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

**Amandeep Singh*and Jasneet Kaur**

*Department of Zoology, Khalsa College Amritsar, India*

***Corresponding author**

Amandeep Singh, Department of Zoology, Khalsa College Amritsar, 143001 Punjab, India, Tel: 91-9855982589; Email: kapooraman22@rediffmail.com

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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

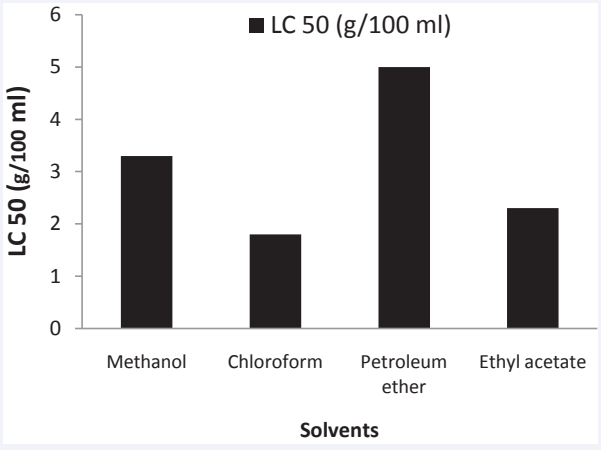


**Figure 1** Toxicity of *R. communis* extracted with different solvents against third instar larvae of *C. bezziana* using Dipping Method.

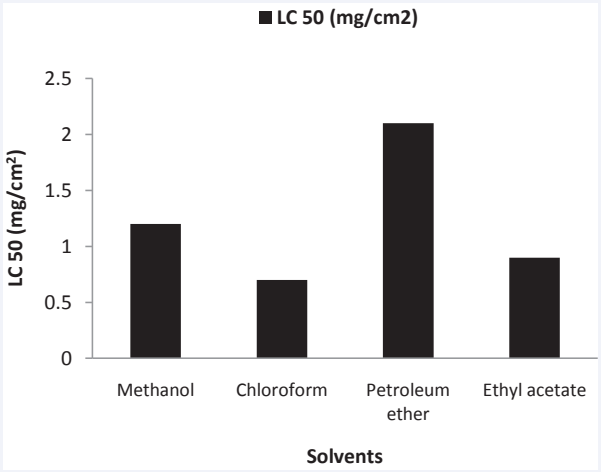


**Figure 2** Toxicity of *R. communis* extracted with different solvents against third instar larvae of *C. bezziana* using Thin Film Technique.

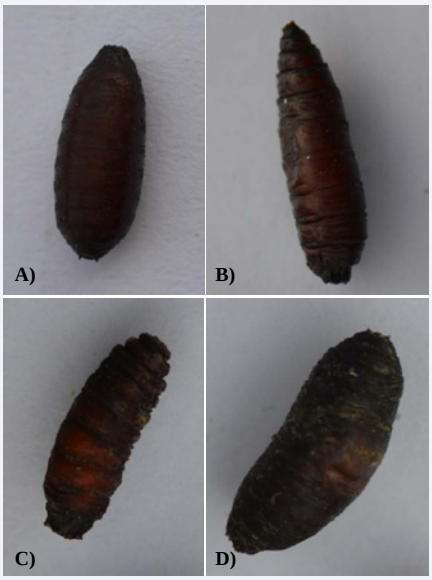


**Figure 3** Different forms of pupae emerged from larvae of *C. bezziana* exposed to leaf extracts of *Ricinus communis* (a) Control (normal) puparium (b) larviform (c) segmented (d) distorted.

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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