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# Antifeeding and Insecticidal Potentials of Verbenaceous Botanicals against grubs of *Henosepilachna vigintioctopunctata* Fabr. (Coleoptera: Coccinelidae)

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**ABSTRACT :** In present study, the insecticidal and antifeedant activity of alcohol extracts of leaves, seed and bark of four verbenaceous plants have been evaluated on the 3rd instar grubs of *Henosepilachna vigintioctopunctata* Fabr. The plants used in this investigation are *Clerodendron siphonanthus*, *Lantana camara* Linn, *Lippia geminate* HBK and *Vitex negundo* Linn. The antifeedant activity was assessed through the feeding protection bioassay. Based on the $EC_{50}$ values, the extracts of *Vitex* seed and *Lantana* unripe fruit showed significant protection power against insects. The $EC_{50}$ values were 0.015 and 0.027 respectively which are statistically significant. *Vitex* seeds extract showed high toxicity within 48 hours with mean mortality rate as 80.86 % where as *L. camara* unripe fruits, *L. camara* leaves and *Clerodendron* leaves produced 78.26 %, 68.83% and 67.90% grub mortality respectively. The toxic percentage of the effects of extract of roots and seeds of *C. siphonanthus*, leaves of *V. negundo* , bark of *V. negundo* and geminating leaves of *L. geminate* were observed as 65.70, 65.50, 64.66, 64.16, 48.93 and 35.90 respectively within the same period of 48 hrs. It is suggested that these plants can be used as bio-control as insecticides.

**Key Words:** Biocontrol, Instar gurb, Antifeedant

## Introduction

India is basically an agro-based country and more than 80% of Indian population still depends on agriculture and Indian economy is largely determined by agricultural production. It is unanimously agreed that insect-pests are the main factors causing damage to crops adversely affects agricultural production. The monetary loss due to feeding by larvae and adult insects alone contributes to billion dollars per annum. Among the Coleopteran, *H. vigintioctopunctata* is the key pest that causes severe damage to crops and brings about significant loss yielding (Chandel *et al.*, 1987; Thakur and Mehta., 2004). Abbaszadeh (2011) very recently suggested that the aqueous fraction of *C. infortunatum* act as insecticidal and antifeedant on *H. armegra.* Chandel(2012) described toxicological compatibility of known biopasticide, *Azadirachta indica* and *Acorus calamus* against mustard aphid, *L. erysimi.*

A considerable concern has been raised about the adverse effects of pesticides affecting environment and also resistance development against pesticide. Hence, there is imperative need for development of safe alternative plant protections by botanical insecticides and antifeedants which have least side effects (Tewari and Moorthy, 1985; Verma *et al.* 1986; Rao *et al.*, 1990; Arivudainambi and Nachiappan, 1993; Meshram and Kulkarni, 1996; Faknath and Kawal , 1993; Dekha *et al.*, 1998).The use of plants for medicinal and insecticidal purposes dates back to antiquity (Sahayaraj, 1998; Vekaria and Patel, 2000; Dwivedi and Garg, 2003; Dubey *et al.*, 2004). Recent studies have focused on natural plant products as alternatives for insect-pest control.

## Material and Methods

Grubs of *H. vigintioctopunctata* were collected from agricultural fields in the vicinity of Kanpur. The grubs were taken to the laboratory, placed individually and reared in groups of 20 grubs in containers. Containers were punched to permit air flow. Each group was fed for 48 hr with fresh leaves of brinjal.

The plant materials used in the present investigation were collected mainly from wasteland and wild areas while a few plants were collected from cultivated fields. The collected materials were dried in shade, made into powder and the extracts were prepared with the help of extraction apparatus using petroleum /alcohol as solvent. Four verbenaceous plant extracts viz., *C. siphonanthus* (seed, root and leaves), *L. camara* leaves and bark), *L. geminate* (leaves and bark) and *V. negundo* (leaves, seed and bark) were used for their biological efficacy against grubs and adults of *H. vigintioctopunctata*.

Antifeeding Test**:** Brinjal leaves of five centimeter square were cut and dipped in the extracts of different concentrations for two minutes. They were dried under clip and left under electric fan for about ten minutes to make a film of the extracts on the leaves for each set of treatment, one was kept as control in which, the leaf pieces were dipped in Benzene + emulsified water only. The treated pieces were kept in Petri dishes on moist filter paper and two third instars grubs of *H. vigintioctopunctata* were released in each Petri dish to feed for 4 hours. Three replicates per treatments were maintained. The area of leaf consumed by two grubs in each replication was measured and results were compared with control.

The data of leaf area consumed by two grubs *of H. vigintioctopunctata* in each replication was bulked in these values and the percentage of leaf area protected over control was calculated. The protection was estimated over damage. The concentrations were converted into log concentrations (100 X). The data were subjected to the Probit analysis. The $EC_{50}$ value in respect to each extract was calculated. The fitness of test was tested by comparing table at respective degree of freedom (df). The variance rate was calculated and the fiducial limits were worked out. Finally, the regression columns were drawn with the regression equation (Abbott, 1925).

**Insecticidal Test:** The 24 hr starved, third instars grubs were used for experimental purpose. The insecticidal test of the plant extracts were performed by dry-film technique. One ml. of solution was sprayed on the Petri-dish. Each concentration was tested in three replications and was kept as control (Benzene + emulsified water). To record the mortality, the sprayed Petri-dishes were gently shaken under an electric fan till the herbal extracts evaporated, leaving behind a uniform dry film of extract on the glass surface. Inside each pair of Petri dish, ten numbers of 24 hrs starved third instars grubs were released and allowed to remain there up to two hours. After this, they were transferred to the fresh Petri dish containing fresh food for feeding. Mean mortality per cent of grubs was observed after 6, 12 and 24 hrs. Laboratory tests were conducted under controlled conditions (27 $\pm$2° C temperatures and 75$\pm$ 5 % humidity).

## Results and Discussions

Table 1 and figure 1 revealed the calculation of log concentration, Probit protection, regression graph of antifeedant test on *H. vigintioctopunctata* Fabr. The $EC_{50}$ value is depicted in table 1. On the basis of $EC_{50}$ values, it clearly indicates that extracts of *Vitex* seed exhibit potent antifeedent activity and shows promising protection towards the grubs of *H. vigintioctopunctata*. **T**he results of the present investigations regarding the feeding deterrents are in conformity with the finding of the earlier investigators (Tripathi *et al.,* 1990; Prakash *et al.,* 1990*;* Rao *et al.,*1990; Yano and Kamimura, 1993; Meshram *et al.,*1994; Huang and Zhou, 1995; Huang and Okamura, 1995; Yasui and Kato, 1998; Joshi and Lockwood, 2000; Juan and Sans, 2000, Pandey and Khan, 2000; Ogendo *et al.* 2003 Kannathasan *et al*., 2007; Perez *et al.,* 2010). Sindhu and Singh (1975) reported that kerne of *Azadirachta indicahas* antifeeding and insecticidal properties. Recently, Chandel *et al.* (2011) studied the compatibility of *Azadirachta indica* against painted bug, *B. cruciferarum* and mustard aphid, *L. erysimi* and held that they good biopesticde for controllong antifeeding and insecticidal agents. The results of the present investigation revealed that verbenaceous plants can be

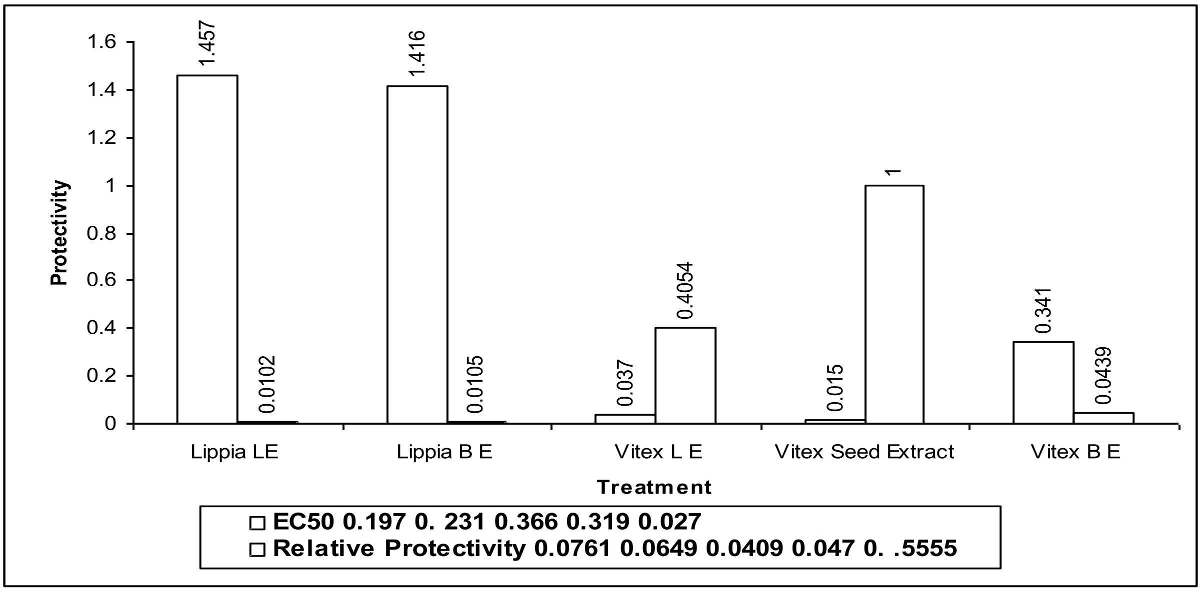


**Fig. 1.** Calculation of log conc. / Probit Protection regression graph.

**Table 1:** Calculation of log conc. / Probit Protection Regression graph (Summary of Antifeedant test on *H. vigintioctopunctata* Fabr.)

| Plant Extracts | Het. | $X^2$ | Regression Equation | $EC_{50}$ | Fiducial Limit |
|---|---|---|---|---|---|
| *Clerodendron* Leaves Extract | 3 | 0.75 | Y = 0.77x + 3.96 | 0.197 | $M_1$= 1.7441<br>$m_2$ = 0.9358 |
| *Clerodendron* Seed Extract | 3 | 0.88 | Y = 0.84x + 3.84 | 0. 231 | $M_1$= 1.7533<br>$m_2$ = 1.0066 |
| *Clerodendron* Root Extract | 3 | 0.78 | Y = 0.69x + 2.38 | 0.366 | $M_1$= 1.5864<br>$m_2$ = 1.1389 |
| *Lantana* Leaves Extract | 3 | 0.17 | Y = 1.00x + 3.49 | 0.319 | $M_1$ = 1.8136<br>$m_2$ = 1.2063 |
| *Lantana* Unripe fruites Extract | 3 | 0.42 | Y = 0.53x + 1.41 | 0.027 | $M_1$ = 1.0234<br>$m_2$ = 1.0202 |
| *Lippia* Leaves Extract | 3 | 1.52 | Y = 2.10x + 1.36 | 1.457 | $M_1$= 1.5144<br>$m_2$ = 0.4061 |
| *Lippia* Bark Extract | 3 | 1.17 | Y = 2.72x + 0.58 | 1.416 | $M_1$= 1.6003<br>$m_2$ = 0.0336 |
| *Vitex* Leaves Extract | 3 | 1.34 | Y = 0.73x + 4.56 | 0.037 | M1= 1.1678<br>m2 = 0.0321 |
| *Vitex* Seed Extract | 3 | 0.58 | Y = 0.61x + 4.84 | 0.015 | $M_1$ = 1.6293<br>$m_2$ = 1.0876 |
| *Vitex* Bark Extract | 3 | 1.48 | Y = 0.58x + 0.32 | 0.341 | $M_1$ = 1.7262<br>$m_2$ = 1.4272 |

In case of $X^2$ was found non significant heterogeneous at P=0.05, Y=Probit Kill, X=Log Concentration X $10^2$
D.F.=Degree of Freedom, $E.C._{50}$= Concentration Calculated at given 50% Protection

used as effective pesticide as important as *Azadirachta.* Ventura and Ito (2000) reported a large number of plants having antifeedint properties against a number of different agricultural pests. Suindararajan and Kumuthakalaralli (2001) evaluated *Gnidia glauca* and *Toddalia aseatica* extracts against *H. armigera* larvae and reported that both extracts showed the high antifeeding action to the larvae.

Kumari *et al.*(2003) described antifeedant and growth inhibitory effects of some neo-clerodane diterpenoids isolated from *Clerodendron* species (Verbenaceae).They have isolated a compounds clerodendrin B, 3-epicaryoptin, 15-hydroxyepicaryoptin and held clerodin as effective antifeedants at 10 ¼g/cm³ (30 ¼g/g) with diet against *E. vitella* and at 10 ¼g/cm² of leaf against *S. litura*. Dwivedi and Bhati (2006) reported the antifeeding response of acetone extracts from four plant viz, *R. communis, E. officinalis T. erecta* and *Z. aungustifolia and claimed that* 100.00, 82.68, 75.48 percent and 77.98 percent protection from *C. chinensis.*

The table 2-3 and 4 and figure 2-5 reveals that the plant extract of *Vitex* seed extract and *Lantana* unripe fruit extracts produced the maximum mortality. It killed

**Table 2 :** Mean mortality percentage of *H. vigintioctopunctata* Fabr. in case of different combination under laboratory conditions :

| **Treatment** (Plant extracts) | **Con. (%)** | **Mean Mortality percent After** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **6 hrs.** | | **12 hrs.** | | **24 hrs.** | |
| | | **$T_1$** | **T.B.V.$_{1}$** | **$T_2$** | **T.B.V.$_{2}$** | **$T_3$** | **T.B.V.$_{3}$** |
| *Clerodendron* leaves | 0.5 | 43.08 | 46.6 | 46.92 | 53.4 | 50.77 | 60.0 |
| *Clerodendron* leaves | 1.0 | 66.15 | 83.3 | 68.85 | 87.0 | 71.56 | 90.0 |
| *Clerodendron* leaves | 2.0 | 83.85 | 98.9 | 90.00 | 100.0 | 90.00 | 100.0 |
| *Clerodendron* seed | 0.5 | 41.15 | 43.3 | 46.92 | 53.4 | 52.80 | 63.5 |
| *Clerodendron* seed | 0.5 | 54.78 | 66.7 | 56.79 | 70.0 | 61.22 | 76.7 |
| *Clerodendron* Seed | 1.0 | 83.85 | 98.9 | 90.00 | 100.0 | 90.00 | 100.0 |
| *Clerodendron* root | 0.5 | 48.85 | 56.7 | 48.85 | 56.7 | 54.78 | 66.7 |
| *Clerodendron* root | 1.0 | 54.78 | 66.7 | 56.79 | 70.0 | 63.44 | 80.0 |
| *Clerodendron* root | 2.0 | 83.85 | 98.8 | 90.00 | 100.0 | 90.00 | 100.0 |
| *Lantana* leaves | 0.5 | 48.85 | 56.7 | 52.78 | 63.4 | 61.22 | 76.2 |
| *Lantana* leaves | 1.0 | 56.79 | 70.0 | 63.93 | 80.7 | 68.85 | 87.0 |
| *Lantana* leaves | 2.0 | 68.85 | 87.0 | 90.00 | 100.0 | 90.00 | 100.0 |
| *Lantana* unripe fruits | 0.5 | 52.78 | 63.4 | 61.22 | 76.8 | 83.85 | 98.8 |
| *Lantana* unripe fruits | 1.0 | 68.85 | 87.0 | 83.85 | 98.8 | 90.00 | 100.00 |
| *Lantana* unripe fruits | 2.0 | 83.85 | 98.8 | 90.00 | 100.0 | 90.00 | 100.0 |
| *Lippia* leaves | 0.5 | 18.44 | 10.0 | 26.56 | 20.0 | 26.56 | 20.0 |
| *Lippia* leaves | 1.0 | 26.56 | 20.0 | 33.21 | 30.0 | 39.23 | 40.0 |
| *Lippia* leaves | 2.0 | 45.00 | 50.0 | 50.77 | 60.0 | 63.14 | 80.0 |
| *Lippia* bark | 0.5 | 26.56 | 20.0 | 40.0 | 40.0 | 45.00 | 43.3 |
| *Lippia* bark | 1.0 | 45.00 | 50.0 | 50.77 | 60.0 | 56.79 | 70.0 |
| *Lippia* bark | 2.0 | 50.77 | 60.0 | 63.14 | 80.0 | 63.14 | 80.0 |
| *Vitex* leaves | 0.5 | 45.00 | 50.0 | 50.77 | 60.0 | 56.79 | 70.0 |
| *Vitex* leaves | 1.0 | 50.77 | 60.0 | 63.14 | 80.0 | 71.56 | 90.0 |
| *Vitex* leaves | 2.0 | 71.56 | 90.0 | 90.00 | 83.7 | 90.00 | 98.9 |
| *Vitex* seed | 0.5 | 63.14 | 79.6 | 71.56 | 90.0 | 71.56 | 90.0 |
| *Vitex* seed | 1.0 | 71.56 | 90.0 | 90.00 | 100.0 | 90.00 | 100.0 |
| *Vitex* seed | 2.0 | 90.00 | 100.0 | 90.00 | 100.0 | 90.00 | 100.0 |
| *Vitex* bark | 0.5 | 39.23 | 40.0 | 45.00 | 50.0 | 63.14 | 80.0 |
| *Vitex* bark | 1.0 | 56.79 | 70.0 | 63.14 | 80.0 | 71.56 | 95.5 |
| *Vitex* bark | 2.0 | 63.14 | 80.0 | 90.00 | 100.0 | 90.00 | 100.0 |
| Control | | 0.00 | 00.0 | 18.44 | 10.0 | 18.44 | 10.0 |

($T_1$, $T_2$, $T_3$ = Treatments and TBV.$_1$, TBV.$_2$, TBV.$_3$ = Transformed Back Values)

C.D. for the treatment combination means = 0.147

C.D. for treatment x period means = 0.048

**Table 3 :** Mean mortality % of *H. vigintioctopunctata* in various plant extracts and periods.

| **Treatment (Plant extracts)** | **Mean mortality percent after** | | | | | | **Mean % Mortality** | |
|---|---|---|---|---|---|---|---|---|
| | **6 hrs.** | | **12 hrs.** | | **24 hrs.** | | | |
| | $T_1$ | $TBV_1$ | $T_2$ | $TBV_2$ | $T_3$ | $TBV_3$ | G.T. | TBV |
| *Clerodendron* leaves | 64.36 | 81.3 | 68.59 | 86.7 | 70.77 | 89.1 | 67.90 | 85.9 |
| *Clerodendron* seed | 59.92 | 74.9 | 64.57 | 81.6 | 68.00 | 88.0 | 64.16 | 81.0 |
| *Clerodendron* root | 62.49 | 78.7 | 65.21 | 82.4 | 69.40 | 87.6 | 65.70 | 83.1 |
| *Lantana* leaves | 64.26 | 81.1 | 68.90 | 87.1 | 73.35 | 91.8 | 68.83 | 86.9 |
| *Lantana* unripe fruits | 68.49 | 88.5 | 78.35 | 95.9 | 87.95 | 99.8 | 78.26 | 95.9 |
| *Lippia* leaves | 30.00 | 25.0 | 36.84 | 36.0 | 42.97 | 46.6 | 35.90 | 34.4 |
| *Lippia* bark | 40.79 | 42.7 | 51.04 | 60.5 | 54.97 | 67.0 | 48.93 | 56.8 |
| *Vitex* leaves | 55.77 | 68.4 | 67.97 | 85.9 | 72.78 | 91.2 | 65.50 | 82.8 |
| *Vitex* seed | 74.90 | 93.2 | 83.85 | 98.9 | 83.85 | 98.9 | 80.86 | 97.5 |
| *Vitex* bark | 53.05 | 63.9 | 66.04 | 83.5 | 74.90 | 93.2 | 64.66 | 81.7 |
| Control | 0.00 | 0.00 | 18.44 | 10.00 | 18.44 | 10.00 | 12.26 | 4.25 |

($T_1$, $T_2$, $T_3$ = Treatments and T.B.V.$_{1}$, T.B.V.$_{2}$, T.B.V.$_{5}$= Transformed Back Values)

C.D. for treatment x period means = 0.075

C.D. for treatment means(plant extract) = 0.032

C.D. for treatment means (control) = 0.160

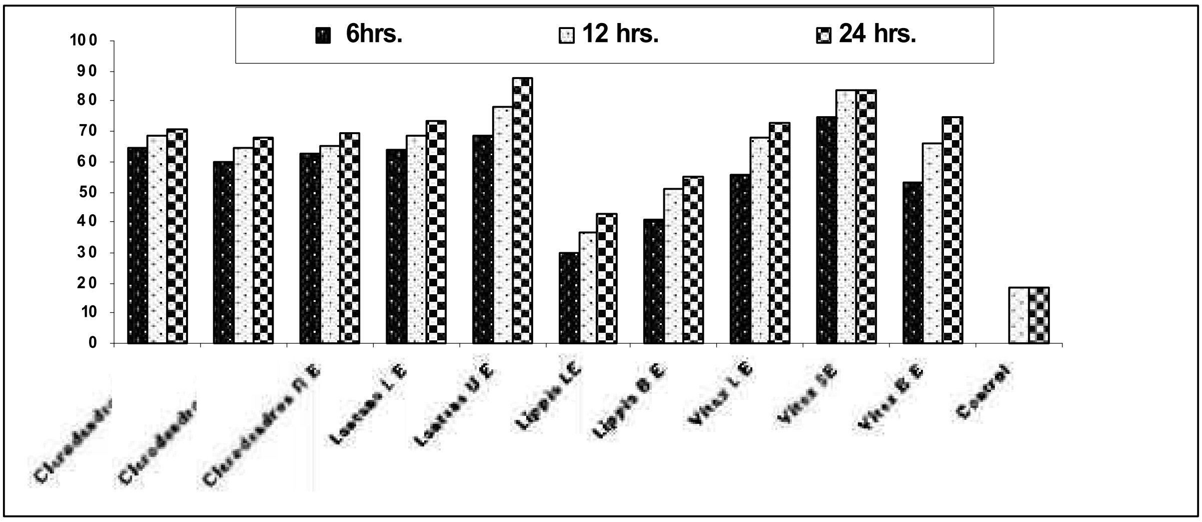


**Fig. 2.** Mean mortality % of *H. vigintioctopunctata* in different periods irrespective treatments

**Table 4** : Mean mortality % of *H. vigintioctopunctata*. in concentrations irrespective of periods in laboratory.

| **Conc.** | **Mean mortality percent after** | | | | | | **Mean mortality %** | |
|---|---|---|---|---|---|---|---|---|
| | **6 hrs.** | | **12 hrs.** | | **24 hrs.** | | | |
| | $T_1$ | $TBV_1$ | $T_2$ | $TBV_2$ | $T_3$ | $TBV_3$ | G.T. | TBV |
| 0.5 | 42.70 | 46.0 | 48.98 | 56.9 | 56.64 | 69.8 | 49.44 | 57.7 |
| 1.0 | 55.83 | 68.5 | 62.94 | 79.3 | 68.42 | 86.5 | 62.39 | 78.5 |
| 2.0 | 72.47 | 90.9 | 83.39 | 98.6 | 84.62 | 9912 | 80.16 | 97.1 |

($T_1$, $T_2$, $T_3$ = Treatments and T.B.V.$_1$, T.B.V.$_2$, T.B.V.$_5$= Transformed Back Values)

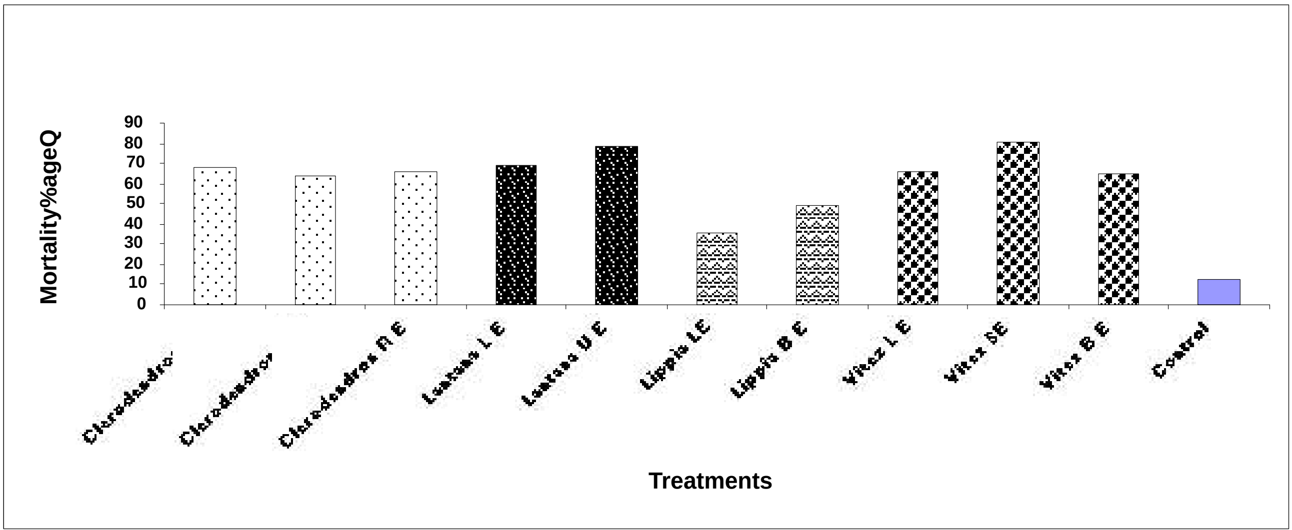


**Fig. 3.** Mean mortality % of *H. vigintioctopunctata* after 24hrs periods.

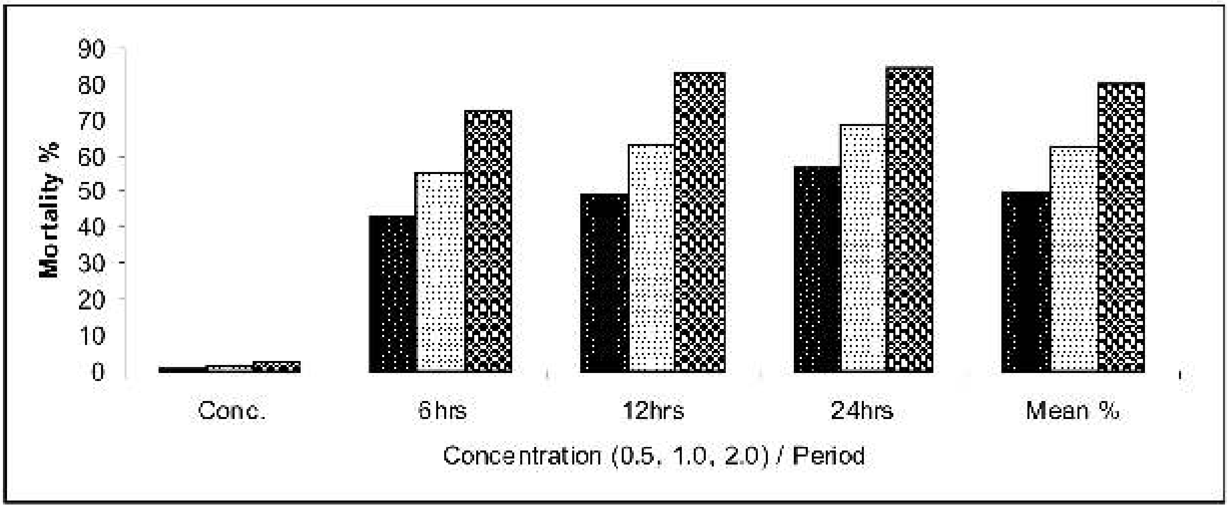


**Fig.4.** Mean mortality % of *H. vigintioctopunctata*. in concentrations and periods.

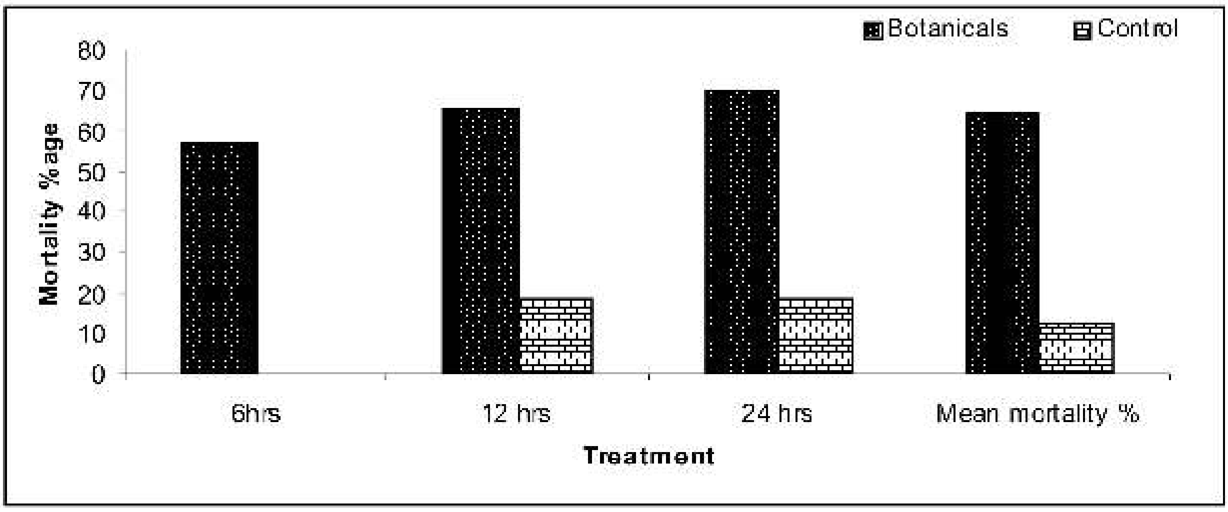


**Fig. 5.** Mean mortality % of *H. vigintioctopunctata*. in botanicals irrespective of control

**Table 5** : Mean mortality % of *H. vigintioctopunctata*. in botanicals irrespective of control.

| Treatments | 6 hrs | 12 hrs | 24 hrs | Mean mortality % |
|---|---|---|---|---|
| Botanicals | 57.00 | 65.10 | 69.89 | 63.99 |
| Control | 00.00 | 18.44 | 18.44 | 12.26 |

80.86 per cent grubs of *H.vigintioctopunctata* followed by extracts of *Lantana* unripe fruit (78.26 %), *Lantana* leaves (68.83%), *Clerodendron* leaves (67.90%), *Clerodendron* root (65.70%), *Vitex* leaves (65.50%), *Vitex* bark (64.66%), *Clerodendron* seed (64.16%), *Lippia* bark (48.93%), *Lippia* leaves (35.90%) respectively.

Similar mortality has also been reported by various workers, notably Bai and Kandaswamy, 1985; David *et al.*, 1988; Buiyah and Quiniones,1990; Raja and Albert, 2000). Kulkarni *et al.*, (1997) reported that reduction in insect-pests was due to the antifeedant properties of the extracts which caused mortality. The anti-insect responses of *Vitex negundo* was reported by Ajiwe and Okeke (1998). Rao *et al*. (2003) isolated from leaves of *V. negundo*, betulinic acid and ursolic acid and tested antifeedant activity against the larvae of castor semilooper *Achoea janata*. They concluded that ursolic acid showed more effective activity than the betulinic acid with larvae of *A. janata regarding mortility.*

Singh and Kanaujia, (2003) evaluated the insecticidal impact of NSKE (5.0 per cent) against the larvae of *Spilosoma obliqua* on castor. Saxena *et al.* (1992) found insecticidal responses of *L. camara* against *C. chinensis*. Ogendo *et al.* (2003) has evaluated the insecticidal and repellent properties of *L. camara* against stored maize grain of *Sitophilus zeamais*. They reported that after 21 days caused 85.0-93.7% insect mortality and repelled 65.0 and 62.5% of insects. These taken plants extract can be more effective as antifeedant and biopesticides for insect-pest management.

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