Evaluation of Larvicidal Activity of *Sapindus Emargintus* (Family: Sapindaceae) Leaf Extracts against the Housefly Larvae (*Musca domestica)* LINN

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Abstract: ***The housefly, Musca domestica L., is a common vector of several bacteria, protozoan, and viral diseases. The botanical pesticides we evaluated the bioefficacy of crude extracts of sapindus emargintus against larvae of Musca domestica. The leaf extract of soapnut disrupt the activity of enzymes of larvae & pupae and inhibits the growth.The mortality was counted up to day 6. Hexane leaf extract of Sapindus emarginatus effective controlling the M. domestica. The percentage mortality rate of M. domestica larva when treated with different concentrations of hexane leaf extract shows the highest mortality 98.75 % was recorded in 2.0µl/100ml.The chloroform leaf extract shows the highest mortality was recorded in 1.5µl/100ml and 2.0µl/100ml at 5th day and 100% mortality found in 5th day and 6th day.The crude extract S. emarginatus will be the best alternative controlling of housefly larvae.***

Keywords: Housefly, Crude extract, Evaluation, *Sapindus emargintus*

1. Introduction

High population densities of the housefly can cause irritation and annoyance to employees, as well as reduction in egg and milk production in poultry farm (Fengsong Liu et al., 2012). India is also on the international map for poultry farming. Raipur-Rani is the second leading poultry belt in India after Tamil Nadu in South India(Vishal Dogra et al., 2010, Raja et al., 2015). In rural areas, houseflies cause irritation to livestock (Chinnaperumal Kamaraj et al., 2011), leading to decreased efficiency in various ways. They also raid urban areas and are a major annoyance in residential areas and business complexes (Axtell & Arend, 1990).

Flies feedstuff and rear on decomposing matter, human wastes and foodstuffs, and exist vectors of pathogens such as bacteria, protozoans, viruses and metazoan parasites and etc. (Barin et al., 2010). However, it is capable of surviving in any area with moderate temperature, water and food supply. It deeds as essential mechanical carriers of pathogenic bacteria, for example Shigella sp, Vibrio cholerae, Escherichia coli, Staphylococcus aureus, and Salmonella sp. However, the undeveloped stages have numerous industrial and medical applications (Feng X et al., 2010).

Chemical control methods have exposed decrease in fly density and the prevalence of fly-associated morbidities in various countries (Fengsong Liu et al., 2012). Among pesticides, organochlorines, organophosphates and, in recent times, pyrethroids have been used for housefly control (P.Kumar et al., 2011). However, houseflies can develop resistance to these pesticides (Scott et al., 2000; Shono & Scott, 2003; Srinivasan et al., 2008).

Housefly management relies heavily on pesticide application (Chintalchere, et al., 2013). However, houseflies hastily progress resistance to these insecticides Moreover, excessive use of synthetic insecticides results in superior pest renaissance as well as ecological/health problems. For instance substitute natural control of housefly could be very auspicious existence biodegradable as well as cost-effective (Bisseleua et al., 2008; Kalpana et al., 2014).

Man and animals, mostly depend on vegetable kingdom for their food many plants are categorized as poisonous plants (Santhosh Kumar et al., 2013). The plant *Sapindus emarginatus* belonging to the Family Sapindaceae is a collective huge tree, extensively distributed in North India, Deccan and Carnatic regions. This plant is chiefly known for its fruits that are in universal use as a substitute for soap (Gabble, 1967). The pericarps contain triterpene saponins, and are used as antifertility, antipruritic and anti-inflammatory mediators in traditional Indian and Thai medicine (Jain, 1976; Kanchanapoom et al., 2001). However, modern scientific medical study has investigated the use of soapnuts in considering migraines.Using soapnut is an operational and natural substitute to chemical insecticides. The present study to investigate the controlling of *Musca domestica* larvae using crude extraction from *Sapindus emarginatus* leaf.

2. Materials and Methods

A collection and rearing of *Musca domestica* larva

The housefly larvae were collected from poultry farm at Marimali Nagar, Kanchipuram district, in Tamil Nadu, India during January 2014 to March 2014. The collected larvae were brought to the laboratory and cultured under the laboratory conditions for experiments. These houseflies were reared in cylindrical boxes (90×140 mm) covered with muslin cloths and maintained at 28±2 ◦C, 65% relative humidity (RH) in a growth chamber. During rearing, flies were fed on a mixture of groundnut oil cake and wheat bran at a ratio of 1 : 3. Eggs were transferred to another box containing the same diet. Hatched larvae were transferred individually to cylindrical vials (28×12 mm) containing a semi-synthetic diet (constituents: 2 g groundnut oil cake, 5 g wheat bran, 2 g milk powder, 1 g honey mixed with 10 mL of water); this diet was changed daily until larvae reached the pupal stage to avoid any contamination. Pupae were transferred to Petri plates containing no diet or moisture. Field-collected flies were used in the repellency bioassays; larvae and pupae obtainedfrom the rearing ofthese field-collected flies were used in the larvicidal bioassays (Kumar et al., 2011). The 2ndintar larvae were used for this experiment.

Collection of *Sapindus emarginatus* leaf

The *Sapindus emarginatus* plant leafs were collected from Divakarapuram, Andhra Pradesh and were brought to the laboratory on December 2014. The collected leaves were shade dried in the laboratory condition at Loyola College, Chennai for extraction (Raja et al., 2015).

Preparation of extracts

Collected leafs were shade dried and finally powdered in a mixture grinder. The powdered material was then packed in paper bags & stored in air tight container until use. The 500 grams of dried powdered material was extracted using approx. The five hundred ml of hexane and chloroform was added to 50g of powdered leafs in a separate 500 ml conical flask. These flasks were placed in a dark place and mixed twice a day to prevent sedimentation. They were then subjected to a rotary extractor to get the respective crude extracts. The crude extracts were collected in a glass vials and stored in dark condition at room temperature. The extract was collected, Hexane and Chloroform was evaporated in a rotary evaporator under reduced pressure. The concentrated dried extract was collected in a glass vials and stored in room temperature for the bioassay (Raja et al., 2015).

Evaluation of leaf extracts

The clean and dried sixty four plastic containers was taken and labelled like, T_1, T_2, T_3, T_4 and control. The stored seed crude extract was taken in a containers and the concentration were prepared. Each one of the prepared crude extracts (i.e. hexane-leaf extract, hexane-seed extract, chloroform-seed extract and chloroform-leaf extract) was taken in four different concentrations of 0.5µl, 1.0µl, 1.5µl, 2.0µl using micropipette and diluted in 100ml of distilled water, respectively. Then, 20ml of each of the diluted extracts were introduced separately in the 64 containers containing the 20 counted larvae, respectively. A control container was set up. A container containing 20 larvae was added with 20 ml distilled water. The mouth of the containers was covered with muslin cloth to prevent larval escape. Four replicates were maintained for each concentration as well as the control. After 48 hours of exposure fresh untreated poultry manure were offered for all the treatment as well as control categories and the observations were made at 24 h intervals upto pupation(Raja et al., 2015).

Corrected mortality was calculated by Abbott's (1925) formulae as in the following formula:

$$\%\ Corrected\ per\ cent\ mortality\ \frac{\%T - \%C}{100\% - \%C} \times 100$$

Where % T= Percentage of dead test organisms.

% C = Percentage of dead control organisms.

For working mortality percentage mortalities were converted to correct per cent mortalities for control.

Data analysis

The collected data were generated and analyzed. Corrected percent mortality data were analyzed separatelywith the LSD test and Two way analysis of variance (ANOVA) (P=0.05) by using the software SPSS 11.5 for windows and Using R language to compare the mean between the concentration by Tukey (HSD) multiple pairwise comparisons of means 95% family-wise confidence level.

3. Results and Discussion

The data were analysed with suitable statistical software (SPSS 11.5) and the corrected per cent mortality using Abbott's formula. The two way ANOVA was analysed data and interpreted. The experiment shows the significant when compare to the different concentrations and averages of larval mortality.

Evaluation of hexane leaf extract against *M. domestica*

The botanical pesticide appear to effective in controlling the domestic insect pest *Musca domestica*. Hexane leaf extract of *Sapindus emarginatus* effective controlling the *M. domestica*. Figure 1 shows the percentage mortality rate of *M. domestica* larva when treated with different concentrations of hexane leaf extract, *viz* 0.5µl/100ml, 1.0µl/100ml, 1.5µl/100ml and 2.0µl/100ml for period of 6 days. There is a proportional increase in mortality with increase in incubation time as well as greater degree of mortality was reported at greater concentrations. At a concentration of 1.0µl/100ml, 1.5µl/100ml and 2.0µl/100ml, the mortality on day 2 was 70% and it shows a improved trend of up to 96.25% and the incubation time prolonged till day 6. The highest mortality 96.25 % was recorded in 2.0µl/100ml.The two way Analysis of variance shows that the larval mortality was highly significant at the given different concentration of hexane extract against *Musca domestica* at ($P \leq 0.05$) 0.5% level of significance (Table 1).

Evaluation of chloroform leafs extract against *M. domestica*

The figure 2 shows that chloroform leaf extract seem to be effective in controlling the domestic insect pest Housefly. The percentage mortality rate of *M. domestica* larva when treated with different concentrations of hexane leaf extract*viz* 0.5µl/100ml, 1.0µl/100ml, 1.5µl/100ml and 2.0µl/100ml for duration of 6 days. There is a proportional increase in mortality with increase in development time as well as greater degree of mortality was reported at higher concentrations. At a concentration of 0.5µl/100ml, the mortality very less associate to other concentration. The highest mortality was recorded in 1.0µl/100ml, 1.5µl/100ml and 2.0µl/100ml at 5th day. The mortality ranges from lower concentration 0.5µl/100ml from 61.25% to 81.25% and 100% mortality found the remaining three concentrations. The mortality was counted up to day 6.The two way Analysis of variance shows that the larval mortality was highly significant at the given dissimilar concentration of entomopathogenic fungi against *Musca domestica* at (P≤ 0.05) 0.5% level of significance (Table 3).

House fly, *Musca domestica L.* is found in all countries of the world but is further adaptable in warm regions. It is considered unique pests which cause health problems in the environment as it accompanies humans during their daily activity ubiquitously, together indoors and outdoors, on work situates or in rest residences causing considerably disruption to them (Albarrak, 2009). Flies interfere with work such as feeding and milking as well as house flies amplified frequency of animal disease transmission, prominent to increased suppository veterinary service costs, and better potential for spread of human diseases. The medical and veterinary pest *Musca domestica* L. has established resistance to most pesticides used against it. For this reason, there is a constant search for new alternative control tools (Douglass and Jesse 2002).

Jesikha (2012) reported that when compare to control all the stages of *Musca domestica* larva shows significant mortality percentage against Fruit extract of *Capsicum annuum*. The extract drastically affected the 1st, 2nd and 3rd instars in dose dependent manner. Begum et al., (2010) study the insecticidal efficacy of *Calotropis procera* and *Annona squamosa* ethanol extracts against 3rd instar larvae of *Musca domestica* and reported as the LC50 values of the extracts of *Calotropis procera* and *Annona squamosa* leaves were found to be 282.5 and 550 mgl-1, individually. The treatment of 3rd instar larvae of *M. domestica* with dissimilar concentrations of the leaf extracts of these two plants exhibited relatively lower percent mortality after shorter period of 24hrs than that at longer period of 48hrs (Sripongpun, 2008), studied the Contact toxicity of the crude extract of Chinese star anise fruits to house fly 2nd instar larvae. The median lethal concentration (LC50) values of the crude extract of Chinese star anise fruits, to the 2nd instar larvae at 24 and 48 hours were 7.4x104 and 4.1x104, respectively.

A massive sum of species showing countless potential as anti-insect agents belong to this genus. *Sapindus saponaria*, a tree widely distributed in Central and South America, is also habitually used as ornamental . Brazilian people commonly formulate household soap from this tree; and use its seeds to make handicrafts. Its wood is approximately used in construction. Its fruits and roots are popularly used as anesthetics, astringents, linctus and diuretics (Ferreira Barreto et al. 2006). Besides, its medicinal potential as healing and anti thrombotic agents has been studied. Research on that area has revealed that flavonoids in the leaf extracts are responsible for those activities (Meyer Albiero et al., 2002). On the other hand, much investigation has been keen to the anti-insect capacity of extracts from diverse organs of this plant. Boiça Junior et al. (2005), on their search for activity against larvae of the cabbage pest *Plutella xylostella* (Lepidoptera: Plutellidae) studied eighteen plant species from a diversity of families, discovery that aqueous leaf extract of *S. saponaria* was unique active products. The extract produced 100 % of mortality in tests where the larvae were offered cabbage foliage disks coated with the extracts to be evaluated as a sole food (Boiça Junior et al., 2005). In a different study, the aqueous fruit extract of this tree showed deterrent properties against alternative cabbage pest, *Ascia monuste orseis* (Lepidoptera: Pieridae). In this case the activity was equivalent to that showed by aqueous extracts of the neem tree, *Azadirachta indica*, the modern botanical pesticide in the market (Isman et al., 1996; Medeiros et al., 2007). The aqueous seeds´ extracts were assessed against another lepidopteran, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), showing strong effect on larvae development and midgut trypsin activity (dos Santos et al., 2008). Not only against lepidopterans has this tree revealed anti-insect potential, but also against other insect orders.

For instance, a saponin extract from fruits from this species showed toxicity against adults of the greenhouse whitefly *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) (Porras & Lopez-Avila, 2009); and complete ethanolic extracts from fruits have shown larvicidal and morphological alterations effects on the mosquito *Aedes aegypti* (Diptera: Culicidae) (Ferreira Barreto et al., 2006). Some other saponins presenting other kinds of biological activity, isolated from the fruits of this species, are shown in Figure 1 (Lemos et al., 1992; Ribeiro et al., 1995).

Sapindus emarginatus, another tree from this genus, widely distributed in India, has also demonstrated larvicidal activity of its fruit extract against three important vector mosquitoes: *A. aegypti*, *Anopheles stephensi* and *Culex quimquefasciatus* (Diptera: Culicidae) (Koodalingam et al., 2009). Later, this group has also investigated the impact of the extracts on the activity of mosquito phosphatases and esterases to gain an insight into the extent of disturbance in metabolic homeostasis inflicted upon exposure to the extract (Koodalingam et al., 2011). Previous reports on this species have shown that the pericarps contain triterpene saponins (Figure 1), which are commonly used as antifertility, antipruritic and anti-inflammatory agents in traditional Indian and Thai medicine (Jain, 1976; Kanchanapoom et al., 2001). Perhaps, anti insect activity may be due to saponins in this plant similarly to the case of *Sapindus saponaria* (Porras et al. 2009). Activity of members of this genus against other insect orders, further than dipterans and lepidopterans, has also been evaluated, including coleoptera and lice. That is the case of the extract from *Sapindus trifoliatus* fruit cortex which showed activity against the red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Mukherjee & Joseph, 2000).

In this case, weight gain was significantly reduced when larvae were fed on diets including the extract at different doses; and females topically treated -upon emergence- with the extract laid fewer viable eggs. (Mukherjee & Joseph, 2001). Another ethanolic fruit extracts, in this case from *Sapindus mukorossi*, also showed anti coleopteran activity against another pest of stored grains, *Sitophilus oryzae* (Coleoptera: Curculionidae) and also against *Pediculus humanus* (Phthiraptera: Pediculidae) (Rahman et al., 2007). Finally, from the methanolic extract of fruits of this species triterpenic saponins (Figure 1) have also been isolated and these natural products demonstrated their potential as growth regulators and antifeedants against *Spodoptera littura* (Lepidoptera: Noctuidae), both as glycosides and as free genines (Saha et al., 2009). In this particular study, it was verified that upon hydrolysis of the saponins, the growth regulatory activity was improved, whereas very little difference was found in regard to the antifeedant activity.

All in all, the genus *Sapindus* contains a variety of species which have been studied on their activity for some insects from different orders. In spite of the fact that not many reports do exist on the action of isolated compounds, the previous ethnobotanical uses of *Sapindus* spp. and the isolation of some active saponins from this genus, may suggest that this group of secondary metabolites might be related to the anti-insect activity. Saponins -glycosides of sapogenins containing a monosaccharide or a polysaccharide unit- reduce the surface tension becoming biological detergents. They are widely distributed secondary plant metabolites, found among almost 100 plant families (Bruneton, 1995). Being effective defenses for some insects (Plasman et al. 2001; Prieto et al., 2007), saponins have been implied in mechanisms of plant resistance against potential herbivores (Nielsen et al., 2010). The genus *Sapindus*, rich in this kind of compounds, may therefore be promissory raw material to develop plant pest control products. Further information can be found at recent works reviewing saponins from *Sapindus* spp. and their activity (Pelegrini et al., 2008; Sharma et al., 2011).

Based of bioactive analysis we conclude that the plant *Sapindus emarginatus* leafs are very effective controlling the insect pest of *Musca domestica*. The four concentration were prepared and treated with 2nd instar larvae all the four concentration was experiential significant mortality. The hexane and chloroform leaf extracts significant mortality. The *Sapindus emarginatus* plant extracts for best controlling the housefly larvae.

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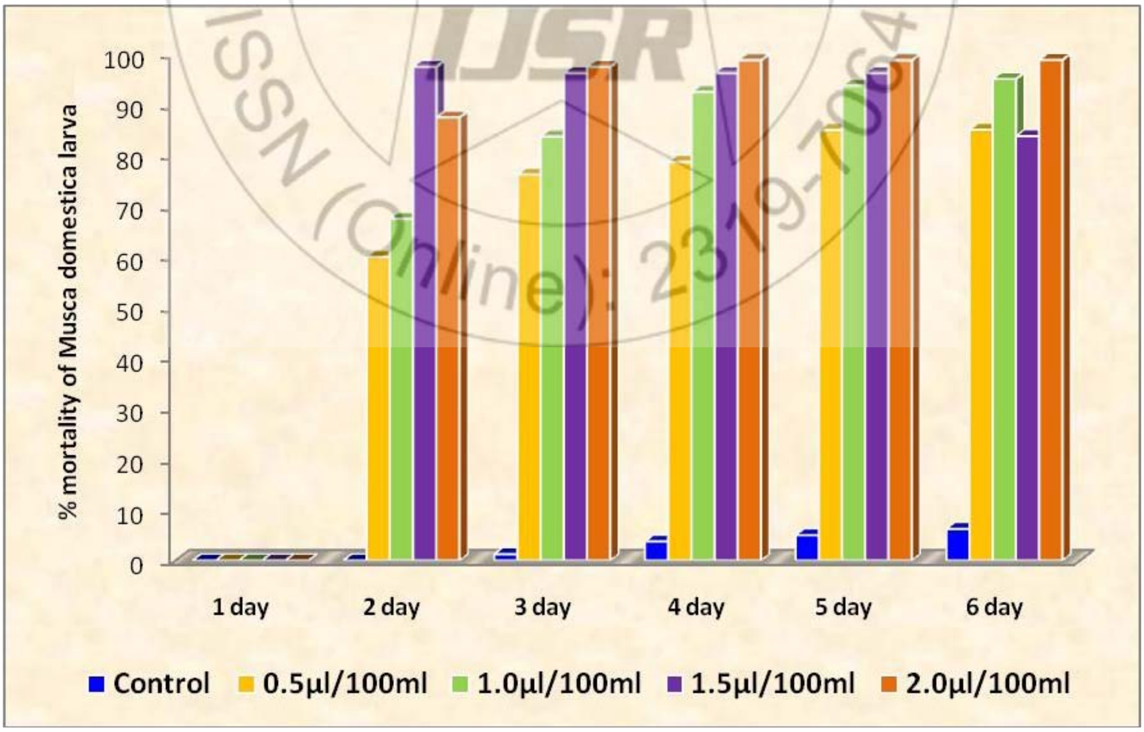

Figure 1: Evaluation of hexane leaf extract against *Musca domestica* larva at different concentration

Table 1: The ANOVA table showing significance of hexane leaf extract against *M. domestica* larva at different concentration

Source of variance	Df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	4	25127	6282	22.12	4.16E-07
Days	5	21789	4358	15.34	2.97E-06
Residuals	20	5680	284		

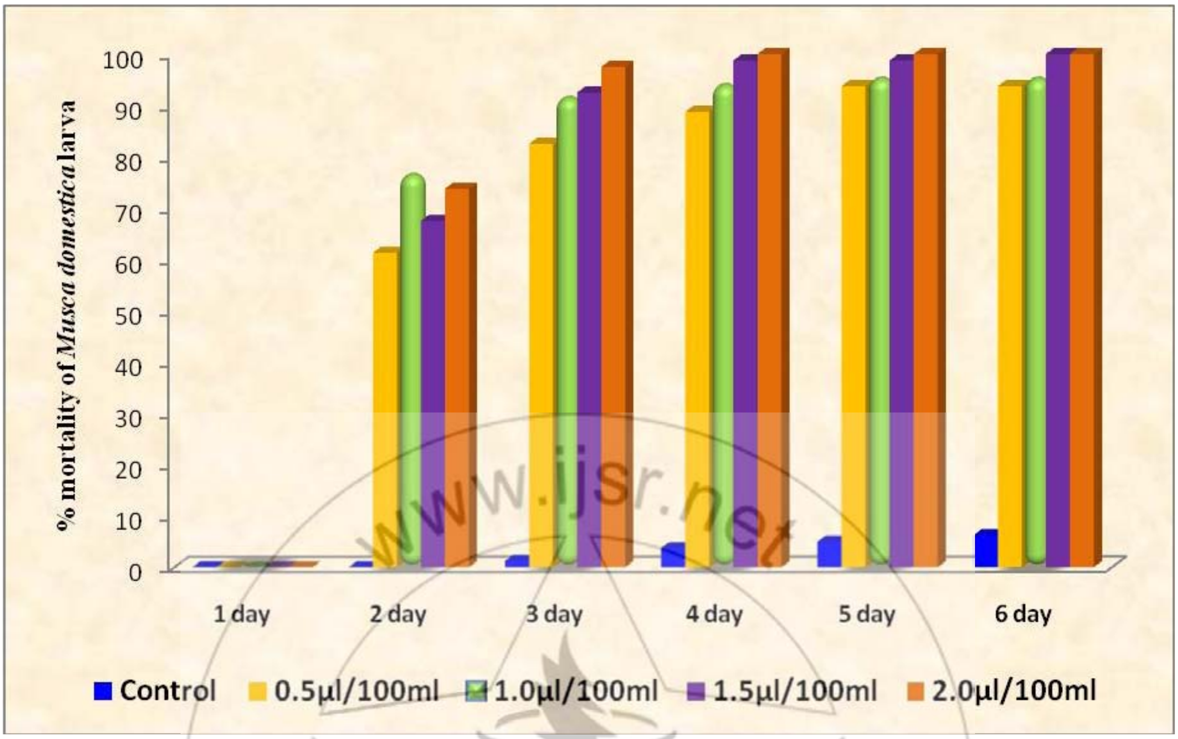

Figure 2: Evaluation of Chloroform leaf extract against *Musca domestica* larva at different concentration

Table 2: The ANOVA table showing significance of Chloroform leaf extract against *M. domestica* larva at different concentration

Source of variance	Df	Sum sq	Mean sq	F value	Pr(>F)
Treatment	4	25320	6330	23.16	2.87E-07
Days	5	23888	4778	17.48	1.08E-06
Residuals	20	5466	273		