The bioefficacy of crude extracts of Azadirachta indica *(Meliaceae) on the survival and development of myiasis-causing larvae of* Chrysomya bezziana *(Diptera: Calliphoridae)*

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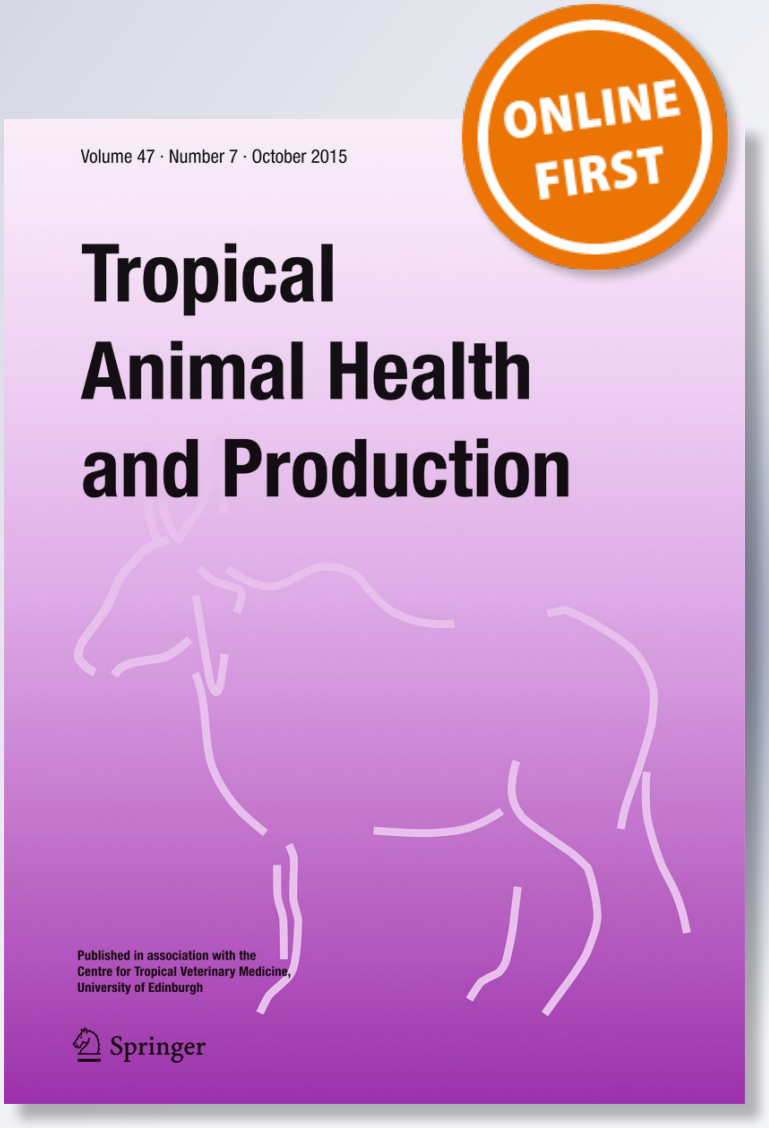

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REGULAR ARTICLES

The bioefficacy of crude extracts of *Azadirachta indica* (Meliaceae) on the survival and development of myiasis-causing larvae of *Chrysomya bezziana* (Diptera: Calliphoridae)

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Abstract Myiasis is a type of parasitosis originating from the invasion of tissues of live humans and other vertebrates by dipteran larvae. The Old World screwworm fly—*Chrysomya bezziana*—is known worldwide in the tropical regions for causing myiasis among man and domestic animals, thereby leading to health hazards and severe economic losses to the dairy farmers. Management techniques for controlling populations of the fly are needed to minimize these losses. Plant-derived materials have been increasingly evaluated these days in controlling the insects of medical and veterinary importance. This study evaluated the efficacy of crude extracts of the plant neem, *Azadirachta indica*, against *C. bezziana*. The dried leaves of the plant were extracted successively with four different solvents viz. petroleum ether, chloroform, ethyl acetate and methanol and were evaluated against the third instar larvae of *C. bezziana* using dipping method and thin film application technique. In the dipping method, larvae were dipped in four different concentrations of plant extracts for 30 s, whereas in the thin film application, they were exposed to a thin film of plant extracts. The results showed that all the extracts had toxic effect on the larvae in both the techniques. In the dipping method, the highest mortalities were recorded in methanol extract followed by chloroform, petroleum ether and ethyl acetate extracts with LC_{50} values 1.07 g/100 ml, 1.7 g/100 ml, 3.39 g/100 ml and 4.9 g/100 ml, respectively. In the thin film application method, methanol extract showed the highest mortalities followed by chloroform, ethyl acetate and petroleum ether with LC_{50} values 0.4 mg/cm^2, 0.6 mg/cm^2, 2.1 mg/cm^2 and 2.5 mg/cm^2. It is concluded that the crude extracts of *A. indica* can be used in controlling the larvae of *C. bezziana* by using the dipping as well as thin film application technique.

Keywords Myiasis · Neem · *Chrysomya bezziana* · Dipping · Thin film

Introduction

Myiasis is the infestation of live humans and other vertebrate animals with dipteran larvae which at least for a certain period feed on the host's dead or living tissue, liquid body substances or ingested food (Zumpt 1965). The diseases of domestic animals lead to considerable reduction in their productive traits and mortality among them which render the poor farmers to face great economic losses. The economic loss to Australian livestock industry due to myiasis had been estimated to be US$200 million a year (Anon 1979). Although no such estimate has been reported in India, similar huge economic loss due to myiasis in domestic animals is apprehended and it poses a major threat to the livestock industry in India as well. The Old World screwworm (OSW) fly—*Chrysomya bezziana* (Villeneuve)—is known to be the predominant fly species responsible for causing myiasis among domestic animals thereby causing significant economic losses to the livestock industry in the tropical regions all over the world. The fly is a worldwide pest of cattle and sheep which occurs throughout Africa, India, Arabian Peninsula, Southeast Asia, Indonesia, Philippines and islands of New Guinea (Norris and Murray 1964; Spradbery and Kirk 1992). The larval infestations impair the animal's physiological functions resulting in economic losses in terms of reduction in milk and meat production (Hall and Wall 1995). The feeding activity of the larvae

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generally causes serious tissue damage, resulting in loss of condition, deficiency of blood, injury to the hide and secondary invasions among domestic animals (Humphery et al. 1980). Parasitized animals often become restless and bite or rub the affected areas. They do not feed properly and become poor in health, debilitated and in severe cases may result in death if left untreated (Guerrini 1988; Schnur et al. 2009). The fly had been reported to cause myiasis in 95 % of the cases among cattle, sheep, horses, dogs and pigs from Australia (Norris and Murray 1964) and 99 % cases from India (Narayan and Pillay 1936). Similarly, *C. bezziana* was reported to cause myiasis in pet dogs from Hong Kong (Chemonges 2003) and other countries (McNae and Lewis 2004). Besides animals, the fly has also been found responsible worldwide for the onset of human myiasis in majority of the cases (Singh and Singh 2015). Reproductively mature flies are attracted to open wounds with foul-smelling purulent discharge, to lay their eggs. On hatching, the larvae invade the broken skin and with their cephalopharyngeal hooks burrow into the dermal layers and start feeding on the tissue, resulting in enlargement of preexisting wounds. The fully grown third instar larvae emerge from the tissue, drop to the soil and then pupate to complete the metamorphosis to the adult stage. The duration of pupal stage varies from 7 to 9 days under tropical conditions, but may last up to 8 weeks during subtropical winter months (Zumpt 1965). After completing the pupal period, the adult flies emerge out of the pupae.

A large number of synthetic products are being used these days to control myiasis, which are non–biodegradable and moreover lead to pollution thereby disturbing the delicate ecological balance. Synthetic antimyiatic agents like ivermectin have been reported to cause contamination of milk and meat products with drug residues which enter the food chain and result in serious side effects among humans (Kaneene and Miller 1997). Plant-derived materials being biodegradable are strongly considered to be the alternate remedy to synthetic products in the control of myiasis-causing larvae. Flora grown in the Indian subcontinent includes a large number of plants which have medicinal importance. The potential of these plants can be harnessed to solve various problems of the country including those of agricultural, health and economic sectors. Neem plant—*Azadirachta indica*—prevalent in India, Bangladesh, Thailand, Nepal and Pakistan, is referred to as "miracle tree" and has been exploited extensively in ecological, medicinal and agricultural sectors (Atawodi and Atawodi 2009).

A. indica has been chosen because it is easily available and contains a number of bioactive compounds which affect the life cycle of the insect pests. Leaves of the plant contain limonoids and tetranortriterpenoids, the most effective of which are azadirachtin, meliantrol, salanin and nimbin (Maheswaran and Ignacimuthu 2012). Out of these, azadirachtin is an important bioactive compound, which shows remarkable insecticidal, antifeedant and repellent activities (Debashri and Tamal 2012). The studies are focussing these days to investigate the activity of plant extracts as an alternative to chemical-based insecticides in controlling the insect pests of medical and veterinary importance. Most of the studies available in the literature regarding the effect of plant extracts on insect pests have been conducted on parasitic arthropods like ticks, mites and mosquitoes, whereas only a few studies were available on myiasis producing flies. Morsy et al. (1998a) reported the larvicidal activity of acetone and chloroform extract of three plants viz. *Cymbopogon citratus*, *Artemisia cina* and *Punica granatum* against the third instar larvae of *Chrysomya albiceps*. Extracts of plant *Nerium oleander* were found to be effective against *C. albiceps* fly (El-Shazly et al. 2000). Volatile oils of *Chenopodium ambrosioides* and *Thymus vulgaris* were reported to be effective against the third instar larvae of *Lucilia sericata* (Morsy et al. 1998b). Although *C. bezziana* is responsible worldwide for causing myiasis among man and domestic animals, no report was available in literature evaluating efficacy of *A. indica* plant extract against it. The only study available in literature was regarding the larvicidal efficacy of essential oil of betel leaf—*Piper betle*—on larvae of *C. bezziana* (Wardhana et al. 2007). The present study aimed at evaluating the efficacy of crude extract of *A. indica* against myiasis-causing larvae of *C. bezziana* using dipping and thin layer application methods.

Material and methods

Larval source and identification

The live larval samples were collected in glass vials containing 70 % alcohol from myiasis-affected dairy animals from various locations and were brought to the Post Graduate Department of Zoology, Khalsa College Amritsar (Punjab), India. So as to identify them, the larvae were processed for preparing permanent mounts of taxonomically important body regions like anterior and posterior spiracles. The larvae were identified as the third instar larvae of *C. bezziana* with the help of keys available in the literature (Zumpt 1965). The anterior spiracles of *C. bezziana* has four to six lobes, whereas the posterior spiracles are surrounded by highly sclerotised peritreme which is incomplete ventrally and contains three oblique slit-like spiracular openings at approximately 45° to the horizontal. The larvae were kept over goat meat in the jar covered with muslin cloth so as to rear them up to the adult stage.

Rearing of flies

The adult flies of *C. bezziana* were reared in the insect cages of 45×45×45 cm size. The adults were fed on sucrose

solution, water and milk powder. Goat meat was kept in the cages as a substrate for egg laying. After oviposition, the egg masses were shifted to fresh meat in a BOD incubator. The larvae were reared on goat meat within the incubator at 30–35 °C.

Plant materials

Leaves of *A. indica* (family Meliaceae) were obtained from the botanical garden in Khalsa College Amritsar (Punjab), India. The plant material (leaves) was spread on muslin cloth sheets and was kept to dry at room temperature for 2 weeks. Dried plant material was powdered using an electric blender and was kept in air tight jars for extraction.

Preparation of crude extracts

Crude plant extracts were prepared using Soxhlet extractor with four different solvents (99 % pure AR) viz. petroleum ether, chloroform, ethyl acetate and methanol. The extracted solvents were filtered using Whatman filter paper no. 1 in case of each solvent. The collected extracts were then evaporated under the reduced pressure using rotary vacuum evaporator at 40–50 °C. So as to obtain completely dried extract, the concentrates were kept at 50 °C in hot air oven. The crude extract of each solvent was weighed and kept in glass vials in deep freezer for further use.

Experimental application

Dipping method

Third instar larvae totalling 1360 from the same batch of eggs of *C. bezziana* were used in batches of 340 for each solvent. Larvae for each solvent were divided into four groups (concentrations) with 80 larvae each i.e. four replicates each with 20 larvae, and a fifth group with 20 larvae was used as control. Different concentrations for each solvent used in this experiment are listed in Table 1 and were prepared by mixing crude plant extracts in ethanol. Third instar larvae were treated by dipping them for 30 s in different concentrations of crude extracts and ethanol alone in case of control group. After treatment the larvae of each replicate were kept in the rearing jar covered by the muslin cloth. The replicates were kept in an incubator at 35 °C and examined daily for seven successive days and mortality rates were recorded. The larvae who survived were observed to demonstrate the effects of extracts on the development of the larvae till fly emergence.

Thin film application method

Third instar larvae totalling 1360 from the same batch of eggs of *C. bezziana* were used in batches of 340 for each solvent and were distributed as mentioned previously. The concentrations in each solvent and control groups (ethanol alone) 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 a thin film of the extract. Larvae were released on thin layer of the extract in petri plates and were covered. Larvae were examined daily for 7 days to record the mortalities and to observe their development till emergence.

Parameters used

The effect of crude extract of neem on the larvae of *C. bezziana* was evaluated using four parameters: larval mortality, % age pupation, pupal mortality and % age adult emergence. Larval mortality was recorded daily for 7 days after the experimental application of the extracts. The larvae were touched with fine-grade "0" brush to check any movement. Moreover, the change in larval coloration was also observed during the period. The % age pupation was recorded by counting the number of viable, turgid and dark brown-coloured puparia after subtracting the dead larvae. The pupal mortality and percentage of adult emergence was recorded daily after 7–10 days of pupation. Percentage larval or pupal mortalities were calculated using the formula:

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae or pupae}}{\text{Number of larvae or pupae introduced}} \times 100$$

Statistical analysis

The data was subjected to statistical analysis by ANOVA to test for the differences between various concentrations and control using SPSS software (version 16.0). LC_{50} values were calculated using probit analysis (Finney 1971). Values with $P<0.05$ were considered to be statistically significant.

Results

The effects of crude extracts of *A. indica* in different solvents on larval survival and development by dipping and thin film application methods in different solvents are shown in Table 1. Figure 1 shows the toxicity in terms of LC_{50} values in four solvents viz. petroleum ether, chloroform, ethyl acetate and methanol of *A. indica* applied with the dipping method. Figure 2 shows the toxicity of crude extract of *A. indica* with all the above solvents applied with the thin film application method. Figure 3 shows the shape of dead larvae and puparia resulted from the treatment with the crude extracts. The dead

Table 1 The effect of crude extracts of *A. indica* on the development of the third instar larvae of *C. bezziana* using the dipping method

Solvent	Conc. (g/100 ml)	Larval mortality (%)	Pupation (%)	Pupal mortality (%)	Emergence of adult (%)
Petroleum ether	4.12	46±1.63	54±1.63	43.28±1.85	56.72±2.15
	2.06	28±2.49	72±2.49	50.34±1.17	49.66±3.24
	1.03	09.33±1.63	90.67±1.63	59.75±1.63	40.25±2.12
	0.515	16±163	84±1.63	34.74±1.63	65.26±3.58
	Control	0.00±00	100±00	0.00±00	100±00
	P value	0.001	0.000	0.000	0.000
Chloroform	2.5	45.34±2.49	54.66±2.50	51.43±2.49	48.57±2.26
	1.25	60±1.63	40±1.63	56.22±2.98	43.78±1.85
	0.625	57.33±1.63	42.67±1.55	31.44±1.63	68.56±1.17
	0.3125	56±01.63	44±1.63	38.1±1.33	61.90±3.01
	Control	0.00±00	100±00	0.00±00	100±00
	P value	0.000	0.000	0.000	0.001
Ethyl acetate	6.5	53.33±2.98	46.67±2.98	38.3±2.49	61.70±2.33
	3.25	26.66±2.11	73.33±2.11	43.76±1.55	56.24±3.50
	1.625	21.33±2.49	78.66±2.49	33.89±2.26	66.11±2.45
	0.8125	32±4.90	68±4.90	57.1±2.50	42.90±1.52
	Control	0.00±00	100±00	0.00±00	100±00
	P value	0.000	0.000	0.001	0.001
Methanol	1.7	46±3.40	54±3.40	80±2.98	20±2.11
	0.85	56±3.40	44±3.40	89.34±2.33	10.66±1.63
	0.425	52±3.89	48±3.89	89.34±1.52	10.66±1.63
	0.2125	82.66±1.63	17.34±1.63	92.01±1.63	7.99±1.33
	Control	0.00±00	100±00	0.00±00	100±00
	P value	0.000	0.000	0.000	0.001

larvae were flaccid with dark brown or black colour, whereas dead puparia appeared to be normal except for the anterior portion that seemed to be as that of third instar larvae.

Dipping method

All the larval mortalities were significantly different ($P<0.05$) when compared with control in all the concentrations of *A. indica* (Table 1). The percentage mortality decreased while percentage emergence increased with decrease in concentrations. The LC_{50} values were recorded as 1.07 g/100 ml, 1.7 g/100 ml, 3.39 g/100 ml and 4.9 g/100 ml in methanol, chloroform, petroleum ether and ethyl acetate extract, respectively. Thus, according to larval mortalities, the effects of the neem extract on the third instar larvae of *C. bezziana* can be arranged as methanol>chloroform>petroleum ether>ethyl acetate. All the larvae who escaped mortality were pupated normally, but all of them did not emerge to adult flies with all the tested concentrations. The percentage emergence of adult flies was significant with all the concentrations as compared with the control ($P<0.05$).

Thin film application method

Laval mortalities were almost higher in all the four extracts using the thin film treatment than the dipping method (Table 2). All the concentrations showed significant differences as compared to control in case of mortality, pupation and fly emergence ($P<0.05$). All the survived larvae pupated but did not emerge completely to the adult flies. LC_{50} values were recorded as methanol 0.4 mg/cm^2, chloroform 0.6 mg/cm^2, ethyl acetate 2.1 mg/cm^2 and petroleum ether 2.5 mg/cm^2. Accordingly, the effect of extracts were in the order of methanol>chloroform>ethyl acetate>petroleum ether.

Discussion

The present study evaluated the efficacy of the crude extract of *A. indica* on the third instar larvae of *C. bezziana* by using the dipping and thin film application methods. The results showed that the extract of *A. indica* in all the solvents had a toxic effect against the third instar larvae of *C. bezziana* in both the methods. The choice of dipping technique is based on

Table 2 The effect of crude extracts of *A. indica* on the development of the third instar larvae of *C. bezziana* using the thin film application method

Solvent	Conc. (mg/cm^2)	Mortality of larvae (%)	Pupation (%)	Pupal mortality (%)	Emergence of adult (%)
Petroleum ether	3	56±2.67	44±1.63	42.67±1.63	57.33±1.63
	1.5	29.33±1.63	70.67±2.11	37.34±1.63	62.66±1.63
	0.75	17.33±1.63	82.67±4.00	26.67±2.11	73.33±1.63
	0.375	22.66±1.63	77.34±1.63	73.34±1.63	26.66±2.98
	Control	0.00±00	100±00	0.00±00	100±00
	P value	0.000	0.001	0.000	0.000
Chloroform	1.2	45.33±2.49	54.67±1.63	36.37±3.54	63.63±5.51
	0.6	37.33±1.63	62.67±4.80	37±2.12	63.0±10.4
	0.3	58.66±2.49	41.34±2.11	33.34±1.55	66.66±2.11
	0.15	67.33±1.63	32.67±1.63	38.1±2.11	61.90±3.01
	Control	0.00±00	100±00	0.00±00	100±00
	P value	0.000	0.656	0.001	0.953
Ethyl acetate	3.3	44.66±3.27	55.34±3.27	75.67±1.63	24.33±1.33
	1.6	86.66±5.58	13.34±17.3	81.67±1.63	18.33±5.00
	0.8	76±4.00	24.00±4.00	89.34±2.20	10.66±1.63
	0.4	90.66±1.63	9.34±1.63	92.01±1.63	7.99±1.33
	Control	0.00±00	100±00	0.00±00	100±00
	P value	0.024	0.000	0.000	0.066
Methanol	0.86	40±2.11	60±2.11	60.00±2.20	40.00±2.11
	0.43	34.80±4.46	65.2±2.50	64.00±2.33	36.00±4.00
	0.21	57.33±3.40	42.67±1.63	80.00±2.59	20.00±2.11
	0.10	67.33±1.63	32.67±1.63	69.34±2.11	30.66±2.67
	Control	0.00±00	100±00	0.00±00	100±00
	P value	0.000	0.000	0.000	0.001

the fact that most of the veterinarians apply extract locally for controlling external parasite in livestock animals. On the other hand, the thin film application technique was selected because the nature of damage caused by the parasite is restricted around the wound of the animals and the portion might be treated with a thin layer of powder or ointment of a particular extract. The active constituents may penetrate into the body of

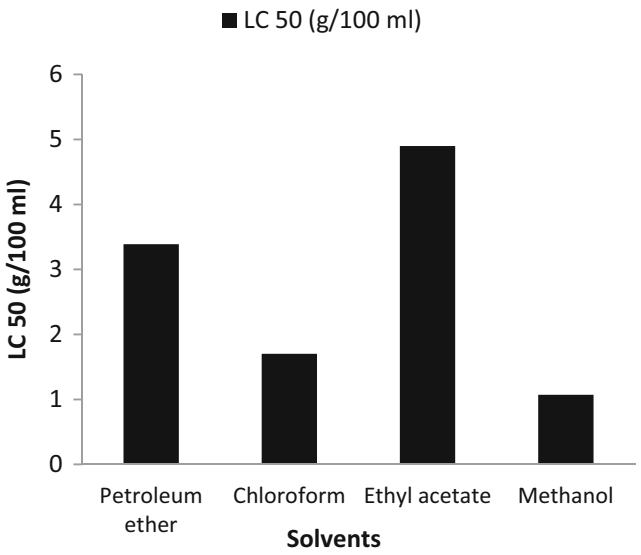

Fig. 1 The toxicity of *A. indica* extracted with different solvents against the third instar larvae of *C. bezziana* using the dipping method

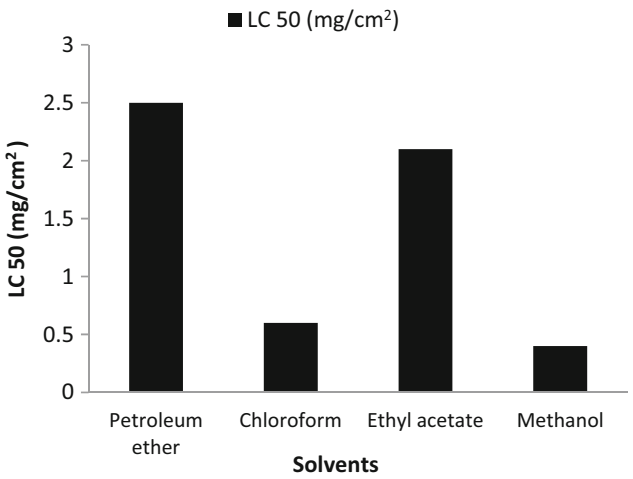

Fig. 2 The toxicity of *A. indica* extracted with different solvents against the third instar larvae of *C. bezziana* using the thin film application method

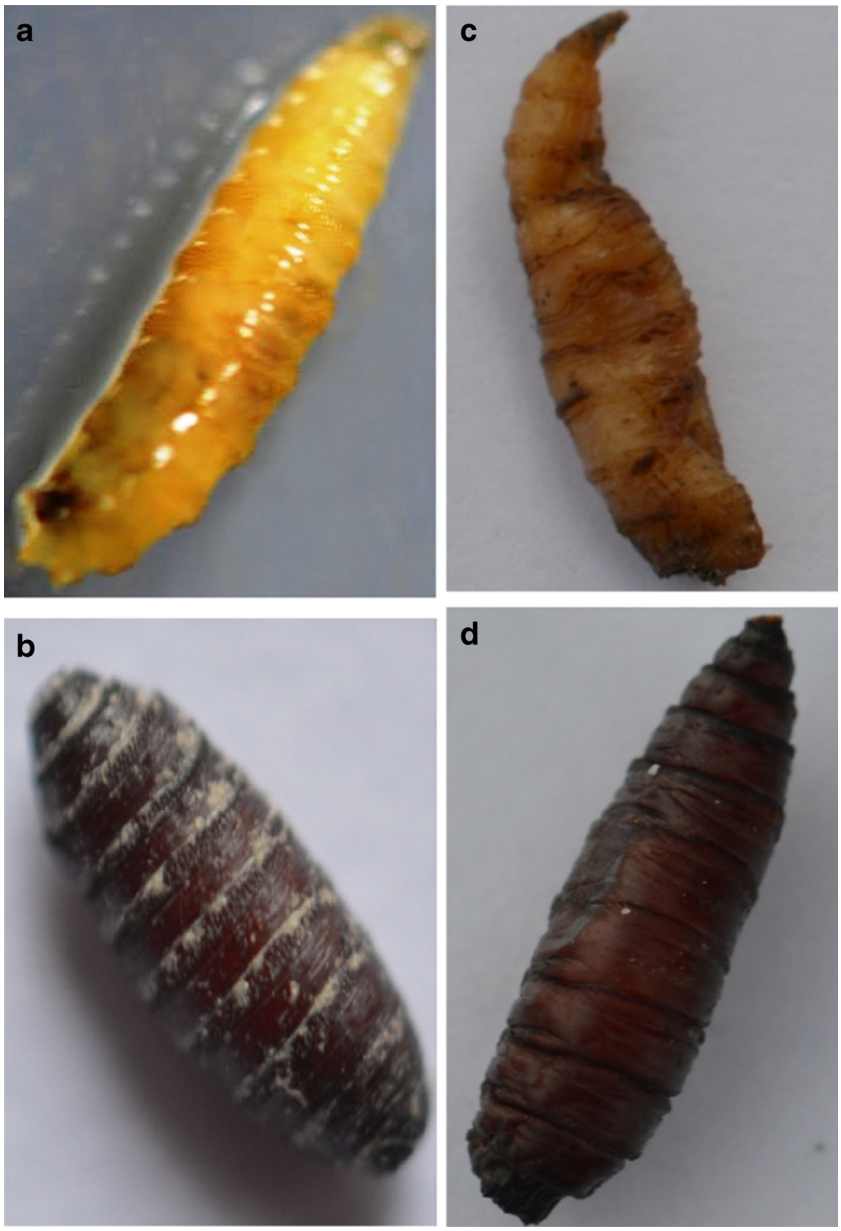

Fig. 3 The effect of plant extracts on the development of the third instar larvae of *C. bezziana*: **a** control larva and **b** control puparium have normal appearance. **c** Treated dead larva, shrunk and dark coloured. **d** Dead puparium with anterior portion resembling normal larva

larvae through ingestion in case of the dipping method or through cuticle in case of the thin film application. Studies have shown that the plant extracts can penetrate to the larval gut thereby damaging its epithelial lining which can either kill them or alter their feeding behaviour. Abdel-Shafy et al. (2009) conducted the histological examination of larval gut after treating them with extracts of wild medicinal plants—*Artemisia herba-alba*, *Artemisia monosperma*, *Euphorbia aegyptiaca* and *Francoeuria crispa*—and reported the damage to the epithelial lining in dead larvae. The present study resulted in mortalities in all the four solvents in the order of methanol>chloroform>petroleum ether>ethyl acetate. The methanol extract showed the highest larval mortality (82.66 %) and pupal mortality (92.01 %) at the lowest concentration of 0.2125 g/100 ml. Similar results were reported in a study evaluating the efficacy of the leaf extract of neem (*A. indica*) on the mosquito larvae of *Culex quinquefasciatus* (Batabyal et al. 2009), where methanol extract showed maximum larval mortality. The maximum toxicity of methanol extract might be due to the reason that azadirachtin, one of the active insecticidal components of *A. indica*, has maximum solubility in methanol (Esparza-diaz et al. 2010). The various components like azadirachtin, salanin and nimbin have been reported in *A. indica* which have shown to posses insecticidal, antifeedant and insect growth inhibitor activities (Evans 2009). These major properties of the components of the plant have made it suitable for the control of insect pest. Instead of killing the pest, these components affect their life cycle. Azadirachtin belongs to a class of organic molecules called tetranortriterpenoids, which is similar in chemical structure to

an insect growth hormone called ecdysone that regulates the pupation and moulting of insects (Mukandiwa et al. 2012). Besides killing, it alters their developmental process in such a manner that the pupated larvae do not emerge into adults. Thus, the larvae which escaped from death pupated normally but all of them did not emerge into the adults in the present study. The chloroform extract showed the highest larval mortality after methanol extract. Based on the polarity of different solvents used, the amount of bioactive compounds dissolved in them also varies. Being a solvent of lower polarity than methanol, chloroform had been reported to dissolve lesser percentage of bioactive insecticidal components of *A. indica*. The chloroform extract of *A. indica* had been reported to contain 15.78 % of azadirachtin (Sinha et al. 1999). The larvicidal activity of the chloroform extract of *A. indica* against *Aedes aegypti* mosquito larvae had been reported which resulted in 100 % mortality (Nour et al. 2012). Ethyl acetate resulted in larval mortalities less than those in methanol and chloroform extracts. Similar results were reported by Kamaraj et al. (2010) while studying the larvicidal activity of *A. indica* against the larvae of *Culex gelidus* and *C. quinquefasciatus* where ethyl acetate extract showed 42 % and 91 % mortality, respectively, for the two species. Petroleum ether extract showed the least mortality out of all the four extracts. Batabyal et al. (2009) reported toxicity of neem extracts against the larvae of the filarial vector, *C. quinquefasciatus*, in which petroleum ether extract exhibited the least toxicity values out of all the extracts with LC_{50} values of 79.17 and 63.17 ppm and LC_{90} values 234.57 and 193.87 ppm after 24 and 48 h of exposure.

A unique phenomenon was observed during the dipping method in chloroform and methanol extracts. It was observed that the highest concentration of the extracts resulted in mortalities lower than those in the lowest concentration in these solvents. Similar results were reported by Abdel-Shafy et al. (2009) while studying the efficacy of wild medicinal plant extracts on the survival and development of *C. albiceps*. It may be due to the fact that the larvae were stimulated for pupation promptly in higher concentrations in order to avoid lethal damage from the extracts, whereas the lowest concentration might not have stimulated them to form pupae immediately and hence more active constitutes were absorbed resulting in higher mortalities. As a result, larvae treated with high concentrations may produce more percentage of normal flies than treated with lower concentrations.

It is concluded that the crude extracts of *A. indica* tested in the present study can be useful in controlling myiasis-causing larvae of *C. bezziana*. The crude extracts of the plant can be applied locally to myiatic wounds among domestic animals to evacuate and kill the maggots present therein. The multiple applications would be useful to develop a prolonged effect, thereby resulting into an effective control strategy. It is further recommended that the neem extract can prove to be a better alternative to synthetic antimyiatic agents which are being used conventionally and are known to contaminate the dairy products like milk and meat with their residues. It will be useful for farmers both at controlling infestations of *C. bezziana* on their livestock under normal working conditions as well as not causing problems of contamination of dairy products with drug residues. Further studies should be conducted to validate the efficacy of *A. indica*-based products on myiasis-causing flies.

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Compliance with ethical standards

The present research work was carried out in compliance with the ethical standards.

Conflict of interests The authors declare that they have no competing interests.

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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

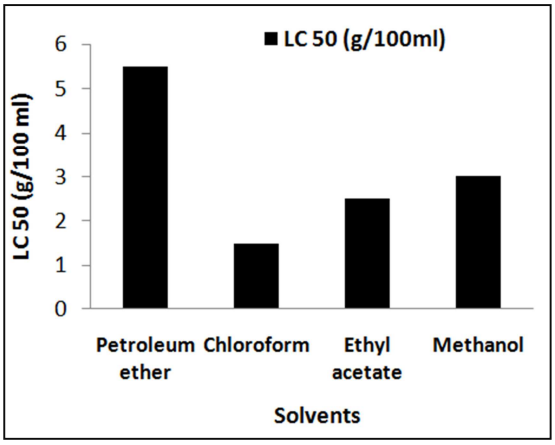

Fig. 1. *Toxicity of R. communis extracted with different solvents against third instar larvae of M. domestica using Dipping Method.*

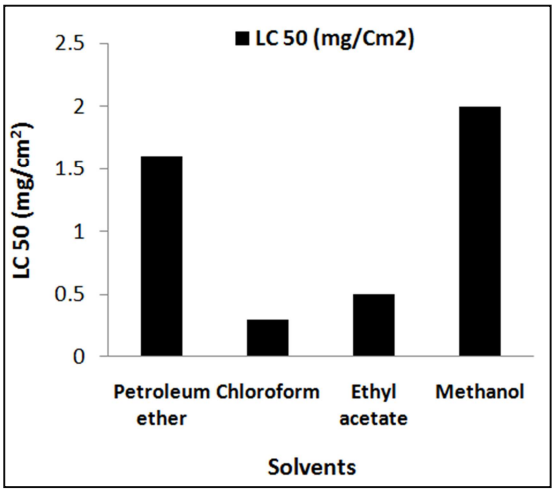

Fig. 2. *Toxicity of R. communis extracted with different solvents against third instar larvae of M. domestica using Thin Film Technique.*

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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Research Article

Activity of Foliage Extracts of Ricinus communis L. (Euphorbiaceae) Against Myiasis Causing Larvae of Chrysomya bezziana Villeneuvae (Diptera: Calliphoridae)

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Abstract

Myiasis, the infestation of live vertebrate animals with dipteran larvae, is a common parasitic problem of livestock industry leading to massive economic losses to dairy farmers across the globe. *Chrysomya bezziana* (Diptera: Calliphoridae) is a predominant fly species responsible for myiasis among domestic animals in tropical regions in the Old World. Synthetic compounds being used to control myiasis generally contaminate the dairy products with their residues leading to severe health hazards among humans. The increasing concern of pesticide accumulation in the environment has prompted researches to develop safer alternatives. Plant-derived materials being biodegradable have been currently evaluated as an alternate remedy in controlling arthropods of medical and veterinary importance. The present study evaluated the efficacy of crude leaf extracts of *Ricinus communis* against larvae of *C. bezziana* by using dipping and thin film technique. The results indicated that the extracts had toxic effects on the larvae in both the techniques. It was concluded that the extracts of *R. communis* can effectively be used in bio-safe management of myiatic infestations among domestic animals caused by the larvae of *C. bezziana*.

INTRODUCTION

Myiasis is the condition where larvae of certain fly species use the tissues or body fluids of a living vertebrate host as a food source for their growth and development. Myiasis is one of the most common parasitic infestations among livestock and is considered as a major problem worldwide in animal raising countries [1]. Dairy farmers have to face huge economic losses due to reduction in the productive traits and mortality as a result of disease among domestic animals. Before the eradication of the screwworm fly in USA, the losses to livestock industry due to myiasis were estimated to be US$ 140 million a year [2]. The average annual cost of fly strike to the Australian sheep industry is estimated at A $ 280 million [3]. The Old World Screwworm fly- *Chrysomya bezziana* Villeneuve (Diptera: Calliphoridae**)** has been reported to be the principal fly species responsible for causing myiasis among sheep and cattle, leading to economic losses to livestock industry in the tropical regions [4]. The fly has also been reported to cause myiasis among humans from developing countries [5]. The neglected open wounds generally predispose animals to myiasis infestations but skin and hairs soiled by excreta are particularly attractive to ovipositing flies. Any slightly bleeding wound, even as small as that of tick bite is liable to become infested [6].

Parasitic infestations among livestock are of particular interest because they results in wide range of pathological effects leading to decline in productivity in terms of reduced milk production, loss of body mass and poor hide quality [7]. Cutaneous lesions established by larval feeding lead to inflammation and significant blood loss, hence become favourable predisposing sites for onset of bacterial infections. Death may result due to toxemia and septicemia in case of non treatment [8]. Though myiasis had been recognized as the major factor leading to economic

losses, the disease is still poorly controlled in many animal raising countries. Over the decades, the control of myiasis was largely dependent on the use of synthetic chemical compounds such as macrocyclic lactones, carbamates and organophosphate insecticides. Ivermectin, a synthetic anti-myiasis agent which is generally administered through intramuscular route among domestic animals, has been reported to cause contamination of milk and meat with drug residues resulting in serious health hazards among humans [9]. The use of chemical compounds has continued despite their potential toxicity and contamination of dairy products by their residues. Due to increasing concern about the pesticide accumulation in the environment and development of resistance among insect pests, the need of alternatives becomes of paramount importance. Plant extracts have been emerging as potent biocontrol agents with low cost and risk free properties, as an alternative to synthetic compounds and can be used successfully in the control of insects of medical and veterinary importance.

Castor, *Ricinus communis* L. (Euphorbiaceae) though originated in Africa, but is now widely distributed throughout the tropical and subtropical regions of the world like Asia, Australia, Brazil and Russia [10]. Although the plant grows wild it is also cultivated for medicinal values of the oil obtained from its seeds. The leaf extract of the plant has proven toxicity against various insect pests. Leaves of *R. communis* contain a toxic component called ricinine which has potent insecticidal activity [11]. Studies evaluating the efficacy of plant extracts on insect pests have been conducted mainly on parasitic arthropods like ticks, mites and mosquitoes whereas limited studies were available on myiasis producing flies and their larvae. The use of plant extracts in control of myiasis among domestic animals as an alternative to synthetic compounds has been reported [12]. Although *C. bezziana* is reported to be the major causative agent of myiasis among livestock in Old World countries, very few studies have been documented regarding the activity of plant extracts against it. The present study was undertaken to evaluate the bioefficacy of crude leaf extracts of the plant *R. communis* against third instar larvae of *C. bezziana*. The study is assumed to be the first report on the effects of the above said plant on survival and development of third instar larvae of *C. bezziana,* with implications to control myiasis.

MATERIALS AND METHODS

Preparation of plant extracts

Leaves of *R. communius* were obtained from Botanical Garden of Khalsa College Amritsar (Punjab) India, dried at room temperature for about two weeks and then powdered using an electric grinder. Powdered plant material was extracted successively with four solvents viz. petroleum ether, chloroform, ethyl acetate and methanol using soxhlet extractor. The extracts were evaporated under reduced pressure using rotary vacuum evaporator and were then kept at 40-45° C in hot air oven so as to obtain completely dried concentrates.

Larval source

Live larvae collected from myiasis affected diary animals from local Civil Veterinary Hospital were preserved in 70% ethanol in glass vials and processed to prepare larval mounts of taxonomically important body regions such as anterior and posterior spiracles. The larvae where identified as 3rd instar larvae of *C. bezziana* with the help of available keys [6]. 8-10 larvae were kept over goat meat in a jar covered with muslin cloth so as to rear them up to the adult stage for maintaining a fly colony.

Fly Colony

C. bezziana adult flies were reared in the laboratory at 25°C and 70% relative humidity using insect cages 45x45x45 cm sizes. The adults were fed with a mixture of 10% (w/v) sugar and multi vitamin syrup solution. Goat meat was used as substrate for ovi position as well as larval rearing and the egg masses were incubated at 30–35°C which is preferred range for the eggs of *C. bezziana* to hatch.

Laboratory bioassay

(i) Dipping Method: The larvicidal bioassay as described by Abdel-Shafy et al [13], was used with little modifications to test efficacy of the plant extract. 2040 fully grown third instar larvae of *C. bezziana* from same batch of eggs were used, 510 larvae for each solvent. Larvae for each solvent were divided into four groups with 120 larvae each i.e. four replicates each with 30 larvae and a 5th group with 30 larvae were used as control. Different dilutions of the plant extract, as obtained with various solvents were prepared by mixing crude plant extract in ethanol (Table 1). The third instar larvae were treated by dipping them in different concentrations of extracts for 30 seconds and ethanol alone in case of control group. The larvae of each replicate were kept in the rearing jar covered by muslin cloth in an incubator at 35°C. Mortality rates were recorded daily for seven successive days and viable larvae were observed to demonstrate the effects of extracts on their development till fly emergence.

(ii) Thin film application: Similar numbers of third instar larvae were used in this experiment and were distributed as mentioned previously. The concentrations in each solvent and control groups (ethanol alone) were prepared as above and are listed in (Table 2). The crude plant extract was poured in glass petri plates (4 cm diameter) and left until dryness so as to obtain a thin film. Fully fed third instar larvae were released on the thin film so obtained and were examined daily for seven days to record mortalities and to observe their development till emergence. Four parameters viz. % larval mortality, % pupation, % pupal mortality and % adult emergence were evaluated to study the effect of the extracts. The percentage age pupation was recorded by counting the number of viable, turgid and dark brown colored puparia after subtracting the dead larvae and %age adult emergence was recorded after 7-10 days of pupation.

Statistical analysis

The data on larval mortality, pupation, pupal mortality and adult emergence were subjected to statistical analysis by ANOVA to test for the differences between various concentrations and control using SPSS (16.0) software. LC_{50} values were calculated using probit analysis.

RESULTS

The effects of crude leaf extracts of *Ricinus communis* in four solvents on larval survival and development by dipping and thin

Table 1: Effect of crude extracts of *R. communis* on development of third instar larvae of *C. bezzian*a using Dipping Method.

Solvent	Conc (g/100 ml)	Larval Mortality (%)	Pupation (%)	Pupal mortality (%)	Adult emergence (%)
Petrolem ether	10	21.33±3.40	78.67±3.40	83.67± 1.55	16.33 ± 1.63
	5	57.23± 2.11	42.77± 3.40	74.00 ± 3.40	26.00 ± 3.40
	2.5	66.66± 1.63	33.34 ± 1.63	38.10±2.11	61.90 ± 3.01
	1.25	71.86±3.40	28.14 ± 1.63	89.34±2.20	10.66 ± 1.63
	Control	0.00 ± 00	100 ± 00	0.00 ± 00	100 ± 00
	P value	0.001	0.000	0.000	0.002
Chloroform	5	16.33 ± 1.63	83.67± 1.55	87.33 ± 1.63	12.67±2.49
	2.5	17.00 ± 4.00	83.00± 4.00	89.34±2.20	10.66 ± 1.63
	1.25	28.66 ± 1.63	71.34± 1.63	31.44±2.11	68.56 ± 1.17
	0.625	56.00±3.40	44.00± 1.63	38.10±2.11	61.90 ± 3.01
	Control	0.00 ± 00	100 ± 00	0.00 ± 00	100 ± 00
	P value	0.000	0.000	0.000	0.001
Ethyl Acetate	4.5	26.66 ± 2.11	73.34 ± 2.11	26.67 ±1.63	73.33 ± 1.63
	2.25	28.00 ± 2.49	72.00± 1.63	22.27±2.59	77.73 ± 1.63
	1.12	56.00 ± 1.63	44.00 ±1.55	42.67±1.63	57.33 ± 1.63
	0.56	87.33 ± 1.63	12.67±2.49	26.67 ±1.63	73.33 ± 1.63
	Control	0.00 ± 00	100 ± 00	0.00 ± 00	100 ± 00
	P value	0.000	0.000	0.001	0.001
Methanol	11.5	16.00 ± 1.63	84.00 ± 1.63	34.74±3.58	65.26 ±2.11
	5.75	26.00 ± 3.40	74.00 ± 3.40	89.34±2.20	10.66 ± 1.63
	2.87	56.33 ± 1.63	43.67 ± 1.55	51.43±2.20	48.57 ± 2.26
	1.43	57.00 ± 2.50	43.00 ± 1.63	66.66± 1.63	33.34 ± 1.63
	Control	0.00 ± 00	100 ± 00	0.00 ± 00	100 ± 00
	P value	0.000	0.000	0.000	0.000

Table 2: Effect of crude extracts of *R. communis* on development of third instar larvae of *C. bezziana* using Thin film Technique.

Solvent	Conc (mg/cm^2)	Larval Mortality (%)	Pupation (%)	Pupal mortality (%)	Adult emergence (%)
Petrolem ether	5	28.33± 1.63	71.67 ± 1.63	42.67±1.63	57.33 ± 1.63
	2.5	42.67± 1.63	57.33 ± 1.63	33.34±1.63	66.66 ± 2.11
	1.25	53.33± 1.63	46.67 ± 1.63	38.10±2.11	61.90 ± 3.01
	0.6	67.34± 1.63	32.66± 1.63	42.67±1.63	57.33 ± 1.63
	Control	0.00 ± 00	100 ± 00	0.00 ± 00	100 ± 00
	P value	0.000	0.001	0.000	0.000
Chloroform	2.5	21.66 ± 1.63	78.34 ± 1.63	92.01±1.63	7.99 ± 1.33
	1.25	27.66 ± 1.63	72.34 ± 1.63	89.34±2.20	10.66 ± 1.63
	0.6	54.66 ± 5.58	45.34 ± 2.11	81.67±1.55	18.33± 2.11
	0.3	57.66± 1.63	42.34± 1.63	74.00 ± 3.40	26.00 ± 3.40
	Control	0.00 ± 00	100 ± 00	0.00 ± 00	100 ± 00
	P value	0.000	0.000	0.002	0.000
Ethyl Acetate	2.2	22.66 ± 1.63	77.34 ± 1.63	26.67 ±1.63	73.33 ± 1.63
	1.1	46.66 ± 1.63	53.34 ± 1.63	81.67±1.63	18.33 ±2.98
	0.55	53.33 ± 2.98	46.67 ± 2.98	89.34±2.20	10.66 ± 1.63
	0.275	57.33± 1.63	42.67± 1.63	92.01±1.63	7.99± 2.33
	Control	0.00 ± 00	100 ± 00	0.00 ± 00	100 ± 00
	P value	0.000	0.000	0.000	0.000
Methanol	5.8	21.33 ± 2.49	78.66±2.49	33.89±2.20	66.11±2.45
	2.9	36.00±1.63	64.00±1.63	56.22±2.33	43.78±1.85
	1.45	37.33±1.63	62.53±1.55	38.10±2.59	61.90±3.01
	0.72	57.67±1.63	32.33 ±2.98	31.44±2.11	68.56±1.17
	Control	0.00 ± 00	100 ± 00	0.00 ± 00	100 ± 00
	P value	0.000	0.000	0.000	0.000

film method are shown in tables 1 and 2. Figures 1and 2 shows the toxicity in terms of LC_{50} values in four solvents viz. petroleum ether, chloroform, ethyl acetate and methanol extracts of *R. communis* applied with dipping and thin film techniques. Larval mortalities were significantly different ($P < 0.05$) for all the four solvents when compared with control in all the concentrations. LC_{50} values in dipping method were 1.8g/100ml, 2.3g/100ml, 3.3g/100ml, and 5g/100ml while for thin film technique were 0.7 mg/cm^2, 0.9 mg/cm^2, 1.2 mg/cm^2 and 2.1 mg/cm^2 in chloroform, ethyl acetate, methanol, petroleum ether extract respectively. Thus, according to larval mortalities the effects of the crude extracts of *R. communis* on third instar larvae of *C. bezziana* were arranged as chloroform > ethyl acetate> methanol > petroleum ether. Surviving larvae pupated normally, but not all of them did emerge to adult flies with all the tested extracts. The larval mortality, pupation and adult emergence rates differed significantly with all the concentrations as compared with the control ($P < 0.05$). Developmental characteristics such as the length of pre pupation period and adult emergence were severely affected. Prolongation of pre pupation stage was noticed in almost all the treated groups. Larvae from the groups treated with crude plant extract pupated after 9-10 days while those from the control group pupated after 6-7 days. Deformed puparia appeared normal except for the anterior portion that seemed to be as that of third instar larva (larviform). Other pupal malformations included- reduced, segmented and distorted puparia followed by exposure to the leaf extracts (Figure 3).

DISCUSSION

The results indicate that extracts of the plant *R. communis* have toxic effect in all the solvents against third instar larvae of *C. bezziana* in both the techniques. The outcomes of this study are promising as these point towards the potential of the plant to be used as a natural control agent for the management of myiasis causing fly populations of *C. bezziana* by hindering the growth and development of their larval stages. Selection of dipping method was based on the fact that most of the preparations are applied locally by dairy farmers for controlling various external parasites among livestock animals whereas thin film application technique was selected because the damage caused by parasite is restricted

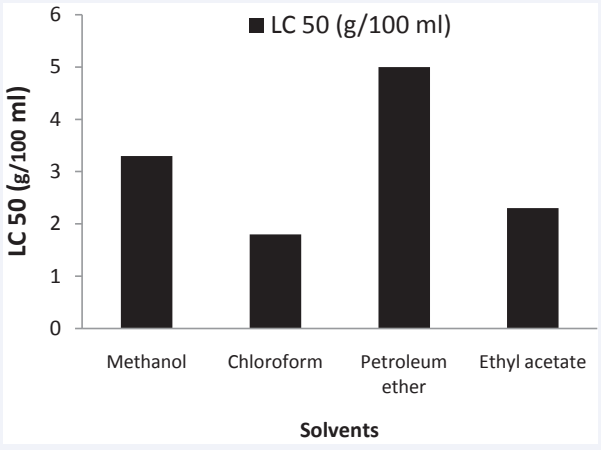

Figure 1 Toxicity of *R. communis* extracted with different solvents against third instar larvae of *C. bezziana* using Dipping Method.

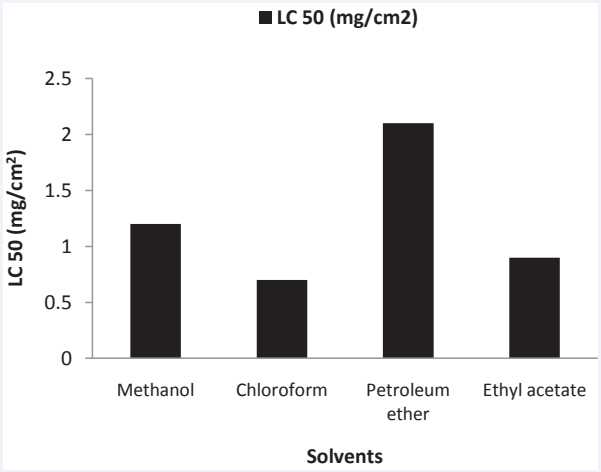

Figure 2 Toxicity of *R. communis* extracted with different solvents against third instar larvae of *C. bezziana* using Thin Film Technique.

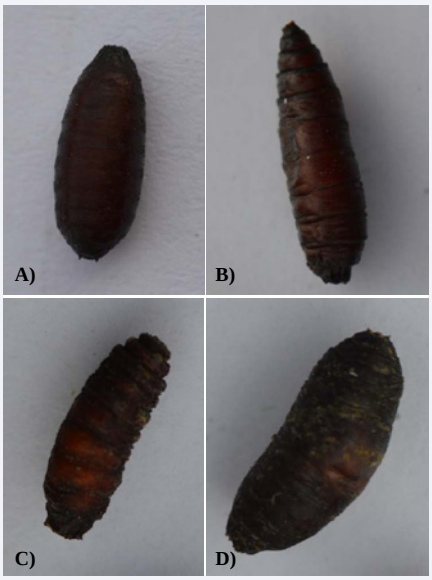

Figure 3 Different forms of pupae emerged from larvae of *C. bezziana* exposed to leaf extracts of *Ricinus communis* (a) Control (normal) puparium (b) larviform (c) segmented (d) distorted.

around the wound and hence can be treated by applying a thin layer of powder or ointment of the particular extract. The active components of the extracts generally penetrate into larval body through oral route in case of dipping method or through the body wall in thin film application. Studies have reported that some active constituents of the plant extracts penetrate through larval gut thus damaging its epithelial lining which results into mortality or alteration in their feeding behavior [14]. Abdel-Shafy et al. [13], conducted histological examination of larval gut after treating them with extracts of four plants- *Artemisia herba-alba*, *Artemisia monosperma*, *Euphorbia aegyptiaca*, *Francoeuria crispa* and reported the damage to the epithelial lining in dead larvae.

The chloroform extract showed highest larval and pupal mortality at lowest concentration (Table 1). Similar results

were reported in a study evaluating the toxicity of extracts from castor plant on the adult grass grub *Costelytra zealandica* where chloroform extract showed maximum mortality and main toxic substance was identified as ricinine by mass spectrometry [15]. The maximum toxicity of chloroform extract might be due to the fact that ricinine, one of the active insecticidal components of *R. communis* has maximum solubility in chloroform [16]. Ricinine belongs to a class of organic molecules known as 3-pyridinecarbonitriles, is a neurotoxic alkaloid which can paralyze and kill insects [17].

Toxicity of ricinine is due to presence of cyanide group in the molecule. Studies have revealed that ricinine inhibits cellular respiratory chain enzymes other than cytochrome oxidase which proves fatal to the insect pest after ingestion or penetration through body wall [18]. The ethyl acetate extract showed the second highest impact on larval mortality after chloroform extract. Based on the polarity of different solvents used, the quantities of active components dissolved in them show considerable variation. Basheer [19] studied larvicidal efficacy of *R. communis* against mosquito larvae of *Anopheles arabiensis* and reported that ethyl acetate leaf extract gained the lowest LC_{50} 0.390g/l while hexane extract was second followed by ethanol extract. Methanol extract showed larval mortality after ethyl acetate extract. Lopez et al. [20], studied that the methanol leaf extract of *R. communis* showed 100% mortality rate 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. Toxicity of *R. communis* had been reported 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 [21].

The physiological and developmental anomalies observed in the present study had been reported in a number of insect pests after exposure to plant extracts. Various mechanisms of action have been put forward to explain these effects caused by plant components. The prolonged larval or pupal periods followed by exposure to plant extracts indicate that they interfere with the hormonal control of moulting. It has been reported that the plant compounds cause progressive degeneration of neuro endocrine glands of the larvae, resulting into generalized dysfunction of the hormonal system leading to prolonged larval and pupal periods [22]. Flavonoids are the secondary metabolites found frequently in plants that play a key role in their defense system against pests and pathogens [23]. Kaempferol-3-O-β-D-xylopyranoside, kaempferol-3-O-β-D-glucopyranoside, quercetin-3-O-β-D-xylopyranoside, quercetin-3-O-β-D-glucopyranoside, kaempferol-3-O-β-rutinoside and quercetin-3-O-β-rutinoside are some of the flavonoids reported in aerial parts of *R. communis* that have revealed insecticidal activity against insect pests [24]. These compounds have been found to affect the activity of an enzyme ecdysone-20-monoxygenase, which is responsible for the biosynthesis of 20-hydroxyecdysone, an important precursor of insect growth hormone, ecdysone [25]. This hormone is secreted by endocrine glands of the insects and initiates the moulting through which larva grows into adult. Hindrances in synthesis of the hormone largely affect the prepupation length and adult emergence. Prolongation of prepupation stage and non emergence of adults noticed in case of treated larvae in the present study might be due to interference with the synthesis of ecdysone by flavonoids components present in the leaf extracts of *R. communis*.

CONCLUSION

It is concluded that the crude leaf extracts of the plant *R. communis* can prove beneficial in controlling myiatic infestations among domestic animals caused by *C. bezziana*, thereby reducing economic losses to farmers. This preliminary study indicates that the plant contains certain active components that can cause larval mortalities and developmental anomalies in the myiasis causing fly *C. bezziana.* The crude plant extract can be applied locally to the myiasis affected wounds by the dairy farmers, so as to kill the maggots present therein and hence alleviate the process of healing. Although a weed, the plant may serve as an efficient, low cost larvicidal agent and a suitable bio control strategy in the future.

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