Bioactivity of some indigenous plants for the control of hadda beetle, *Henosepilachna vigintioctopunctata* infesting brinjal

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ABSTRACT

The present study was conducted to find out natural and cheaper source for the control of brinjal pest, *Henosepilachna vigintioctopunctata*, by the extraction of locally available plants. All the extracts prepared from different parts of the plants proved efficacious when first instar larvae were fed on leaves treated with 1.0% concentration and gave 80.0 to 93.3% larval mortality as against 6.7 ± 6.7% in control set. Flower extract of *Eucalyptus globulus* at 0.5% and seed extract of *Nerium indicum* at 0.2% increased total developmental period to 22.5 ± 1.5 and 22.7 ± 0.6 days respectively as compared to control in which larvae took 20.2 days to complete their development. The highest concentration (1.0%) of all the extracts also showed drastic effect on adult emergence while some abnormal adults with deformed wings, elytra and appendages were also observed in lower concentrations of *N. indicum* and *E. globulus* extracts. The LC_{50} values of these plant extracts also been calculated to compare their efficacy. The results show the insecticidal potentiality of *N. indicum* and *E. globulus* in the control of brinjal pest.

Key words. Emergence, *Henosepilachna vigintioctopunctata*, mortality, plant extracts

INTRODUCTION

Vegetables play a vital role in providing essential protective nutrients like vitamins and minerals and are used as selective diets by everybody. Brinjal or egg plant (*Solanum melongena* Linn.) is one of the most important vegetable crops grown all over India. Brinjal is heavily infested by a number of insects/pests among which *Henosepilachna vigintioctopunctata* (Coleoptera: Coccinellidae), locally known as hadda beetle, is also one of the most destructive pests extensively found all over India and in other countries (Anam *et al*., 2006; Rahaman *et al*., 2008). It is a polyphagous pest which shows its presence on brinjal and other economically important solanaceous and cucurbitaceous crops. Henosepilachna beetle causes considerable economic losses to many crops including brinjal depending on place and season for variations of prevailing environmental conditions (Rajgopal and Trivedi, 1989; Bhagat and Munshi, 2004; Islam *et al*., 2011). It is highly destructive at both, adult and larval stages which feed on the epidermal tissues of leaves, flowers, and fruits by scrapping the chlorophyll content and cause a big yield loss (Imura and Ninomiya, 1978; Srivastava and Butani, 1998; Ghosh and Senapati, 2001). The affected leaves of the plant become skeletonized, gradually dry and drop down. The larvae confine their attack to the lower surface while adult beetles usually feed on the upper surface of the leaves (Prodhan *et al.,* 1990; Khan *et al*., 2000). The management of *Henosepilachna* beetle was based on synthetic pesticides due to their quick and knock down action (Jagan Mohan, 1985; Ghosh, 1986; Samanta *et al.*, 1999; Das *et al.*, 2002; Liu *et al.*, 2003). The frequent and indiscriminate application of these pesticides in the vegetable fields has resulted into widespread development of resistance, undesirable effects on non-target organisms, presence of toxic residues in food, environmental and health hazards (Subramanyam and Hagstrum, 1995; Kranthi *et al*., 2002). These problems have highlighted the need for development of new, safer and eco-friendly pest control measures.

Naturally occurring plant products may play an important role to replace or minimize the excessive use of pesticides as they constitute a rich source of bioactive components (Wink, 1993). A number of plant products or botanicals with a series of important properties such as; insecticidal, antifeedant, repellent, growth inhibitory, chitin synthesis inhibitor property and environmental

friendly nature, attracted the attention of researchers in the direction of pest control programme (Satpathi and Ghatak, 1990; Chitra *et al.,* 1992; Venkataramireddy *et al.,* 1993; Prajapati *et al.,* 2003; Lee *et al.,* 2004; Murugesan and Murugesh, 2008; Swaminathan *et al.,* 2010; Ghosh and Chakraborty, 2012). A number of researchers have reported various properties like larvicidal, antifeedant, reproductive and growth inhibitory properties against different insects of economic importance (Singh and Saratchandra, 2005; Rahman and Talukdar, 2006; Sharma and Rajguru, 2009). In some rural areas, people burn dried leaves of *Eucalyptus* to keep mosquitoes away. As these botanicals possess more than one active components, there will be less chance of development of resistance and easily biodegradable in the environment. Keeping view in mind the extracts of *A. arabica*, *E. globulus* and *N. indicum* were evaluated to investigate their bio-efficacy for the control of *H. vigintioctopunctata*.

MATERIALS AND METHODS

Plant materials

To develop environmental friendly pest control measures, some plants i.e. *Acacia arabica, Eucalyptus globulus* and *Nerium indicum,* were selected from the literature and potential knowledge gained by local people. *A. arabica* (Mimosaceae), commonly known as babool, is a large perennial tree with fruits in the form of legumes. The plant has medicinal importance and grows wildly on roadsides and near ponds. *Eucalyptus globulus,* locally named as safeda belonging to Myrtaceae family, is a perennial tall tree which is also grown by farmers around their fields. It has commercial importance and may be grouped under pharmaceutical or medicinal, industrial and perfumery oils. However, *Nerium indicum* (Apocynaceae)**,** popularly known as kaner or pink kaner is a large evergreen shrub with milky juice producing red or pinkish flowers. It is an ornamental plant with medicinal value cultivating in gardens and homes. The fruit is composed of a pair of follicles. The seeds are oblong, with a plume of hairs at one end.

Organic extraction

For the preparation of organic extracts, green leaves and fruits of *A. arabica,* seeds of *N. indicum* and flowers of *E. globulus* were collected from their natural habitats and washed thoroughly with tap water to remove dust and other particles. After washing, different parts of these plants were kept for shade drying for 10-20 days depending on the nature of parts and finally ground to coarse powder. The powdered material was extracted with petroleum ether (60°-80°C) as solvent in Soxhlet Apparatus for 8 hrs and extracted material in semi-liquid form was kept in a Petridish for overnight to evaporate extra solvent (Mehta *et al*., 1995). The semi-solid crude extract collected from petridishes was then transferred in glass vials and stored in refrigerator for future use. To assess the efficacy of extracts, different concentrations *viz.* 1.0, 0.5, 0.2 and 0.1% were prepared in distilled water from this crude extract stock (Mehta *et al.,* 1995) and tested against first instar larvae of *H. vigintioctopunctata.*

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 culture of test insect was run and maintained as per the method described by Mehta *et al.* (1995) and Saxena and Sharma (2007). Different stages of *H. vigintioctopunctata* were reared on fresh and tender leaves of brinjal by changing them regularly in plastic jars (12.5cm x 25cm) and stalks of leaves were dipped in glass tubes filled with water and corked with thermacole to avoid drying as larvae do not prefer dried leaves. The glass tubes with leaves were kept in plastic jars covered with muslin cloth. The plastic jars were placed in Biological Oxygen Demand (BOD) incubator maintained at 28 ± 1^0C and 65 ± 5% relative humidity (RH). The nucleus culture was continuously maintained to supply different life stages of the insect for evaluation of various herbal extracts.

Bioassay

To evaluate the insecticidal activity of herbal extracts, 2 mL. of each concentration was topically applied on fresh and tender brinjal leaves of equal size which were embedded in water filled glass tubes corked with thermacole and fed to newly hatched or one day old first instar larvae of *H.*

vigintioctopunctata for 24 hrs in plastic jars covered with muslin cloth (Balasubramanian *et al*., 1980). After 24 hrs feeding, larvae were fed with untreated fresh leaves by changing them regularly upto pupation. The control experiment with distilled water was also run simultaneously. The entire experiment was studied at 28 ± 1^0C and 65 ±5 % relative humidity in BOD incubator. The experiments were carried out with five first instar larvae in each concentration and were replicated thrice to collect the data on mortality, developmental period, adult emergence and any other morphological deformity in hadda beetle. Observations on larval mortality were recorded regularly after the treatment.

Statistical analysis

Data were statistically analyzed by Graph Pad Prism-4 software and significant values of entomological data were determined using one-way ANOVA. The LC_{50} values of extracts were calculated with probit analysis by transforming values into probit (Finney, 1962).

RESULTS AND DISCUSSION

The data obtained in the present study have been documented in table 1 and 2 which revealed that a highly significant mortality ($p<0.001$) of first instar larvae of *H. vigintioctopunctata* was recorded with 1.0% *N. indicum* seed extract which was highly significant at $p<0.001$ level. Similarly, a highly significant mortality was recorded in 1.0% concentration of *A. arabica* fruit and *E. globulus* flower extracts as compared to control where larval mortality was noticed. A comparative study was carried out on insecticidal activity of *N. indicum* leaves extract against first instars of *H. vigintioctopunctata* by Saxena and Sharma (2005) while Satpathi and Ghatak (1990) have noted 90% mortality of the same beetle with same concentration of root extract of *T. nerifolia* which was very close to the present findings and confirm the insecticidal activity of the plant. The authors showed that the extract was larvicidal causing mortality with 1.0% concentration while the study made by Saxena *et al.* (2004) using petroleum ether leaves extract of *E. globulus* against the same larval instars revealed 93.3% larval control with similar emergence pattern by feeding on treated leaves of food plant which is in conformity with the present observations using different parts of these plants.

It was important to note that all the extracts of different parts of the plants at 0.5% concentration significantly ($p<0.01$) reduced more than 50% larval population. However, the lower concentrations of all these extracts did not show any significant effect on larval survivality exhibiting mortality. Extracts of *E. globulus* flowers and *N. indicum* seeds at tested concentrations showed adverse effect on feeding, survivality and developmental period of test insect. This might be due to the presence of some bioactive components that disturb the normal biology of the insect. Earlier, Rao *et al.* (1990) reported high anti-feeding effect of different plant extracts including *E. globulus* and noted cent per cent protection against second instar larvae of *H. vigintioctopunctata*. Derwich *et al*. (2010) identified 34 compounds including neriin, digitoxigenine amorphane, 1,8-cineole, α-pinene, calarene, limonene and terpinene-4-ol from the flowers of *Nerium oleander* and registered antimicrobial activity.

When probit regression lines of different extracts were calculated, they showed a linear relationship between mortality percentage and extract concentration. The dose-mortality response bioassay revealed the highest slope value of 2.07 ± 0.50 with *N. indicum* seed extract (LC_{50}=0.261) and R^2 value of 0.90 followed by *A. arabica* fruit extract (LC_{50}=0.367) having very steep slope and R^2 values as 1.98 ± 0.48 and 0.90 respectively. On the other hand, *E. globulus* flower and *A. arabica* leaf extracts exhibited comparatively low slope values with their LC_{50} values as 0.304 and 0.336 respectively (Table 2).

Data on development period indicated that *E. globulus* and *N. indicum* exerted some remarkable effect on longevity of *H. vigintioctopunctata*. The development of larvae was delayed by *E. globulus* and *N. indicum* extracts as against 18.7±0.7 days of untreated larvae while 0.1% of these extracts

Table 1. Different plant extracts on the developmental period (days), adult emergence (%) and cumulative mortatlity (%) of first instar larvae of *Henosepilachna vigintioctopunctata*

Extracts		Concentration (%)	Developmental period (days) (Mean ± SE)	Adult Emergence (%)(Mean ± SE)	Mortality (%) (Mean ± SE)
Plant	Parts				
A. arabica	Leaves	0.1	18.2 ± 0.4	66.7 ± 6.7	33.3 ± 6.7
		0.2	20.0 ± 1.0	73.3 ± 6.7	26.7 ± 6.7
		0.5	19.8 ± 0.9	46.7 ± 6.7[b]	53.3 ± 6.7[b]
		1.0	19.5 ± 1.3	20.0 ± 0.0[c]	80.0 ± 0.0[c]
	Fruits	0.1	19.7 ± 1.8	80.0 ± 11.5	20.0 ± 11.5
		0.2	20.0 ± 1.1	80.0 ± 0.0	20.0 ± 0.0
		0.5	20.2 ± 1.3	46.7 ± 6.7[b]	53.3 ± 6.7[b]
		1.0	19.0 ± 1.0	13.3 ± 6.7[c]	86.7 ± 6.7[c]
E. globulus	Flowers	0.1	21.7 ± 0.7	66.7 ± 6.7	26.6 ± 13.3
		0.2	22.5 ± 1.5	66.7 ± 6.7	33.3 ± 6.7
		0.5	21.3 ± 0.8	40.0 ± 11.5[b]	53.3 ± 17.6[b]
		1.0	19.2 ± 1.4	13.3 ± 6.7[c]	86.7 ± 6.7[c]
N. indicum	Seeds	0.1	21.7 ± 0.3	73.3 ± 6.7	26.7 ± 6.7
		0.2	21.0 ± 0.8	66.7 ± 6.7	33.3 ± 6.7
		0.5	22.7 ± 0.6	33.3 ± 6.7[c]	60.0 ± 0.0[b]
		1.0	21.3 ± 1.7	6.7 ± 6.7[c]	93.3 ± 6.7[c]
		control	18.7 ± 0.7	93.3 ± 6.7	6.7 ± 6.7

[a] significant at p<0.05; [b] significant at p<0.01; [c] significant at p<0.001

elongated normal life cycle by 3.0 days. In contrast to the present findings, Srivastava *et al.* (1995) mentioned a significant shortening of development period of *Dysdercus koenigii* nymphs when treated with *Eucalyptus* oil. However, Verma and Yadava (2003) have reported that *E. globulus* in the form of leaf extract inhibited the growth and development of *D. koenigii.* The extracts prepared from leaves and fruits of *A. arabica* could not affect normal life cycle of treated larvae. During the routine experimental observation, prolongation in fourth instar larvae and pupae were also recorded with *N. indicum* and *E. globulus* extracts in comparison with control larval and pupal period.

Biological data on adult emergence of hadda beetle as presented in table 1 indicated that all the survived larvae in different concentrations of *A. arabica* extracts successfully emerged as healthy adult beetles. As higher concentrations of all the extracts were proved highly toxic to the larvae showing mortality and resulted into significant inhibition in adult emergence. While the extracts of *Eucalyptus* and *Acacia* were effective in checking pest population by inhibiting emergence of healthy adults as few abnormal adults with deformed wings, legs and elytra were developed from the treated larvae which were unable to fly and could not survive. Here, some diapaused pupae were also observed in lower concentration which might be due to some interaction of active components of the extracts with developmental physiology of insect. *Eucalyptus* and *Nerium* extracts at 0.5% dilution reduced the adult emergence where difference of means with control data was significantly different at p<0.01.

Table 2. LC_{50}, slope and R^2 values of different plant extracts against first instar larvae of *H. vigintioctopunctata* delete the table and include the value in the text

Extracts		Slope ± SE	R^2	LC_{50} (95% CL)
Plant	Parts			
A. arabica	Leaves	1.34 ± 0.46	0.81	0.336 (0.186-0.608)
	Fruits	1.98 ± 0.48	0.90	0.367 (0.245-0.550)
E. globulus	Flowers	1.66 ± 0.39	0.90	0.304 (0.177-0.384)
N. indicum	Seeds	2.07 ± 0.50	0.90	0.261 (0.188-0.492)

In support, Gehlot *et al.* (2005) noticed *E. globulus* leaf extract to cause significant inhibition in adult emergence of *Callosobruchus maculatus.* Similar findings on mortality and adult emergence were also explored by Saxena and Sharma (2007) when they tested insecticidal activity of different plants including *Nerium* and *Eucalyptus* against third instar larvae of the same beetle.

Thus, on the basis of LC_{50} values, comparative effectiveness of the extracts tested against first instars of hadda beetle was *N. indicum* seeds > *E. globulus* flowers > *A. arabica* leaves > *A. arabica* fruits. Results of this exhausted study indicate that some plant extracts could be useful for developing new types of insecticides and biorational control agents for controlling pests of agricultural importance.

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