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Influence of Petroleum Ether Leaf Extract of Vitex Negundo On the Oviposition Deterrence of Adult Uzifly(Exorista Bombycis)

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ABSTRACT:- Considering the importance of botanicals in the *Silkworm*(*Bombyx mori*)pest management as an eco-friendly approach, neem oil and petroleum ether leaf extract of *Vitex negundo* has been tried to control Uzifly in the present study. Sixty fifth instar *Silkworm*(*Bombyx mori*)larvae were reared in the wire mesh cage after treating with 0.5, 1 and 1.5 µg of *petroleum ether leaf extract of Vitex negundo* / 1µl of acetone. Then a pair of two day old Uzi fly were released into the cage and allowed to oviposite for 48 hrs to observe oviposition deterrence. Controls and Carrier controls were maintained to compare the results and the experiments were replicated six times. The effect of 0.5µg, 1µg and 1.5µg of *Vitex negundo* leaf extract /1µl of acetone on the Uzifly oviposition on the fifth instar *Silkworm*(*Bombyx mori*)larvae recorded on an average was 136.67±0.715, 127.00 ±0.632 and 109.50±0.764 respectively. In the control and carrier control it was recorded as 169.33±0.615 and 164.67±0.333 respectively. From these results it is evident that petroleum ether leaf extract of *Vitex negundo treated* resultants reduced the oviposition of Uzifly on the fifth instar *Silkworm*(*Bombyx mori*)larvae compared to the controls.

Key words- *Vitex negundo, Uzifly, Silkworm(Bombyx mori)(Bombyx mori).*

I. INTRODUCTION

Among current alternative strategies aiming at decreasing the use of classical insecticides, ecochemical control based on plant-insect relationships is one of the most promising methods. For centuries, plants and insects have followed a parallel and interdependent evolution. Insects cannot live without plants and vice versa. Chemical mediators are used in interspecies communication, especially allelochemicals. These non-nutritional molecules, produced by an organism, modify the behaviour or the biology of an organism from another species (Mulatu *et al.*, 2000).

Consequently, plant allelochemicals exert a wide range of influences on insects: they can be repellent,deterrent or antifeedant; they may inhibit digestion, enhance pollination and capture with their attractive properties; they may increase oviposition or, contrarily, decrease reproduction by ovicidal and larvicidal effects. These molecules generally act at weak doses and have a specific action. Very few are toxic for mammals. Most of them are classed as secondary plant products and therefore have chemical structures that classify them as alkaloids, polyphenolics, terpenes and isoprenoids or cyanogenic glucosides (Strebler, 1989).

The use of plant extracts including allelochemical compounds with known affects on insects, could be a useful complementary or alternative method to the heavy use of classical insecticides. This could improve the biodegradability of insecticide treatments and therefore decrease the quantity of toxic insecticide residues, increase insecticide selectivity and develop a better respect for the environment (Heinmenberg, 1992).

Some plant extracts showed that a majority of the inventions focused on household uses. A cleaning solution including clove essential oils and pyrethroid would cleanse and destroy eggs and larvae, and leave a residue to prevent reinfestation by Blattaria. Several formulations were proposed to control mosquitoes and flies; some of them associated essential oils to pyrethroids (Kono *et al*., 1993) although Eucalyptus essential oil was used as a synergistic insecticide in addition to growth inhibitors (Narasaki *et al*., 1987). Spearmint, bitter almond and birch (*Betula lenta* (Betulaceae)) bark essential oils were incorporated into a mixture showing acaricide, insecticide and insect repellent properties (Matsumoto *et al*., 1987).

Vitex negundo is a much branched shrub with quadrangular tomentose, densely whitish tomentose branchlets up to 5 m tall or sometimes a small, slender tree. Its bark is thin and gray leaves are palmately compound, with three to six foliolate; leaflets are lanceolate, entirely or rarely crenate, terminal leaflets 5–10×1–3 cm, lateral leaflets smaller, all nearly glabrous above, whitish tomentose beneath. Flowers are bluish-purple and small, in peduncled cymes, forming large, terminal, often compound, pyramidal panicles. Fruit is a succulent drupe, black when ripe, 5–6 mm in diameter. Seed is 5–6 mm in diameter, invested at the base with enlarged calyx.

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The scented flowers are hermaphrodite (have both male and female organs) and are pollinated by insects and very effective pests (Ram *et al.*, 2001).

Some indigenous plant materials have been known for their effectiveness to reduce oviposition, egg hatchability and adult emergence of pulse beetle among them Indian privet (*Vitex negundo*) and neem tree (*Azadirachta indica*) have contact toxicity against insect pests (Rojesus *et al*., 1989).

Therefore, in the present study an attempt has been made to study the oviposition deterrence activity of the leaf extract of Vitex negundo against Uzifly Exorista bombycis which is a serious pest of Silkworm(Bombyx mori).

II. MATERIAL AND METHODS

A. *Collection and insect culture*

For the present investigations the Uzi fly maggots were collected from the silkworm cocoon market, kadiri, Ananapur (Dist.) immediately after they pierced out from their host body through cocoon shell (Fig.2.3.1). Some maggots were used for the treatments and some were allowed to metamorphose into pupa for treatments.

The untreated /treated resultants of third instar of maggots and zero hour pupae of Uzifly emerged as adult flies. They were fed with 10% glucose solution soaked in cotton swab (Sriharan et al., 1980) under natural light and temperature to observe the fecundity of Uzifly (Fig.2.3.2.1).

B. Silkworm rearing

Disease free eggs of the cross breed (PMxCSR2) silkworms were used in the present investigation. Silkworms were reared at room temperature. The mulberry leaves harvested from the irrigated mulberry garden were used as food for silkworm. Silkworms were fed four times daily .

C. Plant material Collection

The leaves of Vitex negundo were collected from Muddireddy palli village adjacent to Kadiri. Anantapur (District) and authenticated with vochur No. 987 (K. Madhava Chetty, Asst. Professor, S.V.University, Tirupati).

D. Vitex negundo Leaf extract

The collected leaves were washed thoroughly with running tap water and the excess moisture was removed. The plant material was shade dried at room temperature. Dried leaf powder (200g) of Vitex negundo was extracted with 1000 ml of Petroleum ether for about 48 hours at room temperature (Saxena and Yadav, 1983). Later solvent was evaporated to yield a dark greenish mass. The residue was kept separately in air tight container and stored in a Refrigerator until its use for experiments.

0.5, 1 and 1.5 µg of petroleum ether leaf extract of Vitex negundo was dissolved in 1 µl of acetone and used for the treatments.

LC50 value of neem oil against maggot, zero hour pupae, adult Uzifly and fifth instar silkworm larvae were determined as 1.7, 2.0, 2.4 and 3.5µl / µl of acetone and in case of petroleum ether leaf extract of Vitex negundo LC50 value recorded against maggot, zero hour pupae, adult Uzifly and fifth instar silkworm larvae were as 1.9, 2.2, 2.6 and 3.8 µg/ µl of acetone.

Sixty fifth instar silkworm larvae were reared in the wire mesh cage after treating with 0.5, 1 and 1.5 µg of petroleum ether leaf extract of *Vitex negundo* and then a pair of two day old Uzifly were released into the cage and allowed to oviposite for two days to observe oviposition deterrence. Controls and Carrier controls were maintained to compare the results and the experiments were replicated six times. Treated silkworm larvae were observed for Uzifly oviposition deterrence by counting number of eggs laid by the Uzifly on the treated silkworm larvae.

E. Statistical analysis

The results of the ovipositonal deterrence were expressed as mean ± standard Error (SE) and the data was subjected to ANOVA using sheffee Dunkan and Dunnet as post hoc test using SYTAT version 2011 ($P<0.05$) and ($P< 0.01$) .

III. RESULTS

The fifth instar larvae of Silkworm(Bombyx mori)were sprayed topically with 0.5µg, 1µg and 1.5µg of petroleum ether leaf extract of Vitex negundo with 1µl of acetone to observe the oviposition deterrence of Uzifly. *Controls and Carrier controls were maintained to compare the results and then two pairs of two day old Uzi fly were released into the wire mesh cage for 48 hrs of oviposition. The experiments were replicated six times.* 0.5µg, 1µg and 1.5µg of petroleum ether leaf extract of Vitex negundo sprayed on fifth instar Silkworm(Bombyx mori)larvae affected the oviposition of Uzifly and reduced the oviposition when compared to the control. It was observed that the oviposition of Uzifly on the treated Silkworm(Bombyx mori)was reduced with increase in the concentration of petroleum ether leaf extract of Vitex negundo. (Graph:4.1.2).

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A. Control and carrier control

The oviposition of Uzifly on the fifth instar Silkworm(Bombyx mori)larvae of the control and carrier control *was observed and recorded* on an average as 169.33±0.615 and 164.67±0.333 respectively. Uzifly oviposition deterrence was reduced on the fifth instar *Silkworm*(*Bombyx mori*)larvae treated with petroleum ether leaf extract of *Vitex negundo* when compared to the controls (Fig:3.1.1 & 3.1.2))

B. Treated

The effect of 0.5µg of petroleum ether leaf extract of *Vitex negundo* /1µl of acetone on the oviposition of Uzifly Oviposition of Uzifly on the fifth instar Silkworm(*Bombyx mori*)s treated with 0.5µg petroleum ether leaf extract of *Vitex negundo* /1µl of acetone was observed. It was noted that 136.67±0.715 eggs were laid by the Uzifly on the treated Silkworm(*Bombyx mori*)s.

The effect of 1µg of petroleum ether leaf extract of *Vitex negundo* /1µl of acetone on the oviposition of Uzifly 1µg of petroleum ether leaf extract of *Vitex negundo* /1µl of acetone sprayed on the fifth instar *Silkworm*(*Bombyx mori*)larvae were affected the oviposition of Uzifly and the viposition was reduced to 127.00 ±0.632 when compared to the control and carrier control Uzifly oviposition.

The effect of 1.5µg of petroleum ether leaf extract of *Vitex negundo* /1µl of acetone on the oviposition of Uzifly Oviposition of adult uzifly was affected due to spraying of 1.5µg of petroleum ether leaf extract of *Vitex negundo* /1µl of acetone on the fifth instar *Silkworm*(*Bombyx mori*)larvae. The eggs laid by the Uzifly on the treated Silkworm(*Bombyx mori*)s was observed and recorded as 109.50±0.764.

IV. DISCUSSION

The fifth instar larvae of *Silkworm*(*Bombyx mori*)were topically treated with 0.5µg, 1µg and 1.5µg of petroleum ether leaf extract of *Vitex negundo* with 1µl of acetone affected the oviposition of Uzifly over the c*ontrol and Carrier control (Fif:3.11, Fig:3.1.2)*

The oviposition is one of the most important events in the life cycle of insects. If oviposition is prevented, the insect life cycle is disrupted. In the present study the leaf extract of *Vitex negundo* act as oviposition deterrent against Uzifly, *Exorista bombycis* and Uzifly might be sensitive to phytochemical stimuli response to the odour of the petroleum ether leaf extract of *Vitex negundo,* this might have reduced the oviposition of Uzifly on the *Silkworm*(*Bombyx mori*)larval body as also observed by Rajkumar and Jebanesan(2009) working with oviposition activity of *Cassia obtusifolia* Linn (Family:Leguminosae) leaf extract against *Anopheles stephensi*.

The Uzifly deterred from the oviposition on the *Silkworm*(*Bombyx mori*)larvae treated with *Vitex negundo* leaf extract. The presence of secondary metabolites/ phytochemicals in petroleum ether leaf extract of *Vitex negundo* may be the cause for the reduction of oviposion of Uzifly. These results are in agreement with reports of Mani, (1989) *and* Zettler(2002).

In the present study, results of oviposition deterrency of Uzifly on the petroleum ether leaf extract of *Vitex negundo* treated fifth instar *Silkworm*(*Bombyx mori*)larvae reduced over the control. These results are in agreement with the reports of Murugesh *et al.,* (2010) where in they reported that the oviposition behaviour of the uzi fly was very much affected due to spraying of plant extracts i.e, *Eucalyptus citriodora* (59.53) *and Tridax procumbens* (60.34%) and *Parthenium hysterophorus* (62.03%). Loke *et al*., (1992) also reported that the plant extracts reduce the oviposition by *Plutella xylostella* with aqueous extract of neem.

The bioactive compounds present in the petroleum ether leaf extract of *Vitex negundo* may be the cause for the ovipositon deterrence of Uzifly on the treated fifth instar *Silkworm*(*Bombyx mori*)larvae as also reported by Liu *et al.*, (2006) working with *Chrysanthemum morifolium* on *Plutella xylostella*, by Elango *et al.,* (2009) and by Rajkumar & Jebanesan (2009) working with *Vitex negundo* against *Culex quinquefaciatus*.

Petroleum ether leaf extract of *Vitex negundo* may have showed fumigant activity against the Uzifly resulting in its oviposition detrrency on the treated fifth instar *Silkworm*(*Bombyx mori*)larvae as also reported by Zhao and Hou (2006) *Juniperus chinensis* against dimond black moth.

Allelochemicals present in the petroleum ether leaf extract of *Vitex negundo* could have reduced the oviposition of Uzifly on the treated fifth instar Silkworm(*Bombyx mori*)larvae as also reported by Saxena and Basit (1982) working with *Eucalyptus globulus* (Myrtaceae) and *Coriandrum sativum* (Umbelliferae) on *Amrasca devastans* (Homoptera: Cicadellidae).

Oviposition deterrence of the Uzifly on the petroleum ether leaf extract of *Vitex negundo* treated resultants of fifth instar *Silkworm*(*Bombyx mori*)larvae may be due to the presence of deterrence compounds present in the petroleum ether leaf extract of *Vitex negundo*. According to Raja *et al*., (2003), plant extracts on *Spodoptera litura* , Gajendran and Gopalan (1981) with *Parthenium hysterophorus* on

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Spodoptera litura, Veeranna and Nirmala (2001) with *Pongamia glabra* against Uzifly and Miah *et al.*,(1993) on *Callosobruchus chinensis* with nishinda (*Vitex negundo*) leaf powder are effective in reducing number of eggs laid by the insect pests.

The chemical components present in the petroleum ether leaf extract of *Vitex negundo* could have prevented the uzi fly from egg laying on the treated *Silkworm*(*Bombyx mori*)larvae by acting as deterrent as also reported by Barman *et al.,* (1990) working with eucalyptus oil on oviposition of *Exorista bombycis* by Urs and Srilatha (1990) with eucalyptus oil against *Corcyra cephalonica* and by Sathyaseelan and Bhaskaran (2010) with *Andrographis* leaf extract (52.3%), *Leucas aspera* leaf extract (49.2%) and Neem Seed Kernel Extract (47.5%) against mealy bugs.

The active fractions from petroleum ether leaf extract of *Vitex negundo* might have exhibited oviposition deterrent activity against Uzifly, *Exorista bombycis* on the treated fifth instar *Silkworm*(*Bombyx mori*)larvae as also reported by Hermawan *et al.,* (1994).

The active biomolecules present in petroleum ether leaf extract of *Vitex negundo* might have caused ovipositon deterrency against Uzifly on the treated *Silkworm*(*Bombyx mori*)larvae. These results are in agreement with the reports of Kannathasan *et al*.(2007), Upadhyay, *et al., (*2006), Sathyaseelan *et al.,* (2009) and Kannan *et al*., 2009).

The petroleum ether leaf extract of *Vitex negundo* may have modified the micro-environment there by discouraging the Uzifly to deter the oviposition on the treated fifth instar *Silkworm*(*Bombyx mori*)larvae as also reported by Kim *et al*., (2003) working on cinnamon (*Cinnamomum cassia*) bark against the pulse beetle.

The petroleum ether leaf extract of *Vitex negundo* has been found to possess oviposition deterrent activity due to the biological activity of extract and various compounds, including phenolics, terpenoids, and alkaloids present in it, these compounds may jointly or independently contribute to produce oviposition deterrent activity against Uzifly as also reported by Vijaya kumar *et al.,* (2011).

Present study results of ovipositon deterrency on the treated fifth instar *Silkworm*(*Bombyx mori*)larvae confirms that petroleum ether extracts compounds, having lower polarity are highly volatile and might have remained as effective deterrent and thereby reduced the oviposition of Uzifly on the treated fifth instar *Silkworm*(*Bombyx mori*)larvae with the petroleum ether leaf extract of *Vitex negundo* as also reported by Sadia Kanvil *et al.,* (2006).

The various phytochemicals of petroleum ether leaf extract of *Vitex negundo* could have reduced Uzifly oviposition on the treated *Silkworm*(*Bombyx mori*)larvae. The results of the present study are in agreement with the reports of Valterova, *et al*., (1997).

In present study, all the doses of petroleum ether leaf extract of *Vitex negundo* brought significant reduction in oviposition of Uzifly *Exorista bombycis* when compared to the control as also reported by Meena (2000) working on *Callosobruchus chinensis,* Gehlot and Singhvi (2006) with Eucalyptus leaf extract on *Callosobruchus maculatus* and by Rahman and Talukder (2006) with *Vitex negundo,* on *Callosobruchus maculates.*

From these results it is evident that petroleum ether leaf extract of *Vitex negundo* shows an effective ovipositon deterrent activity. This reduction in the oviposition of Uzifly on *Silkworm*(*Bombyx mori*)larvae reduces the formation of pierced cocoons and thereby improves the quality and quantity of the cocoons.

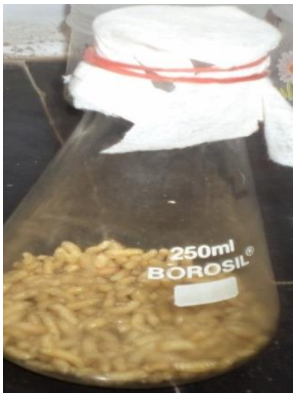

Fig: 2.3.1 - Third instar uzifly maggots collected from the Cocoon Market

Fig.2.3.2.1 – Uzifly pupae were allowed for emergence

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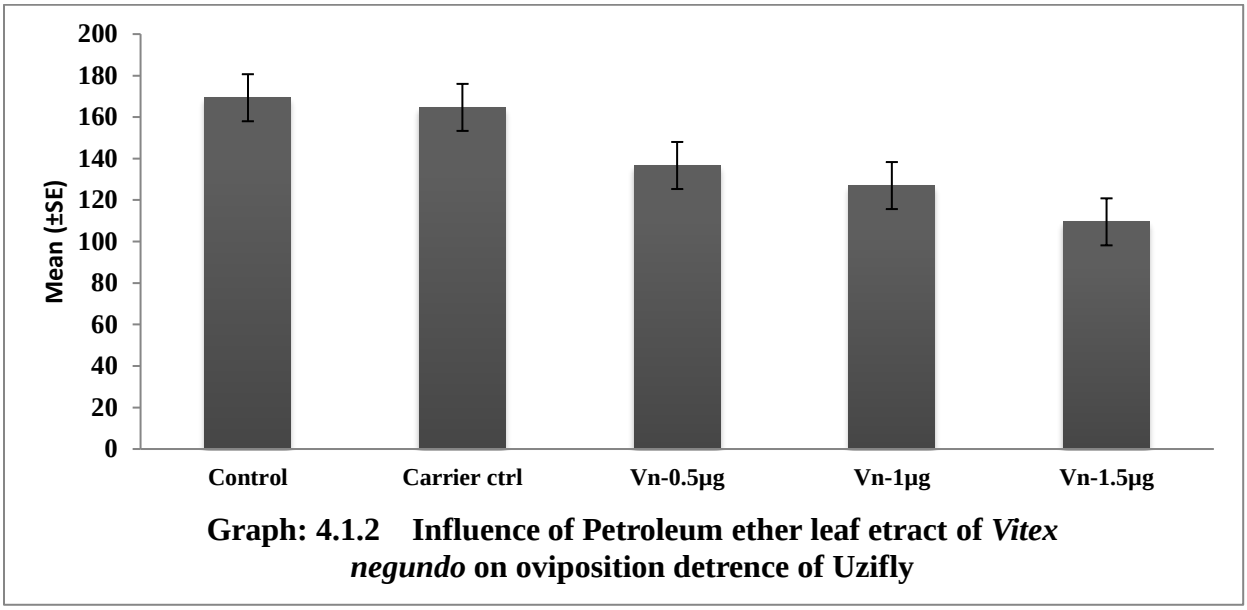
Fig. 3.1.1 - Oviposition of Uzifly on the fifth instar Silkworm larvae (Untreated)

Fig. 3.1.2 - Oviposition deterrence activity of Uzifl on the treated silkworm larvae with Petroleum ether leaf extract of *Vitex negundo*



Graph: 4.1.2 Influence of Petroleum ether leaf etract of *Vitex negundo* on oviposition detrence of Uzifly

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