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Toxicity of Leaf Extracts of *Ricinus communis* L. (Euphorbiaceace) Against the Third Instar Larvae of *Musca domestica* L. (Diptera: Muscidae)

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Abstract: The housefly, *Musca domestica* is a ubiquitous insect that has potential to spread wide variety of pathogens to humans and livestock animals leading to diseases and huge economic losses in developing countries. Flies have resisted human attempts to control them since antiquity and the problem of fly resistance to synthetic insecticides had resulted in need to develop biopesticides as an alternative management tool. Plant product are the most promising sources and under extensive trials for their insecticidal activity against various insect species. This study evaluated the efficacy of crude extracts of the castor plant *Ricinus communis* against *Musca domestica* by using dipping and thin film technique. The laboratory bioassay in both techniques resulted in considerable larval and pupal mortalities indicating toxicity of plant extract against the fly. Besides, the larval mortalities the extracts induced developmental aberrations such as reduced pupations and non emergence of adults. The results indicate that the plant extracts contain certain active principles which interfere with the hormonal control of development affecting the life cycle of the fly. It can be concluded that crude extract of *R. communis* can be effectively used as in controlling fly populations of *M. domestica* as the safer, ecofriendly and economic alternative to synthetic insecticidal agents.

Keywords: *Ricinus communis, Musca domestica*, Dipping, Thin Film

1. Introduction

The common house fly, *Musca domestica* L. is a worldwide pest of veterinary and public health importance throughout the recorded history [1]. The ability of the fly to flourish on the vast variety of organic substratum has enabled it to exploit virtually any area inhibited by humans and animals. The fly, being the vector of various pathogens such as bacteria, virus, protozoa etc. is reported to be menace to human as well as livestock [2]. Various communicable diseases like cholera, typhoid, poliomyelitis, typhus fever and dysentery among humans are the result of oral-fecal contamination due to activity of the housefly [3]. Recent concern about the food born human diseases have endorsed the role of housefly in spreading disease causing organisms such as *Salmonella typhi*, *Vibrio cholerae*, *Shigella* spp. [4, 5].The larvae of the fly can also be myiasis producing agents in human and animals leading to huge economic looses particularly in livestock industry [6-8]. Apart from disease transmission, high population density of *M. domestica* causes annoyace and food spoilage [9].

Over the decades, synthetic insecticides such as organophosphates, carbamates and pyrethroid insecticides have been used in short term control of this fly [10-12]. Housefly quickly develops resistance to these pesticides, leading to global problem and is proving havoc due to their ability to develop metabolic and behavioral mechanisms to avoid chemical insecticides [13]. *M. domestica* had developed resistance to DDT within the few years after its introduction [14, 15]. Moreover the use of synthetic anti-myiatic agents like avermectins among livestock animals has been found to cause contamination of dairy products like milk and meat with drug residues resulting in serious health hazard among humans [16]. Continuous increase in biomagnifications of these synthetic insecticides at each trophic level in the target and non target organism and high cost of chemical insecticides has provoked researchers to develop plant based insecticides [17]. The co-evolution of

plants with insects has equipped them with the surplus bioactive components, which can be used against insects. The use of plant extracts as an alternative to synthetic products to control housefly populations could be very promising since these are eco-friendly, biodegradable as well as cost effective. A large number of plants have shown the remarkable insecticidal activities against a large number of insect pests [18-20].

Ricinus communis- member of family Euphorbiaceace, is a weed widely distributed in countries like Asia, South Africa, Brazil and Russia [21]. The plant was selected for its easy availability and presence of reported bioactive components which interfere with the life cycle of the insect pests [22]. Studies of aerial parts of the plant have reported the presence of active constituents like ricin, ricinine, N-demethylricinine, and flavonoids [22, 23]. Ricin is the most toxic bioactive component present in seeds but ricinine which is an effective insecticide is located in all parts of the plant. These compounds have shown remarkable insecticidal, antifeedent and repellent activities [23, 24]. Studies have reported toxic effects of *R. communis* extract against arthropod vectors like ticks, mites and mosquitoes. Brahim et al. [25] studied the toxicity of aqueous extracts of the plant against mosquito larvae of *Culex pipiens, Aedes caspius, Culiseta longiareolata* and *Anopheles maculipennis* (Diptera: Culicidae). The leaf extract of *R. communis* has been shown to posses insecticidal properties against insect pests like *Spodoptera frugiperda* [26]; *Callosobruchus chinensis* [27] and *Cosmopolites sordidus* (Coleoptera: Curculionidae) [28]. Several studies have reported the toxic effects of various plant extracts in control of fly populations of *M. domestica* [29-32]. However no study was available regarding toxicity of *R. communis* against the fly. The present study was therefore undertaken to evaluate the efficacy of crude extracts of *R. communis* on the third instar larvae of *M. domestica* using dipping and thin film technique.

2. Materials and Methods

2.1. Collection and Preparation of Plant Extracts

Leaves of *Ricinus communis* were obtained from waste lands near Khalsa College Amritsar (Punjab) India. The collected plant material was given a dip in water to remove dust and then kept to dry at room temperature for about two weeks. Completely dried plant material was powdered using electric mill and was kept for extraction. Powdered plant material was further extracted successively with four different solvents viz. methanol, ethyl acetate, chloroform and petroleum ether using soxhlet extractor. The extracts were filtered over sodium sulphate using Whatman filter paper in case of each solvent. The collected extracts were evaporated under reduced pressure using rotary vacuum evaporator. So as obtain completely dry extract, the concentrates were then kept at 40 – 45°C in hot air oven. The crude extracts of each solvent were weighted and kept in vials in deep freezer for further use.

2.2. Fly Culture

M. domestica flies were collected from nearby areas with the help of a sweep net and reared in the laboratory using insect cages of 45x45x45 cm size. Adult flies were fed on a mixture of 10% (w/v) sugar and multi vitamin syrup solution. Goat meat was kept in separate petri plates as substrate for oviposition. The egg masses were incubated at 25-30°C and the larvae were reared on goat meat till pupation.

2.3. Experimental Application

2.3.1. Dipping Method

1020 third instar larvae of *M. domestica* were used in this experiment, 255 for each solvent. Larvae for each solvent were divided into four groups with 60 larvae each i.e. four replicates each with 15 larvae and a 5th group with 15 larvae were used as control. 3rd instar larvae were treated by dipping them in different concentrations of extract for 30 seconds and ethanol alone in case of control group. Concentrations for each solvent used in this experiment were prepared by mixing crude plant extract in ethanol (Table 1). The larvae of each replicate were kept in a rearing jar covered by muslin cloth. The replicates were kept in an incubator at 35°C and mortality rates were recorded daily for seven successive days. The survived larvae were observed to demonstrate the effects of extracts on their development till fly emergence.

2.3.2. Thin Film Technique

1020 third instar larvae of *M. domestica* were used in batches of 255 larvae for each solvent and distributed as mentioned previously. The concentrations in each solvent were prepared as above and are listed in Table 2. The crude plant extract was poured in petri plates (4 cm diameter) and left until dryness so as to obtain thin film of the extract. Larvae were released on the thin film so obtained and were covered thereafter. Larvae were examined daily for seven consecutive days to record the mortalities and to observe their development till adult emergence.

2.4. Parameters Used

The effect of *R. communis* extract on development of *M. domestica* larvae was evaluated by using following four different parameters viz. % larval mortality, % pupation, % pupal mortality and % adult emergence. Larval mortality was recorded daily for 7 days. The % age pupation was recorded by counting the number of viable, turgid and dark brown colored puparia after subtracting the dead larvae. The %age adult emergence was recorded daily after 7-10 days of pupation.

2.5. Statistical Analysis

The data collected from larval mortality, pupation, pupal mortality and adult emergence were analysis of variation (ANOVA) and LC_{50} values were calculated using Probit analysis [33]. SPSS (16.0) software is used to test the differences between the various concentrations.

3. Results

Table 1 shows the percentage larval and pupal mortalities in *Musca domestica* following exposure to crude extract of *R. communis* in four different solvents viz. methanol, ethyl acetate, chloroform and petroleum ether both in dipping and thin film technique. The lethal concentration (LC_{50}) values in different solvent of *R. communis* in the methods are shown in Fig 1 and 2. The results show that there were significant differences ($P<0.05$) in mean mortality for all the four solvents when compared with control. The LC_{50} values recorded in case of dipping method were 3g/100ml, 2.5g/100ml, 1.5g/100ml, 5.5g/100ml in methanol, ethyl acetate, chloroform and petroleum ether extract respectively. Thus, according to larval mortalities the effect of extracts of *R. communis* on larvae of *M. domestica* can be arranged as chloroform> ethyl acetate> methanol> petroleum ether. Larvae, who escaped mortality, pupated normally but all of them did not emerge to adults in various concentrations showing pupal mortality. Similarly, the LC_{50} values in case of thin film technique were recorded as 2 mg/cm^2, 0.5 mg/cm^2, 0.3 mg/cm^2, 1.6 mg/cm^2 in methanol, ethyl acetate, chloroform and petroleum ether extracts respectively. According to LC_{50} values the effects of the extracts were in the order chloroform > ethyl acetate > petroleum ether > methanol. In thin film technique the larval mortalities were almost higher as compared to dipping method as shown in Table 2.

Developmental characteristics such as the prolongation of prepupation period and adult emergence were severely affected and noticed in almost all the treated groups. Larvae from the groups treated with crude plant extract pupated after 9-11 days while those from the control group pupated after 6-7 days. Pupation and adult emergence rates were found to be reduced to as low as 24% and 12.75% in dipping method and 10% and 25.55% in thin layer technique both in chloroform extracts.

Table 1. *Effect of crude extracts of R. communis on development of third instar larvae of M. domestica using Dipping Method.*

Solvent	Conc. (g/100ml)	Larval Mortality (%)	Pupation (%)	Pupal mortality (%)	Adult Emergence (%)
Methanol	10	72.66±1.63	27.34±1.63	57.60±2.50	42.33±1.22
	5	66.66±1.63	33.34±1.63	53.34±1.63	46.66±2.98
	2.5	56.55±1.33	43.45±1.33	51.36±1.63	48.64±3.12
	1.25	44.66±1.63	55.34±1.63	47.13±1.63	52.87±1.58
	Control	0.00±00	100±00	0.00±00	100±00
	P value	0.000	0.000	0.000	0.000
Ethyl Acetate	4	53.33±2.98	46.67±2.98	63.34±2.49	36.66±1.33
	2	46.66±2.11	53.34±2.11	54.25±1.63	45.75±1.50
	1	38.00±2.90	62.00±2.90	48.12±2.26	51.88±2.45
	0.5	34.80±4.90	65.20±4.90	44.45±2.50	55.55±1.58
	Control	0.00±00	100±00	0.00±00	100±00
	P value	0.000	0.000	0.001	0.001
Chloroform	6	76.00±4.00	24.00±4.00	87.25±2.49	12.75±2.26
	3	60.00±1.63	40.00±1.63	66.67±2.98	33.33±1.85
	1.5	52.00±2.98	48.00±2.26	61.23±1.63	38.77±1.17
	0.75	46.00±1.63	54.00±1.63	58.56±1.33	41.44±1.58
	Control	0.00±00	100±00	0.00±00	100±00
	P value	0.000	0.000	0.000	0.001
Petroleum ether	7.5	58.00±1.63	42.00±1.63	77.78±1.63	22.22±1.63
	3.5	46.03±2.49	53.97±2.49	73.34±1.63	26.66±2.98
	1.75	21.33±2.49	78.66±2.49	65.35±1.63	34.65±2.12
	0.875	16.00±1.63	84.00±1.63	55.56±1.63	44.44±2.58
	Control	0.00±00	100±00	0.00±00	100±00
	P value	0.001	0.000	0.000	0.000

Table 2. *Effect of crude extracts of R. communis on development of third instar larvae of M. domestica using Thin Film Technique.*

Solvent	Conc. (mg/cm^2)	Larval Mortality (%)	Pupation (%)	Pupal mortality (%)	Adult Emergence (%)
Methanol	5	68.45±2.11	31.55±2.11	60.00±2.20	40.00±2.11
	2.5	54.00±2.11	46.00±2.20	64.00±2.33	36.00±4.00
	1.25	48.66±1.63	52.00±2.33	55.56±2.59	44.44±1.63
	0.625	40.00±2.33	60.00±2.59	42.67±1.63	57.33±1.63
	Control	0.00±00	100±00	0.00±00	100±00
	P value	0.000	0.000	0.001	0.000
Ethyl Acetate	2	75.67±2.49	24.33±1.27	66.67±1.63	33.33±2.11
	1	67.33±1.63	32.67±1.63	60.00±2.11	40.00±1.63
	0.5	58.66±2.49	41.34±2.00	43.34±1.63	56.66±2.11
	0.25	46.66±1.63	53.34±1.63	37.66±1.63	62.34±1.85

Solvent	Conc. (mg/cm^2)	Larval Mortality (%)	Pupation (%)	Pupal mortality (%)	Adult Emergence (%)
Chloroform	Control	0.00±00	100±00	0.00±00	100±00
	P value	0.000	0.001	0.000	0.000
	3	90.00±5.58	10.00±17.3	71.45±1.31	28.55±1.63
	1.5	86.66±5.58	13.34±17.3	67.55±1.63	32.45±1.63
	0.75	66.66±1.63	33.34±1.63	55.56±1.85	44.44±1.63
	0.375	48.34±1.63	51.66±2.11	43.33±2.49	56.67±1.31
Petroleum ether	Control	0.00±00	100±00	0.00±00	100±00
	P value	0.000	0.000	0.001	0.000
	3.8	66.66±2.67	33.34±1.63	66.37±1.54	33.63±1.63
	1.9	59.99±1.63	40.01±2.11	51.43±2.49	48.57±2.26
	0.95	47.67±1.63	52.33±4.00	56.22±2.98	43.78±1.85
	0.47	38.99±1.63	61.01±1.63	36.37±1.54	63.63±1.63
	Control	0.00±00	100±00	0.00±00	100±00
	P value	0.000	0.001	0.000	0.000

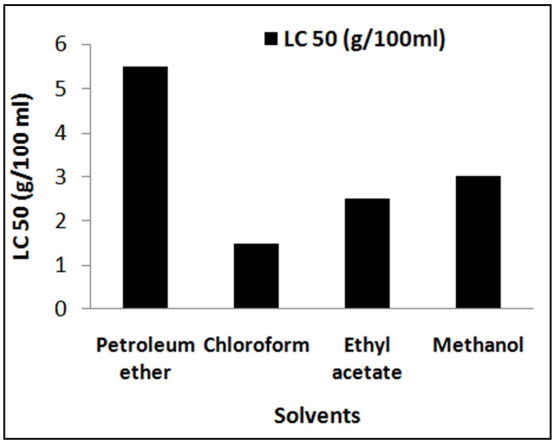

Fig. 1. *Toxicity of R. communis extracted with different solvents against third instar larvae of M. domestica using Dipping Method.*

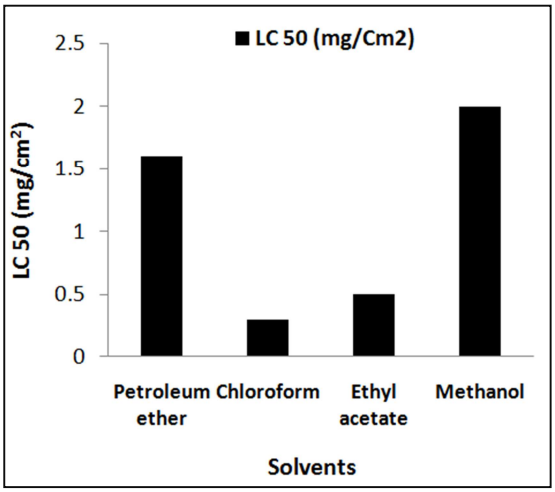

Fig. 2. *Toxicity of R. communis extracted with different solvents against third instar larvae of M. domestica using Thin Film Technique.*

4. Discussion

Laboratory bioassays in present study evaluated the efficacy of crude extracts of *R. communis* on the third instar larvae of *M. domestica* using dipping and thin film technique. The results showed that the extracts of *R. communis* in all the solvents had toxic effects against the third instar larvae of *M. domestica* in both the methods. Larval mortality may be due to penetration of bioactive components of the plant extracts into the larval body through oral route or body wall in dipping and thin film method respectively. It has been reported that feeding behavior of the larvae was altered due do injurious effects caused by the active plant components that damaged epithelial lining of the gut [34]. The present study resulted in mortalities in all the four solvents in the order of chloroform > ethyl acetate > methanol > petroleum ether.

The chloroform extract of *R. communis* showed the highest larval and pupal mortality. Similar results were reported in a study evaluating the toxicity of castor plant against the adult grass grub- *Costelytra zealandica* showing highest activity in chloroform extract. Ricinine was identified as main toxic substance by mass spectrometry [35]. The maximum activity of chloroform extracts might be due to the fact that ricinine, the potent insecticidal component of *R. communis* has been reported to have maximum solubility in chloroform [36]. Ricinine is a neurotoxic alkaloid that can paralyze and kill the insects [37]. It has been reported to have insecticidal activity against insect pest like *Spodoptera frugiperda* (Lepidoptera: Noctuidae) [38], *Atta sexdens rubropilosa* (Hymenoptera: Formicidae) [39] and *Myzus persicae* (Homoptera: Aphididae) [40]. The ethyl acetate extract showed the highest larval mortality after chloroform extract. Similar results were studied where ethyl acetate extract of *R. communis* showed highest mortality rate at lowest LC_{50} 0.390g/l while hexane extract was second followed by ethanol extract against *Anopheles arabiensis* [41]. Methanol extract showed lesser larval mortality than ethyl acetate extract. Lopez et al. [38] studied that the methanol leaf extract of *R. communis* showed 100% mortality against larvae of *Spodoptera frugiperda* at 24,000 ppm whereas the activity initiated at 560 ppm. Petroleum ether extract showed the least mortality among all the four extracts. Batabyal et al. [42] reported the toxicity of *R. communis* against *Culex quinquefasciatus* in which the carbon tetrachloride extract was observed to be most effective with LC_{50} 144.11 ppm, followed by methanol extract with LC_{50} at 91.62 ppm. The petroleum ether extract was the least efficient with LC_{50} 390.26 ppm.

The developmental anomalies observed in the present study had been reported in the number of insect pests following exposure to plant extracts. Azadirachtin, one of the active ingredients of *A. indica* has been reported to have disrupting effects on insect growth and development including prolongation of larval or pupal stages and inhibition of moulting [43]. Various mechanisms of action have been put forward to explain these effects. The prolonged larval or pupal periods generally observed followed by exposure to plant products indicate that they interfere with the hormonal control of moulting [44]. Flavonoids are the phytochemicals constituting 5-10% of the known plant secondary metabolites. These are involved to exert toxic effects on insects which include insecticidal, antifeedent, antimicrobial, ovicidal and oviposition deterrent activity. Flavonoids isolated from the *R. communis* have demonstrared considerable insecticidal activities against *Callosobruchus chinensis* [27]. Insecticidal activity is mainly due to inhibition of certain vital enzymatic pathways, in which flavonoids block hydroxylase enzyme by action of cytochrome- P450 which is involved in regulation of moulting process of insects [27]. Flavonoids have also been reported to affect the insect ecdysone-20-monooxygenase, which is responsible for the synthesis of 20-hydroxyecdysone, a vital precursor of insect growth hormone- ecdysone. The hormone is responsible for regulating the life cycle of the insects since it initiates moulting and hence they grow into adults. Any obstruction in synthesis of the hormone affects the duration of prepuation period and adult emergence rates. Prolongation of prepupation period and non emergence of adults among the treated larvae in the present study can be attributed to the hindrance in biosynthesis of ecdysone by flavonoids present in leaf extract of *R. communis.*

5. Conclusion

It can be concluded that leaf extracts of *R. communis* tested in present study can be useful in controlling fly population of *M. domestica.* The results indicate that the plant extract can cause larval mortality and developmental anomalies in the life cycle of the fly and can prove to be a safer alternative to conventional synthetic insecticides which are known to contaminate food chain leading to severe ailments among humans. Since easily accessible, the *R. communis* extracts can prove to be cost effective and eco-friendly pest control agents. There is great potential for the plant to be taken up for development of biopesticides in the near future.

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