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Lethal effect of *Thevetia peruviana* leaf extract on larval stages of *Musca domestica* (L)

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Abstract

The common house fly, *Musca domestica* L. (Diptera, Muscidae) is a major domestic insect pest, particularly in tropical countries. House fly management uses several integrated methods to control house fly populations. Chemical insecticides gives many problems such as insect resistance, toxic side effects to human or non-target organism and residues in the environment. Hence in the present study a new effort has been made to assess the larvicidal activity of *Thevetia peruviana* leaves, extracted with acetone and evaluated against the developmental stages of housefly *Musca domestica*. The result revealed on the age and concentration and is based on the effect of plant extract against the housefly larvae. LC_{50} and LC_{90} were noticed and the values are 153.169, 208.642, 251.332, 479.836, 587.287 and 614.775 ppm against I-III instar larvae respectively. The toxic effect were noticed in the order of I>II>III instar larvae respectively. The larvicidal activity may be due to the presence of phytochemicals alkaloids, steroidal glycosides, phenol, chlorogenic acid, flavonoid and tannin. The phytochemicals individually or synergistically cause the larval mortality.

Keywords: *Musca domestica*, *Thevetia peruviana*, Phytochemicals and LC_{50}.

Introduction

Housefly, *Musca domestica* L. is an important medical and veterinary pest that causes irritation, spoils food and acts as a vector for many medical and veterinary pathogenic organisms or may cause annoyance to humans and agronomic livestock, resulting in considerable economic loss in livestock business. The order Diptera presents an array of insects which more than any other group poses the greatest challenge to human and veterinary health as vectors of diseases. House flies can be found worldwide, except Antarctica. Therefore, they become very important in public health concern. House flies feed on liquid or semi-liquid substances beside solid material which has been softened by saliva or vomit. Because of their high intake of food, they deposit feces constantly, one of the factors that make the insect a dangerous carrier of pathogens. It acts as important mechanical carriers of pathogenic bacteria, such as *Shigella* sp, *Vibrio cholerae*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* sp. Some may also be the vectors of variety eggs of worm parasites (Ugbogu *et al*., 2006). Apart from disease transmission, *M. domestica* soils man's food and usually constitutes a nuisance, particularly the adult stage (Ande, 2001).

Control measure against this insect in the short-term is the use of conventional insecticides. Many insecticides such as organochlorines and organophosphates and more recently pyrethroids and spinosad, have been used for housefly control. The indiscriminate use of chemical insecticides has given rise to many well-known and serious problems, such as the risk of developing insect resistance and insecticidal residual for humans and the environment. The house flies not only resisted insecticides which were used as spray, but also the insecticides mixed in the baits (Kim *et al*., 1997). In order to search out alternate measures against this insect, biocontrol agents like parasitic

wasps *Pteromalids* gave satisfactory results in the control of house fly in the poultry shed, however, these have not proved successful in other situations (Crespo *et al*., 1998). These problems coupled with acute neuro-toxicity to man and his domesticated animals have stimulated the search for biologically based alternatives. Accordingly, botanical insecticides based on natural compounds from plants, are expected to be a possible alternative. They tend to have broad-spectrum activity, are relatively specific in their mode of action and easy to process and use. They also tend to be safe for animals and the environment (Belmain *et al*., 2001). Several studies have also looked at the possibility of using plant extracts in the control of eggs, larvae, pupae and adults of *M. domestica* (Issakul *et al*., 2004; Malik *et al*., 2007).

Screening of plant extracts for deleterious effect on insects is one of the approaches in the search of novel biological insecticides. Insecticidal activity of many plants against several insects has been demonstrated. Seed as well as foliar extracts of several plants have been reported to have toxic and potent growth reducing activity to insects. The deleterious effect of plant extracts or pure natural/ synthetic compounds on insects can be manifested in several manners including toxicity, mortality, antifeedent, growth inhibitor, suppression of reproductive behavior and reduction in fecundity and fertility (Taskin *et al*., 2004). Plants are well known producers of diverse kind of chemical compounds and many products that are used for defend plant against different kinds of pests (Isman and Akhtar, 2007). Many plants have been reported about their potential insecticidal actions on larvae and/or adults of house flies via crude extracts or extracted active compounds. Some results also showed their effects on metamorphosis or emergence or fecundity or life span of house flies (Russell *et al.*, 1976; Gbewonyo and Candy, 1992; Shalaby *et al.*, 1998; Liao, 1999; Morsy *et al.*, 2001; Singh, 2001; Sukontason *et al.*, 2004; Abdel Halim and Morsy, 2006).

The plant tissues extracted, climatic and growth conditions, variation in cultivation and the methods used for extraction and analysis affects the composition of oils from particular species. For this reason there have been considerable efforts to examine the effects on individual components that are common to those essential oils known to have insecticidal properties (Koul *et al.*, 2008). From all above it is evident that plant originated pesticides can prove great beneficial for pest/vector control. In the present study we investigated the bioactivity of crude extract of *T. peruviana* against the larval stages of housefly, *M. domestica*.

Materials and Methods

Rearing of *Musca domestica*

Houseflies were collected from the fields and were reared at 28± 2^{0}C and 60% - 70% RH in the laboratory. They were transferred to cages (45X45X45cm), covered with mosquito net. Jaggery, water and milk powder with groundnut oil cake were kept an adult meal. Through mixing of groundnut cake with water (200g/100ml) and dry rice bran (100gm) was used as medium for oviposition kept in a separate bowl in the rearing cage. From the bowl the eggs were collected every six hours are transferred to a plastic bowl containing the prepared medium for larval development. The rearing medium was briefly stirred thrice daily to avoid fungal growth. The culture was maintained with proper diet and used for further experiments.

Preparation of plant extract

The leaves of *T. peruviana* were collected from the field and chopped into small pieces with the help of a knife and dried under shade at room temperature (27 ± 2^{0}C) for about 20 days. The completely dried leaves were powdered with an electrical blender and sieved to get fine powder. The powder was stored in airtight containers for further analysis. The plant powder was extracted with acetone by using Soxhlet apparatus for 8 hours. The extracts were concentrated using a vacuum evaporator at 45^{0}C under low pressure. After complete evaporation of the solvent, the concentrated extract was collected and stored in glass vials at 4^{0}C in refrigerator for further experiments. One gram of concentrated extract was dissolved in 100 ml of the acetone, kept as a stock solution. This stock solution was used to prepare the desired concentrations of the extract for exposure of the housefly larvae.

Larvicidal bioassay

The I, II, III instar larval stages of house fly were used for the larvicidal bioassay. From the stock solution 100-500 ppm concentrations were prepared with wetted rice bran and the freshly moulted larvae were introduced. After 24 hours the mortality was calculated and tabulated. Mortality in control was negligible in calculation. The percentage of larval mortality was corrected by Abbot's formula (Abbott, 1925). LC50, LC90 values were calculated from toxicity data by using probit analysis (Finney, 1971).

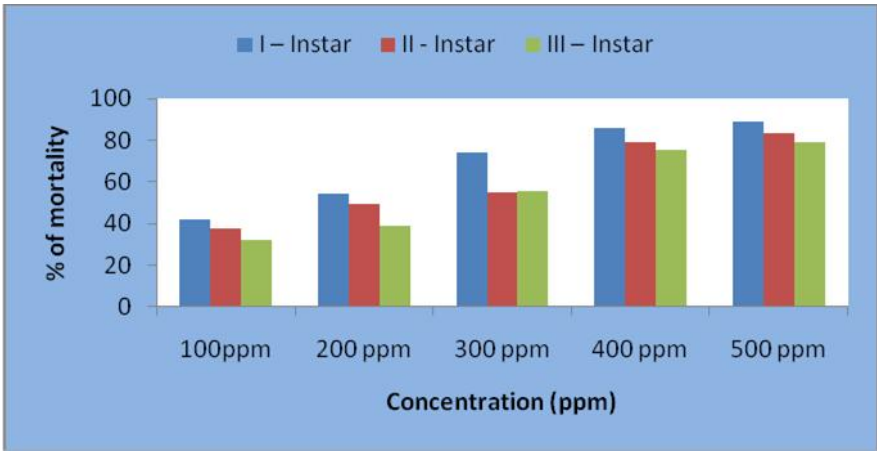
Preliminary phytochemical analysis

The extracts were subjected to preliminary phytochemicals tests to determine the groups of secondary metabolites present in the plant materials as follows alkaloids, carbohydrate, steroidal glycosides, saponis, tannin, phenol, chlorogenic acid, flavonoids, coumarim, anthocyanin and terpenoid (Harborne, 1998).

Results and Discussion

Bioassay tests were conducted to find out the effect of *T. peruviana* plant extract on the larval forms (I, II and III) of *M. domestica* were treated with different concentrations (100-500ppm) for 24hours. The mortality percentages ranging from 41.8-89.1% for I instar larvae; 37.5- 83.3% for II instar larvae and 32.1-78.8% for III-Instar were treated with 100-500 ppm of acetone extract of *T. peruviana* respectively (Fig.1). The noticed LC_{50} (and LC_{90}) values of methanol extract of *T. peruviana* were 153.169 (479.836) ppm for I-instar larvae; 208.642 (587.287) ppm for II-instar larvae; 251.388 (614.775) ppm for III-instar larvae respectively (Table.1). Mortality caused by the plant extract might be due to the presence of differential toxic ingredients in the methanol extract of *T. peruviana*.

Table: 1. Larvicidal effect of acetone leaf extract of *T. peruviana* against different larval instar of *M. domestica* treated with 24 hours.



Values are expressed by mean ± SD of three samples

Table 1. LC_{50} and LC_{90} values acetone leaf extract of *T. peruviana* on I, II, III instar larvae of *M. domestica*

Larval and pupal stages	LC_{50} (ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	Regression equation	Chi-Square (χ^2)
I – Instar	153.169 (103.767-189.375)	479.836 (431.733-552.433)	Y=-0.601+0.140X	1.721
II – Instar	208.642 (162.601-244.432)	587.287 (519.167-696.108)	Y=-0.706+0.137X	3.619
III – Instar	251.382 (213.799-284.143)	614.775 (545.859-722.669)	Y=-0.887+0.138X	2.798

LCL- Lower confidential limit; UCL- Upper confidential limit

Similarly, Eucalyptol is one of the principle constituents in *E. globules*, has been reported to be very toxic to male housefly at LD_{50} of 118µg/fly (Sukontason *et al*., 2004). The essential oil of *Citrus sinensis* was found the most potent among 12 oils against the adult stage of *M. domestica* recording LC_{50} of 3.9mg/dm^3. GC/MS analysis revealed that limonene (92.47%), linalool (1.43%) and β myrcene (0.88%) were the principle components of the essential oils of *C. sinensis* (Palacios *et al*., 2009). Many studies have drawn attention to the toxic effects of plant extracts on Dipterans with respect to the different plant constituents and tolerate levels of the tested insects (Dhar *et al*., 1996; Malik *et al*., 2007). In paralleled studies, ethanolic extract of *P.nigrum, A. indica, C.aegyptiaca* and *C. indicus* were found to possess

the highest potency among the bioassayed candidates against the larval stage of *M. domestica*, in addition to producing different forms of developmental effects to the treated larvae (Mansour *et al*., 2011).

The residual toxicity of a pesticide, for a specific short period, after application is required to achieve higher degree of pest control; especially for insects of frequent visiting to the sprayed area such as houseflies, mosquitoes, cockroaches and other pest of medical importance. Several reports were published on agricultural pest such as *Spodoptera littorallis* (Meisner *et al*., 1981) and the mite *Amblyscius follacis* (Bostanian *et al*., 1985). Chavan (1984) reported the residual activity of neem fraction NP-2 against the mosquitoes found the product was effective upto 9 days (68% Mortality) at 100 ppm. Organophosphorous (eg. Chloropyrifos) and carbomate (e.g. Methomyl) insecticides are toxic to insects and mammals by virtue of their ability to inactivate the enzyme acetylcholinesterase, which is a class of enzymes that catalyze the hydrolysis of the neurotransmitting agent acetylcholine; leading the poisoning (Fukuto, 1990). Synthetic pyrethroids (eg. Deltamethrin) are generally recognized as neurotoxicants that act directly on excitable membranes related to their ability to modify electrical activity in various part of the nervous system. Those effects is caused by a sterioselective and structure-related interaction with voltage dependent sodium channels, the primary target site of pyrethroids (Vijverberg *et al*., 1982).

In the present investigation methanol extract of *T. peruviana* was subjected to preliminary phytochemical analysis. The result showed the presence of alkaloid, glycosides, phenol, chlorogenic acid and terpenoid (Table.2). Studies have also established that the activity of phytochemical compounds on target species varies with respect to plant parts from which they are extracted, solvent of extraction, geographical origin of the plant and photosensitivity of some of the compounds in the extract, among other factors (Sukumar *et al*., 1991). The co-evolution of plants with insects has equipped them with a plethora of chemical defenses, which can be used against insects. Since botanicals are less likely to cause ecological damage, a large number of plants have been screened for their insecticidal activities against different insect pests and some of these have been found to be promising specifically on related Dipterans (Malik *et al*., 2007). Botanical products have become more prominent in assessing current and future pest control alternatives. Over the past two decades, surveys of plant families have discovered sources of new botanical.

Table 2: Preliminary phytochemical analysis of acetone leaf extract in*T. peruviana*

S. No	Phytochemical Constituents	Name of the Test	Plant extract
1	Alkaloid	Mayer's test Dragendroff's test Wagner Test	- + +
2	Carbohydrate	Molish Test Fehling Test Benedicts Test	+ - -
3	Steroidal Glycosides	Libermann's test Salkowaski test	+ +
4	Saponin	Foam Test	-
5	Tannin	Lead Acetate	+
6	Phenol	Phenol reagent	+
7	Chlorogenic acid	Ammonia test	+
8	Flavonoids	Ammonia test	+
9	Coumarin	Sodium chloride test	-
10	Anthocyanin	H_2So_4 test	-
11	Terpenoid	Borntrager's test	+

+ Present of compounds; - Absent of compounds

Most studies on the synergistic, antagonistic and additive toxic effects of binary mixtures involving phytochemicals have been conducted on agricultural pests rather than pests of medical importance. In an attempt to explain synergistic activity involving phytochemicals. Thangam and Kathiresan (1991) summarized that synergism might be due to phytochemicals inhibiting the insect ability to use detoxifying enzymes against synthetic chemicals. Identifying these synergist compounds within mixtures may lead to the development of more effective biopesticides as well as the use of smaller amounts in the mixture to achieve satisfactory levels of efficiency. Indeed, joint action may well prolong the usefulness of synthetic insecticides that will eventually be unusable due to resistance (Shaalan *et al*., 2005). Synergistic action with conventional chemical pesticides determined in the present study could be exploited for integrated Pest Management (IPM) programs. The results of the present investigation reveal the broad spectrum toxic properties of the methanol extract of *T. peruviana* against the developmental stages of *M. domestica.*

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