.223	3.519+0.772x	83 (61–113)
	CLM(LC <sub>50</sub> )	2.818	3.241+0.835x	127 (98–164)
	NAI (I <sub>50</sub> )	2.818	3.241+0.835x	127 (98–164)

In none of the case, the data were found to be significantly heterogeneous at  $P=0.05$ , IGR, Insect growth regulatory activity; CLM, cumulative larval mortality; LC<sub>50</sub>, median lethal concentration; NAI, normal adult emergence inhibition; I<sub>50</sub>, concentration calculated to give 50% normal adult emergence inhibition

Table 2 Comparative efficacy of neem seed kernal extract (NSKE) and extracts of leaves of *Callistemon. lanceolatus* and *Vinca rosea* at various ratio against neonate larvae of *H. armigera* by artificial diet incorporation method

Treatment	*GI <sub>50</sub> 7DAT	RelativeGI <sub>50</sub>	GI <sub>50</sub> 9 DAT	RelativeGI <sub>50</sub>	LC <sub>50</sub>	RelativeLC <sub>50</sub>	I <sub>50</sub>	RelativeI <sub>50</sub>
<i>Individual compound</i>								
NSKE	286	1	315	1	223	1	247	1
<i>C. lanceolatus</i>	119	2.38	129	2.44	189	1.18	72	3.43
<i>V. rosea</i>	92	3.11	105	3.00	183	1.22	76	3.25
<i>Mixtures (C. lanceolatus: V. rosea)</i>								
1:1	70	4.09	77	4.09	101	2.20	40	6.18
1:3	62	4.61	69	4.57	80	2.79	36	6.86
3:1	81	3.53	83	3.80	127	1.76	47	5.26

\*GI<sub>50</sub>, concentration calculated to give 50% larval weight reduction

similarly. The pooled extract was subjected to vacuum distillation at 54°C to obtain blackish methanol extract which kept under room temperature to get the solid methanol extract (23.73 g).

A continuous culture of *H. armigera* was maintained in an insectary having controlled environment room 27±1°C temperature and 70±5% relative humidity, a photo phase of 14 hr and scoto phase of 10 hr. The insectary was exposed once every month to germicidal lamp (UV lamp) for 12 hr to prevent microbial infection.

The neonate larvae were reared on the chickpea-based semi-synthetic diet as reported by Singh (1983) with slight modification. The extracts were weighed accordingly and mixed in diet ingredients and stirred thoroughly by using a mini electric blender. For preparing mixtures, crude extracts were weighed and mixed each other at different ratios (1 : 1, 1 : 3 and 3 : 1) and then incorporated with artificial diet. The treated diet discs (20 mm diameter and 10 mm thick) were transferred individually to clean Petri-dishes (8 cm × 1.5 cm). Each treatment and control with treated and normal diet respectively, replicated 30 times and one (7± 1-day-old) larvae were placed in each Petri-dish. Per cent larval growth reduction was calculated (Abbott 1925) and data transformed to arcsine were analyzed statistically by using completely randomized block design. The means were separated using the Duncan's multiple range test (DMRT) (Gomez and Gomez 1984) and were compared at 5% level of significance. The analyses were carried out using IRRI-STAT software package.

The data recorded for various parameters was subjected to probit analysis for the calculation of LC<sub>50</sub>, GI<sub>50</sub> and I<sub>50</sub> by using GWBASIC and Agres software programme.

## RESULTS AND DISCUSSION

Incorporation of methanolic extract of *C. lanceolatus* and *V. rosea* leaves and their mixtures at different ratios into the artificial diet significantly reduced the larval growth as compared to control. The growth inhibition (GI<sub>50</sub>) values of methanolic extracts of *C. lanceolatus* and *V. rosea* leaves alone and their mixture at 1 : 1, 1 : 3 and 3 : 1 ratio, were recorded after 7 and 9 days of treatment as 119 and 129, 92 and 105, 70 and 77, 62 and 69, 81 and 83 ppm, respectively (Table 1). The larvae that fed on the treated diet with methanolic extracts of *C. lanceolatus* and *V. rosea* leaves alone and their mixtures at 1 : 1, 1 : 3 and 3 : 1 ratios of both the plants required 41.68, 43.21, 47.16, 48.89, 44.47 and 20.61, 22.68, 25.23, 26.84, 24.81% additional days over the control (ADOC) to reach pupal and adult stage respectively (Table 3). Results showed that all the methanolic extracts and their mixtures at different ratios were superior than the neem seed kernel extract when tested against neonate larvae of *H. armigera* by artificial diet incorporation method. Our present study is also in conformity with the findings of Jaiswal and Srivastava (1993). They further reported that

Table 3 Effect of various extracts of *V. rosea* and *C. lanceolatus* alone and their mixtures on the larval and pupal duration of *H. armigera* by artificial diet incorporation method

Stage	Plant material	Duration (days)			
		Average	Control	ADOC	% ADOC
Larval	<i>C. lanceolatus</i>	31.00	25.63	5.37	41.68
	<i>V. rosea</i>	31.88	26.22	5.66	43.21
	1:1	34.50	26.84	7.66	47.16
	1:3	35.63	27.13	8.50	48.89
	3:1	32.75	26.36	6.39	44.47
	Neem oil	34.13	27.22	6.91	46.89
Pupal	<i>C. lanceolatus</i>	17.63	14.46	3.17	20.61
	<i>V. rosea</i>	19.13	15.67	3.46	22.68
	1:1	21.63	14.26	7.37	25.23
	1:3	22.75	15.24	7.51	26.84
	3:1	21.25	14.36	6.89	24.81
	Neem oil	20.88	15.69	5.19	24.77

Per cent additional days over control (% ADOC) = [Larval/pupal duration in treatment/100-Larval/pupal duration in control] × 100

leaf and stem extracts of *Callistemon lanceolatus* bio assayed against cotton stainer, *Dysdercus cingulatus* revealed the presence of juvenile hormone type of IGR activity by producing crumpled wings (so unable to fly) and 3 segmented tarsi. This is a strong indication that members of family Myrtaceae are a good source for IGR.

Based on LC<sub>50</sub> value results showed that all the extracts were superior than the neem seed kernel extract. The LC<sub>50</sub> values of methanolic extracts of *C. lanceolatus* and *V. rosea* leaves and their mixtures at the ratios of 1 : 1, 1 : 3 and 3 : 1 were 189, 183, 101, 80, 127 ppm, respectively and when compared with neem seed kernal extract it was 1.18, 1.22, 2.20, 2.79 and 1.76 times higher than the latter. Sreenivasan *et al.* (2003) evaluated the efficacy of the aqueous leaf extract of *V. rosea* (*Catharanthus roseus*) and recorded 29.2% mortality decrease in growth of *P. xylostella* under laboratory condition. Mohsen *et al.* (2005) also reported antimicrobial and insecticidal activity after evaluating the extracts of the ornamental tree *C. lanceolatus* [*C. citrinus*] against late third instar larvae of the urban mosquito *C. quinquefasciatus*. They observed that 10–1000 ppm treatment of this crude extract, resulted larval and cumulative (larval + pupal + adult) mortalities increased from 8% to 13–84% and 27–100%, respectively. Delayed insect growth regulating activity was also noticed in the fourth instar larvae of the progeny of surviving mosquitoes.

In the present study both the methanolic extracts and their different mixtures significantly reduced the normal adult emergence. Mixture at 1 : 3 ratio was most effective as caused 50% inhibition of normal adult emergence at 36 ppm concentration. All the methanolic extracts and their mixtures were more effective as compared to neem oil.

Various compounds including phenolics, terpenoids and alkaloids exist in plants. Jointly or independently they may contribute to the protection of plants against herbivores, although some herbivores have counter adapted to them. It has been well recognized that plant-derived insect growth regulators could be developed into products suitable for integrated pest management. Much concern has been focused, therefore, on the distribution, nature and practical use of chemical substances having the growth regulating activity for insects in plants. Among them, most potent insect growth regulators are quinone and alkaloids. In East Asia, *V. rosea* has long been considered to have natural medicinal properties, because it is rich in alkaloids, such as vincristine, vincalukoblastine, 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, antifungal (Kshirsagar *et al.* 2004) and nematocidal activity. At 10% concentration *C. lanceolatus* caused 100% feeding deterrence of feeding, and after 4 days exposure of the larvae *C. lanceolatus* to treated food, larval weight gain was lower than in the control. 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. Among the 3 different proportions (1 : 1, 2 : 1 and 3 : 1), azadirachtin-turmeric oil (1 : 1) mixtures recorded pronounced IGR activity ( $EC_{50}$   $1.26 \times 10^{-2} / 2.16 \times 10^{-2}\%$ ) and considerable antifeedant activity ( $EC_{50}$   $2.90^{-2} \times 10 / 1.0 \times 10^{-2}\%$ ) against third and fifth instar larvae, respectively. The apparent enhancement in activity of azadirachtin-turmeric oil mixtures were attributed to the possible additive, synergistic and/or stabilizing effect of turmeric oil. In our present study also 1 : 3 ratio was found to be most effective than 3 : 1 and 1 : 1. Many botanical insecticides including azadirachtin is having diverse mode of action. At higher concentration (75%) of methanolic extract of *Vinca* in mixture might be blocking the other side of action or inhibiting the ion channels.

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