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Oroxylum indicum leaf extract effect as ingestion based method on larvicidal activity of *Musca domestica*

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Abstract

Musca domestica L. (Diptera: Muscidae), is a common insect pest capable of transferring numerous pathogens. The current study evaluated the larvicidal activity of *Oroxylum indicum* leaf extracts against *Musca domestica* using ingestion-based method. Leaf extracts were prepared using five solvents: hexane, chloroform, ethyl acetate, ethanol, and acetone. The larvicidal assay was conducted on second-instar larvae under controlled laboratory conditions. Mortality, pupal emergence, and adult emergence were recorded at 24 and 48 hours post-treatment. Among the extracts, the ethanol extract showed the highest larvicidal activity at 24 hours and 48 hours post treatment. In contrast, ethyl acetate extract exhibited the lowest larvicidal effect at 24 hours and 48 hours post treatment. Hexane, chloroform, and acetone extracts demonstrated moderate larvicidal activity. Larvae treated with hexane, chloroform, ethanol, and acetone extracts failed to develop into the pupal stage, whereas ethyl acetate-treated larvae showed a pupal emergence rate of 20%. No adult emergence was observed in any of the solvent extract treatments. These findings suggest that *Oroxylum indicum* leaf extract, particularly in ethanol, has significant potential for the management of *M. domestica* larvae using ingestion-based method and could be explored as a natural larvicide in integrated pest management programs.

Key words : *Oroxylum indicum*, house fly, ingestion-based method, larvicidal, pupal emergence.

The housefly, Musca domestica (Diptera: Muscidae), is a prominent clinical and veterinarian pest that causes annoyance, spoils food, and serves as a carrier for over 100 different infections. It poses a major hazard to human beings and animals[10]. It has been discovered that both livestock and human myiasis are caused by fly larvae[1,5]. Using synthetic insecticides to get rid of flies and practicing proper cleanliness and sanitation are

important ways to keep people and animals healthy[13]. To control the number of house flies, synthetic pesticides are frequently employed. But today, resistance has emerged to almost every kind of pesticide used to manage it, and it is a worldwide problem[15]. The improper application of these synthetic pesticides has led to a number of major issues, such as toxin persistence in the environment and biomagnifications via trophic levels that have a negative impact on people[16]. Hence, in order to find viable substitutes for traditional pesticides, researchers are always searching for active natural compounds derived from plants[4]. The plants are potential options for managing insect infestations because of their ecotoxicological qualities, which include lesser toxicity to people, affordability, ease of development and decomposition, and less harmful effects on the environment[8]. Globally, a variety of plant species have been used to manage the population of dipteran pests[11]. Among the plants, *Oroxylum indicum* has been widely investigated for its insecticidal activity[3,14], however, similar studies with *Musca domestica* has not been undertaken. The aim of this research is to evaluate the larvicidal activity of *Oroxylum indicum* leaf extracts on second-instar larvae of *M. domestica* using ingestion-based method.

Preparation of plant extract :

Fresh leaves of *Oroxylum indicum* were procured from Sindhudurg, Maharashtra. Plant identification was done by Botanical Survey of India, Pune. The fresh leaves were cleaned, and dried under the shade. The leaves were then kept in hot air oven at 60 °C overnight to completely remove moisture from the samples. Oven dried leaves were ground into fine powder. The powder was used freshly for metabolite extraction. The powdered leaves were extracted using ethanol, ethyl acetate, acetone, chloroform, and hexane separately following a Soxhlet extraction method. The solvent in the extracts was evaporated with air drying[2].

Culture of *Musca domestica* :

The study was carried out at the Department of Zoology, Modern College of Arts, Science and Commerce, Ganeshkhind, Pune. *Musca domestica* strains were captured from four different areas to obtain heterotic population. Identification of Strains of *Musca domestica* was done by Zoological survey of India, Pune. The house flies were reared under controlled laboratory conditions of 28 ± 2 °C temperature and 60 –70% relative humidity. Food grade jar of 30 cm diameter and 40 cm height with its opening enclosed with cloth sleeves at the front were used for breeding. The glass pipette was used to place milk and sugar solutions and oviposition trays. A 3% sugar solution-soaked cotton swab was placed in the cage to provide sustenance. An adult diet consisting of 50% glucose and 50% MacConkey agar powder was prepared and provided daily. Newly emerged flies were given fresh milk-soaked cotton swab for three days to enhance egg production, followed by milk-sugar solution[12]. Once the larvae reached the second instar stage they were collected and used for bioassay.

Bioassay test (Ingestion-based method) :

The botanical extracts were evaluated and tested using standard methods with slight modifications[9]. The larvicidal assay was

conducted on second-instar larvae under controlled laboratory conditions. Bioassays were conducted by exposure of 30 larva to ingestion-based method in petri plate. 500 microliter of the extract solution at a higher concentration of 0.2 mg/mL was prepared and mixed in 3gm of wheat dough for the preparation of extract based larval food. Bioassays were conducted under controlled conditions of 28–30 °C and 65–70% humidity. Mortality, pupal emergence, and adult emergence were recorded at 24 and 48 hours post-treatment. Wheat dough used as the absolute control. Each experiment was performed in triplicate. Pure solvents were used as negative control for each extract. Corrected mortality percentage (Abbott's formula) was calculated 24 and 48 hours post-treatment. Standard error of the mean and one way ANOVA was carried out at 95 % confidence level through IBM SPSS version 24 software.

$$\text{Corrected mortality (\%)} = \left(\frac{\text{\% mortality in treatment group} - \text{\% mortality in control group}}{100 - \text{\% mortality in control group}}\right) * 100$$

The plant extracts showed significant larvicidal activity at 24 and 48 hours post-treatment. Among the extracts, the ethanol extract exhibited the highest larvicidal activity, with a corrected mortality of 90 ± 4.7% at 24 hours and 91.7 ± 3.4% at 48 hours. In contrast, ethyl acetate extract demonstrated the lowest larvicidal effect, with 0 ± 0% mortality at 24 hours and 25 ± 5.9% at 48 hours. Hexane, chloroform, and acetone extracts exhibited moderate larvicidal activity. Larvae treated with hexane, chloroform, ethanol, and acetone extracts failed to develop into the pupal stage, whereas ethyl acetate-treated larvae showed a pupal emergence rate of 20%. No adult emergence was observed in any of the solvent extract treatments. The botanical extracts examined here show strong insecticidal

Table-1. Larvicidal activity of *Oroxylum indicum* leaf extracts against *Musca domestica* by ingestion-based method

Plant extract	Number of larvae	Corrected mortality (%)		Pupa emergence (%)	Adult emergence (%)
		24 hours	48 hours		
Hexane	30	80 ± 4.7	83.3 ± 3.4	0	0
Chloroform	30	10 ± 4.7	16.7 ± 3.4	0	0
Ethyl acetate	30	0±0	25 ± 5.9	20	0
Ethanol	30	90 ± 4.7	91.7 ± 3.4	0	0
Acetone	30	70 ± 4.7	87.5 ± 5.9	0	0
Control (Absolute)	30	0 ± 0	0 ± 0	80	100
Control (Negative)	30	0 ± 0	0 ± 0	80	100

The values followed by ± is the standard error of the mean. All the means are significantly different from each other at $p<0.05$ level of significance by Tukey's HSD post hoc analysis.

efficacy. The larval mortality may be linked to interference with neurophysiological processes, potentially involving acetylcholinesterase inhibition, but this hypothesis needs validation through targeted biochemical assays[6,7] or the active constituents may interfere with insect metabolic processes, potentially affecting enzyme-mediated detoxification or hormone regulation, though the specific pathways remain to be identified[17]. In earlier studies higher antifeedant activity was observed in *Oroxylum indicum V.* (81.66%) against third instar of *Scirpophaga incertulas* (W.)[14]. The methanolic plant extract of *Oroxylum indicum* showed 81.5% repellency and 68.30% mortality at 12% concentration against *C. chinensis* (L.)[3]. However, similar studies with *Musca domestica* has not been undertaken. The results obtained in the present study highlight the activity of *Oroxylum indicum* against larval stage of *Musca domestica*. The current study would certainly suggest *O. indicum* as the potential candidate to manage larval stage of the *Musca domestica*. Hence, it becomes applicable to develop active formulations. In ANOVA post hoc analysis using Tukeys HSD, all means were found to be significantly different from each other at $p<0.05$ level of significance.

In this study, the larvicidal activity of *Oroxylum indicum* leaf extracts was evaluated against *Musca domestica* using ingestion-based method. The findings of the present investigation suggest that *Oroxylum indicum* leaf extract, particularly in ethanol, has significant potential for the management of *M. domestica* larvae and could be explored as a natural larvicide in integrated pest management programs. Further studies with a range of concentrations and identification of active phytochemicals are recommended to elucidate the dose-response relationship and mechanism of action

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Conflict of Interest :

Authors have no conflict of interest.

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