Herbal Preparations Of *Ageratum Conyzoides* And *Nerium Indicum* As Potential Insecticides Against *Henosepilachna Vigintioctopunctata*, A Polyphagous Pest Of Brinjal

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Abstract
In the present study, solvent guided leaf extracts of *Ageratum conyzoides* (Goat weed) and *Nerium indicum* (Pink kaner) were evaluated on the Ist instar larvae of *Epilachna 28 punctata* Fab. to observe its growth inhibition, larval mortality, developmental period and adult emergence. 1% leaves extracts of both *A. conyzoides* and *N. indicum* showed 100 and 93.3±3.3% larval mortality respectively till emergence. Whereas 0.2 and 0.1% of *A. conyzoides* extract had shown only 33.3±6.7% larval mortality and that of *N. indicum* extract exhibited only 22.2±2.6 and 16.7±6.7% mortality when compared to control (13.9±4.6%). The leaf extract of *A. conyzoides* at 0.5% significantly increased the total developmental period (27.4±1.4 days) in contrast to control (22.8±1.3 days) and reduced adult emergence (40±5.2%). The LC_{50} values of both the extracts had also been determined. During the development of hadda beetle, diapause condition and deformed adults were also noticed.

Keywords: Ageratum conyzoides, Brinjal, Extracts, Henosepilachna vigintioctopunctata, Mortality, Nerium indicum

Introduction

Brinjal also known as eggplant (*Solanum melongena* Linn.) is one of the most important vegetable crops grown in India and apart. It has diversified problems of sucking, leaf feeding and borer pests from seedling to fruiting stage. It is severely assaulted by *Henosepilachna vigintioctopunctata* (Fabr.) (Coleoptera: Coccinellidae) which is one of the most destructive pests extensively found all over country and in other parts of the world (Rahaman et al., 2008; Sharma and Saxena, 2012; Wang et al., 2017). It is a polyphagous pest which shows its appearance on brinjal and other solanaceous and cucurbitaceous crops of economic significance. The grubs and adults of *H. vigintioctopunctata* cause heavy damage to this crop. It had been a long dream of biologists to prevent the pest population by inhibiting their growth, moulting and developmental process. But the large scale dependence on insecticide oriented approaches as well as their successive use in the management of hadda beetle has resulted into the environmental pollution, residual toxicity in fruits, vegetables, development of insecticide resistance, decreasing efficacy of the chemicals, etc. (Kannaiyan, 2002; Kumar et al., 2013).

Due to various problems generated by indiscriminate application of insecticides, there is an urgent need to develop ecofriendly pest-specific alternatives with different modes of action for the control of *H. vigintioctopunctata*. A number of plant products or phytochemicals with a numerous properties of pest control interest such as; insecticidal, antifeedant, repellent, growth inhibitory, chitin inhibitor attracted the attention of scientists to develop an ecofriendly pest control programme to minimize the excessive application of synthetic insecticides in agriculture (Ghosh and Chakraborty, 2012; Ghosh, 2020). In the process, a number of researchers have worked to identify plant species having larvicidal, antifeedant, reproductive and growth inhibitory properties against different insects (Swaminathan et al., 2010; Jeyasankar et al., 2014; Kalaiyarasi and Ananthi, 2015). Keeping view in mind, the present study was conducted to develop a biocontrol schedule by using methanolic leaf extracts from *Ageratum conyzoides* (Goat weed) and *Nerium indicum* (Pink kaner) to save the crop from this pest ravages.

2. Materials and methods

2.1. Plant materials and extraction

To develop environmental friendly pest control measures, two plants, *Ageratum conyzoides* (Goat weed) and *Nerium indicum*, (pink kaner) were selected from the literature and potential knowledge gained by local people. The fresh leaves of *A. conyzoides* and *N. indicum* were collected at their peak stages from the local areas. The collected plant materials were thoroughly washed to remove dust, kept in shade for drying for 8-10 days. The dried plant materials were coarsely

ground to powder for extraction process. The extract was prepared using methanol as solvent in Soxhlet apparatus for 8 h. The extracted material in semi-liquid form was then dried in rotary evaporator to remove solvent from the crude extract (Mehta *et al*., 1999). The solvent free crude extract thus obtained was transferred in glass vials and stored in refrigerator and used for the present study. To evaluate the bioactivity, different concentrations *viz.* 1.0, 0.5, 0.2 and 0.1% were prepared in distilled water from crude extract (Mehta *et al.*, 1999).

2.2. Test insect

The experimental insect, *H. vigintioctopunctata*, was originally collected from the brinjal fields of Nariawal village located at Bareilly district and was continuously reared in Pests and Parasites Research Laboratory in the Department of Zoology, Bareilly College, Bareilly. The nucleus culture of the beetle was maintained in laboratory as per the method of Saxena and Sharma (2007). The stock culture was maintained in the laboratory at 28ºC and 65 ± 5% relative humidity (RH) to supply different life stages of the beetle for experimentation.

2.3. Bioassay

For conducting the experiments, first instar grubs of *H. vigintioctopunctata* were procured from the nucleus culture reared and maintained in the laboratory. Various concentrations of the extracts viz., 0.1, 0.2, 0.5 and 1.0% were prepared in distilled water for evaluation. To work out antilarval activity of the extracts, the first instar grubs of hadda beetle were fed on brinjal leaves treated with different concentrations of these extracts for 24 h and kept in plastic jars covered with muslin cloth as per the method of Saxena and Sharma (2007). Thereafter, normal fresh leaves were provided to them upto the pupation. The control experiments were conducted with the same manner with water. The experiment was replicated thrice having six grubs in each to evaluate the bioactivity of extracts in terms of larval mortality, developmental period, adult emergence, diapause condition and any other morphological deformity.

2.4. Statistical analysis

The entomological data was statistically analyzed using GraphPad Prism-5 computer software and significant differences among the mean values of different groups were analyzed by one-way ANOVA. The LC_{50} values of the compound were estimated performing probit analysis of dose response data converting the log doses and probit transformed mortality data (Finney, 1962).

3. Results and discussion

The plant extracts may have a crucial place in the management of pest control program due to their ecofriendly nature and numerous active principles which reduce the probability of developing resistance in targeted pests. In the present study, it was observed that methanolic herbal extracts from identified two plant species exhibited bioactivity against first instar grubs of hadda beetle. The entomological data revealed that 0.5 and 1.0% leaf extract of *A. conyzoides* killed 66.7±6.7 to 100% treated grubs and *N. indicum* killed 55.6±5.8 to 94.4±3.3 %, respectively which is in agreement of the findings obtained by Satpathi and Ghatak (1990) with 1.0% *N. oleander* leaf extract in this beetle. However, a mortality of 33.3% was recorded with 0.2 and 0.1% concentrations of *A. conyzoides*, whereas in case of *N. indicum* extract, it was found 22.2±2.6 and 16.7±6.7%, respectively, as compared to control (13.9±4.6%). It indicated that the lower concentrations of *N. indicum* possessed comparatively lower toxic effect on the grubs. It was noticed that the mortality occurred mainly at first and second instar stages of *H. vigintioctopunctata*. Analysis of dose response data revealed the LC_{50} values of 0.22 (95%CI= 0.19-0.25) and 0.32 (95%CI= 0.27-0.37) for *A. conyzoides* and *N. indicum* extracts, respectively against first instar grubs (Fig. 1). Workers like Singh and Rao (2000) have noticed 35.15% mortality in *Spodoptera litura* with 716.8µg dose of *A. conyzoides* leaf extract which is very close (33.3%) to the present findings with 0.2 and 0.1% extract of the same plant species. The antifeedant and insecticidal properties of yellow kaner (*Thevetia nerifolia*) against *H. vigintioctopunctata* (Bai and Koshy, 1999) while antifeedant activity of *N. indicum* plant against pulse beetle, *Callosobruchus chinensis,* was reported (Patil *et al.*, 2000).

Table 1. Effect of methanolic extracts of *A. conyzoides* and *N. indicum* leaf on first instar grubs of *H. vigintioctopunctata*

Plants	Conc. %	Developmental Period (days) of grubs					Total Dev. Period (days)	Inhibition of Adult Emergence %	Larval Mortality %	LC_{50} (95% CI)
		Ist Instar	IInd	IIIrd	IVth	Pupa				
A. conyzoides	1.0	5.0	5.50	2.33	-	-	12.8±0.9	-	100±0^{c}	0.22 (0.19-0.25)
	0.5	4.33	5.00	5.33	7.0	5.75	27.4±1.4	40±5.2^{b}	66.7±6.7^{c}	
	0.2	4.50	4.33	4.17	5.67	5.33	24.0±1.1	14.3±5.7	33.3±3.3^{a}	
	0.1	4.83	4.00	4.50	5.83	4.67	23.8±1.2	5.0±5.0	33.3±6.7^{a}	
N. indicum	1.0	5.17	4.67	2.67	2.33	2.00	16.8±0.9	15.0±5.0	93.3±3.3^{c}	0.32 (0.27-0.37)
	0.5	4.67	5.33	4.00	4.33	4.00	22.3±0.8	15.0±3.3	55.6±5.8^{c}	
	0.2	4.50	4.83	4.83	5.50	4.17	23.8±0.8	2.2±1.2	22.2±2.6	
	0.1	4.00	4.17	4.67	4.50	4.17	21.5±1.2	100±0.0	16.7±6.7	
Control	D.W.	4.42	4.17	4.50	5.00	4.67	22.8±1.3	100±0.0	13.9±4.6	

Superscripts showing significantly different mean values from control at [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$

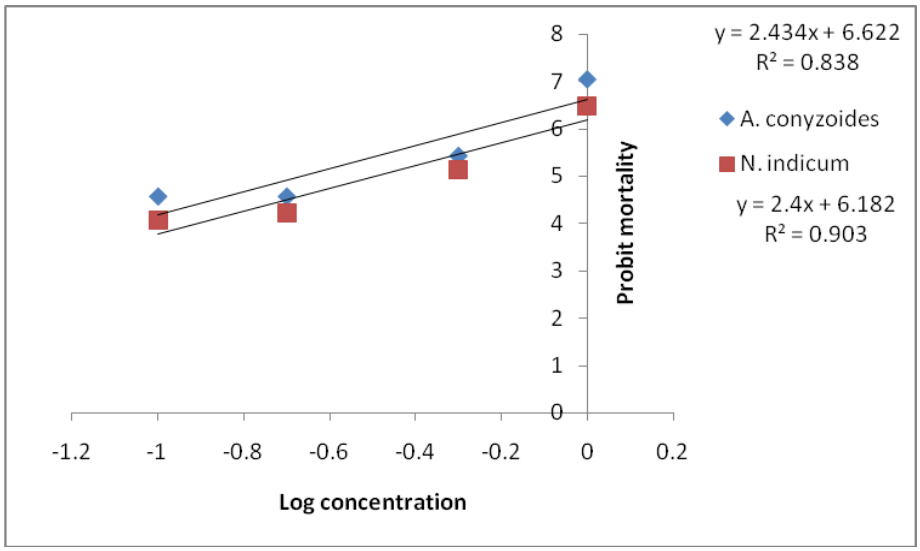

Fig. 1. Log-probit mortality graph of methanolic leaf extracts of *A. conyzoides* and *N. indicum* against first instar grubs of *H. vigintioctopunctata*

Data on development period of treated larvae indicated that *A. conyzoides* extract at 0.5% exerted a significant impact on total developmental period of hadda beetle showing 27.4±1.4 days as compared to control (22.8±1.3 days), whereas no lower concentrations could not show any effect on developmental period (Table 1). However, the extract at 1% concentration of *A. conyzoides* showed no complete development of the treated grubs as they died in third instar stage and could not reach at fourth stage of their development. In contrast, Mehta *et al.* (1999) reported positive impact on longevity i.e. 12.83 days with 1.0% treatment of *A. haustonianum* leaf extract. Herbal preparation of *N. indicum* leaf at 1% reduced the development of the grubs and completed their growth in 16.8±0.9 days. Sharma and Saxena (2012) reported an elongation in the total period of 2.3-4 days with seed extract of *N. indicum* when tested against first instar grubs of *H. vigintioctopunctata* while no such prolongation in this period was noticed in the present study with leaf extract of the same plant species. A prolongation of 2 days in fourth instar grubs was recorded with 0.5% extract of *A. conyzoides* as compared to control where it took 5 days to complete its fourth stage development.

The entomological data on inhibition of adult emergence in hadda beetle revealed an inhibition of 2.2±1.2 to 6.7±3.3% from the pupae in different concentrations of *N. indicum* leaf extract as compared to control pupae where 100 percent pupae emerged into adults (Table 1). The leaf extract of *A. conyzoides* at 0.5% concentration showed significant ($p<0.01$) inhibition of emergence (40±5.2) while 0.2% inhibited only 14.3±5.7% emergence. During the experiments, which were unable to fly and could not survive. Here, some diapaused pupae were also observed in 0.5 and 0.2% extract of *A. conyzoides* leaf which might be due to some interaction of active components of the extracts with developmental physiology of insect. Similarly, some pupae emerged as deformed adults having deformed wings, legs and elytra and were incapable to fly properly. The extract of *A. conyzoides* was reported to possess juvenile hormone activity against hadda beetle leading to the malformed adults (Saxena and Sharma, 2007). From the present exhaustive study, it can be concluded that *A. conyzoides* may be a good alternative to chemical control method and can be employed in the development of an ecofriendly herbal control approach of resistant insect pests.

Acknowledgement

The authors earnestly thank Dr. Somesh Yadav, Principal, Bareilly College, Bareilly, for providing agricultural land for brinjal plantation and Dr. Kamal Kumar Saxena, Head, Zoology Department, Bareilly College, Bareilly, for all the necessary facilities that were made available to carry out the research work. One author acknowledges the Division of Research & Innovation, Uttaranchal University, Dehradun, India for valuable support.

Conflict of interest

Authors declare that they have no conflict of interest in the data provided in the manuscript.

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