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LABORATORY EVALUATION OF SOME PLANT EXTRACTS AS TOXICANTS ON EPILACHNA BEETLE (*Henosepilachna vigintioctopunctata*) (F.) (COLEOPTERA: COCCINELLIDAE)

Research Article

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ABSTRACT

Experiments were carried out to evaluate the toxicity of six botanicals viz. Custartd apple, Oleander, Parashi, Neem, Ramtulsi and Marigold against epilachna beetle. The extracts from leaves and shoots were prepared by using methanol as a solvent. The results showed that extracts from all the six plants had direct toxic effect on epilachna beetle. LC_{50} values of all six plant extracts were compared from 24 to 120 hours after treatment (HAT). Their order was found with respect to decreasing toxicity in the following way: Parashi (1.29705) > Ramtulsi (1.31635) > Custard apple (1.34735) > Marigold (1.85334) > Neem (2.40967) > Oleander (2.54763) and Ramtulsi (0.33442) > Custard apple (0.36948) = Oleander (0.36948) > Parashi (0.41420) > Neem (0.47818) > Marigold (0.62597) at 24 and 120 hours after treatment respectively.
Abbreviations: HAT: Hours after treatment, LC_{50}: Lethal concentration

Introduction

India is an agriculture-based country; a major part of its economy is being contributed by vegetables. Among them, brinjal is an affordable common vegetable in all sectors of the society. 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* L.) is one of the most important vegetable crops grown all over India. It is heavily infested by a number of pests among which *Henosepilachna vigintioctopunctata* (Coleoptera: Coccinellidae) is one of the most destructive insects extensively found all over India and in other countries (Sharma and Saxena, 2012). It is a polyphagous pest which shows its presence on brinjal and other economically important solanaceous and cucurbitaceous crops.

Indiscriminate use of pesticides for insect pest management results in various environmental and ecological problems such as pest resistance and outbreak of secondary pests (Hagen and Franz, 1973), long persistence, bioaccumulation and health hazards (Bhaduri *et al*., 1989) and environmental pollution (Devi *et al*., 1986; Fishwick, 1988). Substitutes for synthetic pesticides are being strongly conceived whereby researchers are now paying much emphasis on the biologically active indigenous plant products because they are environmentally safe, biodegradable and cost effective (Saxena *et al*., 1983; Ghani, 1998). With the use of such botanicals as an insecticide, the production of the crop can be enhanced without any residual toxicity. Plant extracts contain botanical insecticides or phytochemicals that could be used to repel, deter feeding, limit reproduction and survival of various insect pest species including ladybird beetles (Mondal and Ghatak, 2007; Swaminathan *et al*., 2010). The present paper deals with the insecticidal effect of plant extracts against *Henosepilachna vigintioctopunctata* (F.). Keeping those above facts in view, an attempt has been made to find out the effective substitute of synthetic chemicals by botanicals.

Materials and Methods

Collection and destruction of plant materials

Different parts (shoots and leaves) of six plant species of Custard apple (*Annona squamosa* L.), Nerium (*Nerium indium* L.), Neem (*Azadirachta indica* A. Juss), Ramtulsi (*Ocimum gratissimum* L.) and Marigold (*Tagetes erecta* L.) were collected from the medicinal plants garden of Ramakrishna Mission Ashrama, Narendrapur, West Bengal and Parashi (*Cleistanthus collinus*) from Bankura. They were washed in running water. Afterwards, the plant materials were kept in shade for air drying. The shoots and leaves were oven dried for at least 15 minutes

within temperature range of 45-50^0 C. When the plant parts become crispy, those were taken out and crushed in a mixer grinder. The crushed powder was shifted in a conical flask containing methanol and was kept for 24 hours allowing the methanol to extract the toxicants of the leaves and shoots of different plant species. The conical flask containing methanol with powders derived from leaves and shoots was stirred for 10 minutes by a magnetic stirrer for every samples and left to stand for next 24 hours. After 24 hours, the extract was then filtered through a fine cloth and again through filter paper (Whatman No. 1). The filtrated materials were taken in a round bottom flask and condensed to 50 ml by evaporation of solvent in a water bath maintained at 55^0 C. After the evaporation of solvent, the condensed or dried extract was scrapped out with the help of a sharp knife and weighted. About 0.5, 1.0, 1.5, 2.0 and 2.5 gm scrapped dried materials were dissolved in each of 100 ml of water as to get the desired concentrations of the extracts of 0.5, 1.0, 1.5, 2.0 and 2.5 % respectively.

Rearing of epilachna beetle

The adults were collected from brinjal field at Agricultural Training Centre farm, Ramakrishna Mission Ashrama, Narendrapur, Kolkata-700103, West Bengal and reared in laboratory condition within temperature range of 28 ± 2°C and at 70-75% relative humidity during the year 2013. These laboratory reared adults were used for bioassay and the culture was maintained throughout the study period.

Insect bioassay / Insecticidal activity

A laboratory test for direct toxicity by topical application method was conducted according to the method of Mamun *et al.,* 2004 with slight modification. Five different concentrations (0.5, 1.0, 1.5, 2.0 & 2.5%) of six plant extracts were used which were prepared previously. One microlitre (ml) of the prepared solution was applied to the dorsal surface of the thorax of each insect using a micropipette syringe. In each replication 20 insects (10 males & 10 females) were treated and each treatment was replicated thrice. In addition, the same numbers of insects were treated with water only for control treatment. After the treatment, the insects were transferred into petridishes having 9 cm diameter (20 insects/ petridish) containing brinjal leaves and covered with muslin cloth. Petioles of the brinjal leaves were tied with wet cotton plug to avoid early drying of leaves. Insect mortalities were recorded at 24, 48, 72, 96 and 120 hours after treatment (HAT). Original data were corrected by Abbots (1987) formula.

$$\text{Percentage of corrected mortality} = \frac{\text{Observed mortality} - \text{Control mortality}}{100 - \text{Control mortality}} \times 100$$

Data analysis was carried out using Microsoft excel-2007. The experimental data were statistically analyzed by using MSTAT statistical software with the help of a computer. LC_{50} values were calculated by using Probit analysis (Finney, 1971).

Results and discussions

The results of the Probit analysis for the estimation of LC_{50} values and their fiducial limits, the regression equation and relative toxicity after 24, 48, 72, 96 & 120 hours for mortality of epilachna beetle of six plant extracts are presented in the tables (Tables 1- 6). The LC_{50} values of Custard apple, Oleander, Parashi, Neem, Ramtulsi and Marigold at 24 hours after treatment (HAT) indicated that Parashi (1.29705) was the most toxic and Oleander (2.54763) was the least toxic and their decreasing toxicity order were as the following Parashi > Ramtusi> Custard apple> Marigold> Neem> Oleander.

The LC_{50} values of Custard apple, Oleander, Parashi, Neem, Ramtulsi and Marigold after 120 HAT indicated that Ramtulsi (0.33442) was the most toxic and Marigold (0.62597) was the least toxic and their decreasing toxicity order also are as follows Ramtulsi > Custard apple = Oleander > Parashi > Neem > Marigold.

When the LC_{50} values of all six plant extracts were compared from 24 HAT to 120 HAT (Fig-1 and Fig-2), only Parashi shows same LC_{50} value at 96 HAT and 120 HAT. At 120 HAT Custard apple and Oleander also shows the same LC_{50} value. In case of other five plant extracts Ramtulsi, Custard apple, Oleander, Marigold and Neem showed decreasing fashion of LC_{50} with increasing order of toxicity accompanied with increasing order of time interval against adults of epilachna beetle. The Chi-square values of different plant extracts of different HAT were significantly same at 5% level of probability and did not show any heterogeneity of the mortality data.

The authors showed that the extracts of Parashi and Ramtulsi were most toxic at 24 and 120 hours after treatment (HAT) while the study made by Prakash and Rao (1997) using the extracts of leaves in methanol @ 20 ml/100 gm of *Cleistanthus collinus* (Karada) indicated larval mortality to coleopteran Sawtooth grain beetle (*Oryzaephilus surinamensis* L.). Also the extracts of *Ocimum gratissimum* and *Mentha aquatica* proved the insecticidal property against *Plutella xylostella (*DBM) and *Formica ruffa* in Democratic Republic of Congo *(*MashiMango *et al.,* 1990). In a laboratory evaluation Ramtulsi (*Ocimum gratissimum*) showed 93.33 % and 73.33 % mortality 120 HAT at 1.5% and 1.0 % concentration which was significantly at par among the five treatments for Mealy Bug (*Ferissia virgata* Cock.) (Gupta *et al.,* 2007). The results are in conformity with the present observations using same type of extracts by Parashi and Ramtulsi.

Tables (1-6): Estimated LC_{50} value, fiducial limit, regression equation and heterogeneity (χ^2) for the methanolic extracts from six plant species

1. Custard apple

Time interval (hrs)	Heterogeneity(χ^2)	Regression equation	LC_{50}	Fiducial limit	Relative toxicity
24	33.274 14.699	Y = 2.23168 × −0.28896	1.34735	1.15643 1.56969	1
48	58.714 14.364	Y = 2.50559 × +0.41355	0.68383	0.54809 0.83553	1.97
72	82.803 22.952	Y = 2.90763 × +0.83404	0.51660	0.39405 0.65619	2.60
96	63.283 2.731	Y = 3.17623 × +1.01769	0.47818	0.37909 0.58920	2.81
120	60.628 17.749	Y = 3.53930 × +1.53041	0.36948	0.28673 0.46300	3.65

2. Oleander

Time interval (hrs)	Heterogeneity (χ^2)	Regression equation	LC_{50}	Fiducial limit	Relative toxicity
24	33.274 14.699	Y = 2.23168X −0.90637	2.54763	2.15609 3.05557	1
48	58.714 14.364	Y = 2.50559 × −0.44456	1.57537	1.30952 1.90260	1.62
72	82.803 22.952	Y = 3.17623 × +0.27263	0.82067	0.68552 0.97027	3.10
96	63.283 2.731	Y = 3.53930 × +0.55100	0.69874	0.58472 0.82226	3.65
120	60.628 17.749	Y = 3.53930 × +1.35481	0.36948	0.28673 0.46300	6.89

3. Parashi

Time Interval (hrs)	Heterogeneity (χ^2)	Regression equation	LC_{50}	Fiducial limit	Relative toxicity
24	33.274 14.699	Y=2.23168 X−0.28896	1.29705	1.11342 1.50934	1
48	58.714 14.364	Y = 2.50559X −0.17032	0.85511	0.69652 1.03504	1.51
72	82.803 22.952	Y = 3.17623 × +0.92582	0.51111	0.40802 0.62585	1.52
96	63.283 2.731	Y = 3.53930 × +1.35481	0.41420	0.32680 0.51235	2.54
120	60.628 17.749	Y = 3.53930 × +1.35481	0.41420	0.32680 0.51235	3.13

4. Neem

Time interval (hrs)	Heterogeneity(χ^2)	Regression equation	LC_{50}	Fiducial limit	Relative toxicity
24	33.274 14.699	Y=-2.2168 ×-0.85241	2.40967	2.04476 2.87993	1
48	58.714 14.364	Y = 3.53930 × +0.31516	0.81462	0.69245 0.94693	2.96
72	82.803 22.952	Y = 2.50559 ×-0.60158	0.68383	0.54809 0.83553	3.52
96	63.283 2.731	Y = 2.90768 × −0.29247	0.51660	0.39405 0.65619	4.66

120	60.628 17.749	Y = 3.17623 × +0.02810	0.47818	0.37909 0.58920	5.04

5. Ramtulsi

Time interval (hrs)	Heterogeneity(χ^2)	Regression equation	LC_{50}	Fiducial limit	Relative toxicity
24	33.274 14.699	Y=2.23168 × −0.26640	1.31635	1.13060 1.53198	1
48	58.714 14.364	Y = 2.50559 × +0.04308	0.68383	0.54809 0.83553	1.92
72	82.803 22.952	Y = 2.90763 × +0.76725	0.54466	0.41933 0.68672	2.41
96	63.283 2.731	Y = 3.17623 × +1.48372	0.34109	0.25692 0.43915	3.86
120	60.628 17.749	Y = 3.53930 × +1.68138	0.33442	0.25514 0.42677	3.93

6. Marigold

Time interval (hrs)	Heterogeneity(χ^2)	Regression equation	LC_{50}	Fiducial limit	Relative toxicity
24	33.274 14.699	Y=-2.2168 × -0.28896/ -0.59799	1.85334	1.58888 2.17873	1
48	58.714 14.364	Y = 2.50559 × +0.04308	1.39573	1.16318 1.16762	1.32
72	82.803 22.952	Y = 2.90763 × +0.76725	1.03144	0.84389 1.25099	1.79
96	63.283 2.731	Y = 3.17623 × +1.48372	0.88457	0.74368 1.04082	2.09
120	60.628 17.746	Y = 3.53930 × +1.68138	0.62597	0.52634 0.73976	2.96

A comparative study was carried out on insecticidal activity of *Nerium indicum* leaves extracts against first instar of *H. vigintioctopunctata* by Saxena and Sharma (2005) while Satpati and Ghatak (1990) have noted 90% mortality of same beetle with same concentration of *T. neriofolia.* Those findings had close proximities with the present findings and confirm the insecticidal activity of the plant. The yield of sunflower was increased by reducing epilachna attack on foliage and capitulum of the crop by virtue of spraying 10% aqueous extract of *Ricinus communis* leaves (Ahmed, 2007). The aqueous seed extracts of *Annona squamosa* (5ml/L), *Azadirachta indica* (6 ml/L) and petroleum ether extract of *Acorus calamus* (2 ml/L) reduced population build-up of *H. vigintioctopunctata* infesting cucumber up to 53.24%, 41.67% and 33.16% respectively (Mondal and Ghatak, 2007), while 10% turmeric and neem seed kernel dusts were found most effective against epilachna on brinjal (Sankari and Narayanasamy, 2007). Moreover, Mondal and Ghatak (2009) reported excellent suppression of *H. vigintioctopunctata* attacking brinjal and cucumber respectively using extracts of indigenous plants.

In our results also the application of botanical pesticides reduced the population of epilachna beetle at different concentration levels of 0.5, 1.0, 1.5, 2.0 & 2.5 %. Reddy *et al.* (1990) reported that petroleum ether (1%) extracts of *Azadirachta indica* A. Juss and *Annona squamosa* L. reduced the number of *H. vigintioctopunctata* larvae infesting brinjal. Similarly, Rao *et al.* (1990) studied that extracts of *Annona squamosa* L., *Argemone mexicana* L., *Calotropis gigantea* Ait., *Datura stramonium* L., *Eucalyptus globulus* Labill, *Pongamia glabra* Vent and *Ricinus communis* L. (0.5%) showed cent percent protection against second instar larvae of *H. vigintioctopunctata* indicating high anti-feedant effect. Gupta *et al.* (2007) found in a laboratory experiment that Marigold (*Tagetes erecta* L.) showed 93.33 % and 86.66 % mortality 120 HAT at 1.5 & 1.0 % concentrations respectively. It was very difficult to compare the present findings with those of the earlier ones as not enough study was made elsewhere with the test plant extracts of Parashi, Ramtulsi and Marigold against *Henosepilachna vigintioctopunctata*. Specially Parashi is locally available at Bankura and Purulia districts of

West Bengal. They are used against insect pests by homemade preparation in the form of aqueous extracts. It may be observed from the experiment that the botanicals used have direct toxic effect on epilachna beetle. Thus on the basis of LC_{50} values , comparative effectiveness of the six extracts could be useful for developing new type of insecticides and biological control agents for controlling pests of agricultural importance.

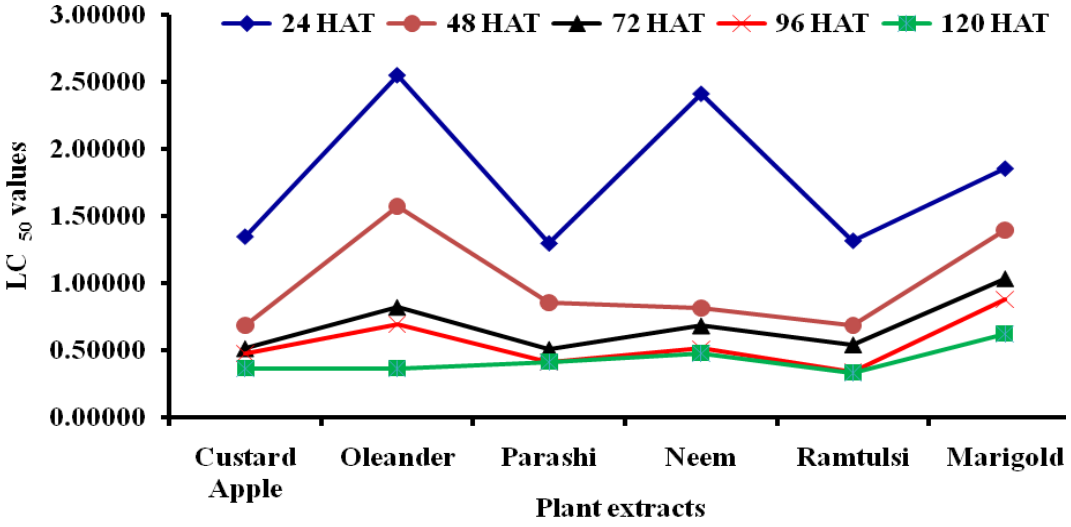

Fig 1. LC $_{50}$ values of six plant extracts from 24 to 120 HAT

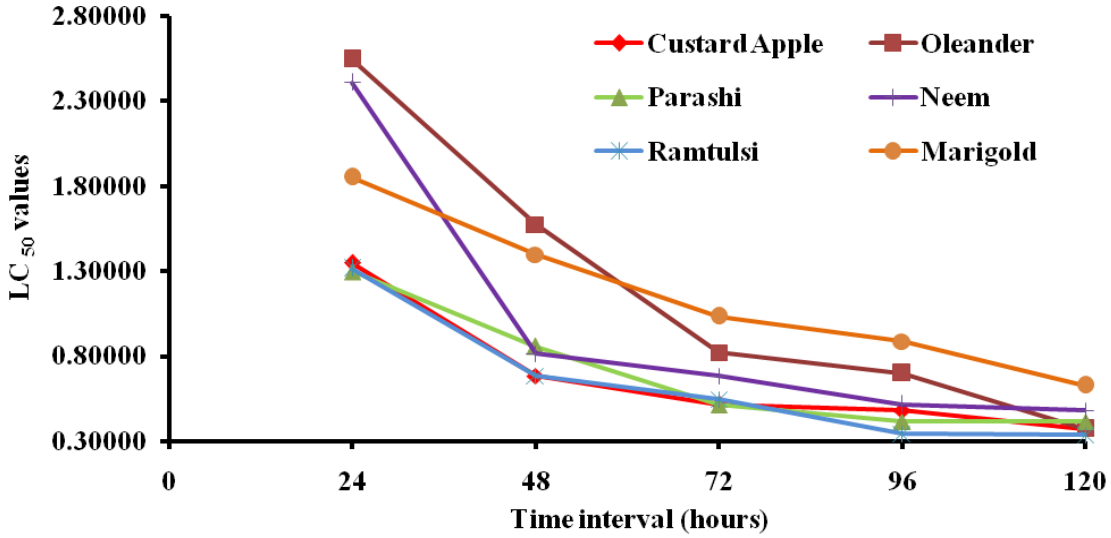

Fig 2. LC_{50} values at different time intervals for different plant extracts

Conclusion

The present data suggest that the methanolic extracts of the six indigenous plants could protect the plants against epilachna beetle. The prime merit of such botanical insecticides is that these could easily and cheaply be prepared by the farmers and / or manufactured by small scale industries as crude or partially purified forms. However, comprehensive investigations at field levels are needed to ascertain the eco-friendly nature of the extracts under study.

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