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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

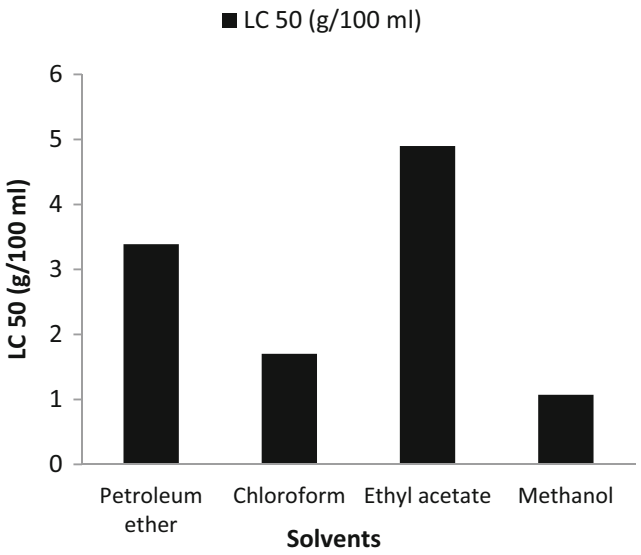

Fig. 1 The toxicity of *A. indica* extracted with different solvents against the third instar larvae of *C. bezziana* using the dipping method

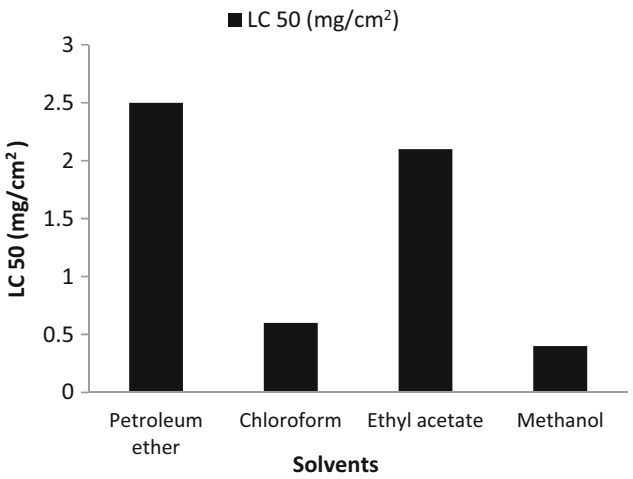

Fig. 2 The toxicity of *A. indica* extracted with different solvents against the third instar larvae of *C. bezziana* using the thin film application method

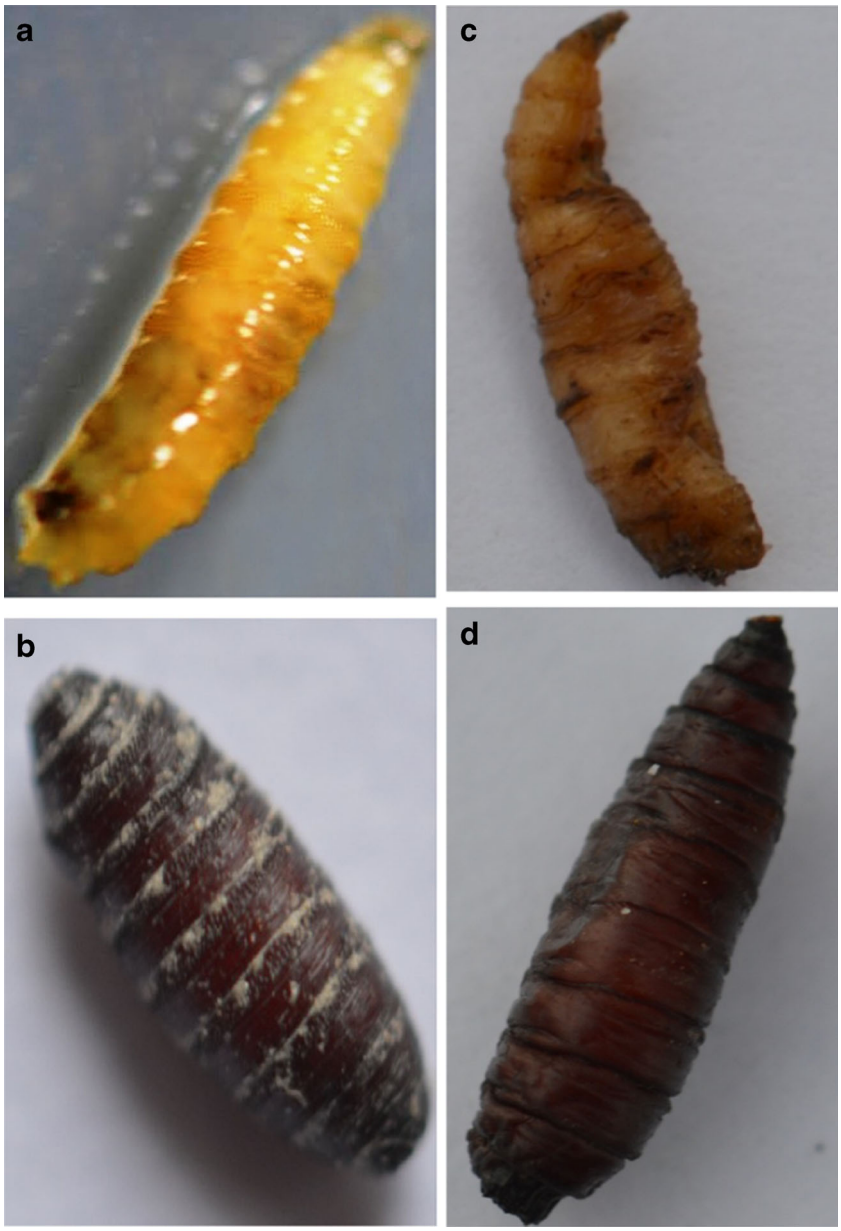

Fig. 3 The effect of plant extracts on the development of the third instar larvae of *C. bezziana*: **a** control larva and **b** control puparium have normal appearance. **c** Treated dead larva, shrunk and dark coloured. **d** Dead puparium with anterior portion resembling normal larva

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.

References

Abdel-Shafy, S., El-Khateeb, R.M., Soliman, M.M. and Abdel-Aziz, M.M., 2009. The efficacy of some wild medicinal plant extracts on the survival and development of third instar larvae of *Chrysomya albiceps* (Wied) (Diptera: Calliphoridae). *Tropical Animal Health and Production*, **41**, 1741–1753.

Anon, 1979. Screw-worm fly. Possible prevention and eradication policies for Australia. Australian Bureau of Animal Health, Canberra.

Atawodi, S.E. and Atawodi, J.C. 2009. *Azadirachta indica* (neem): a plant of multiple biological and pharmacological activities. *Phytochemistry Reviews*, **8**, 601–620.

Batabyal, L., Sharma, P., Mohan, L., Maurya, P. and Srivastava, C. N., 2009. Relative toxicity of neem fruit, bitter gourd, and castor seed extract against the larvae of Filaria vector, *Culex quinquefasciatus. Parasitology Research*, **105**, 1205–1210.

Chemonges, N.S., 2003. *Chrysomya bezziana* in pet dogs in Hong Kong: A potential threat to Australia. *Australian Veterinary Journal*, **81** (4), 202–205.

Debashri, M. and Tamal, M., 2012. A Review on efficacy of *Azadirachta indica* A. Juss based biopesticide: An Indian perspective. *Research Journal of Recent Sciences*, **1** (3), 94–99.

El-Shazly, M.M., El-Zayat, E.M. and Hermersdorfer, H., 2000. Insecticidal activity, mammalian toxicity and mutagenicity of an ethanolic extract of *Nerium oleander* (Apocynaceae). *Annals of Applied Biology*, **136** (2), 153–157. doi: 10.1111/j.1744-7348.2000.tb00020.x

Esparza-Diaz, G., Juan, A.V.J., Lopez-Collado, J. and Daniel, A.R.L., 2010. Azadirachtin Extraction using cold press and soxhlet method. *Biopesticide International*, **6** (1), 45–51.

Evans, W.C., 2009. Trease and Evans' Pharmacognosy. (Saunders Elsevier, London), pp. 433–434.

Finney, D.J., 1971. Probit analysis. (Cambridge University Press, London), pp 68–78.

Guerrini, V.H., 1988 Ammonia toxicity and alkalosis in sheep infested by *Lucilia cuprina* larvae. *International Journal of Parasitology*, **18**, 79–81.

Hall, M. and Wall, R., 1995. Myiasis in humans and domestic animals. *Advances in Parasitology*, **35**, 257–334.

Humphery, J.D., Spradbery, J.P. and Tozer, R.S., 1980. *Chrysomya bezziana*; Pathology of Old World Screw-worm fly infestations in cattle. *Experimental Parasitology*, **49** (3), 381–397.

Kamaraj, C., Rahuman, A.A., Mahapatra, A., Bagavan, A. and Elango, G., 2010. Insecticidal and larvicidal activities of medicinal plant extracts against mosquitoes. *Parasitology Research*, **107**, 1337–1349.

Kaneene, J.B. and Miller, R., 1997. Problems associated with drug residues in beef from feed and therapy. *Revue Scientifique Et Technique De L'Office International Des Epizooties,* **16** (2), 694–708.

Maheswaran, R. and Ignacimuthu, S., 2012. A novel herbal formulation against dengue vector mosquitoes *Aedes aegypti* and *Aedes albopictus. Parasitology Research*, **110**, 1801–1813.

McNae, J.C. and Lewis, S.J., 2004. Retrospective Study of Old World screw-worm fly (*Chrysomya bezziana*) myiasis in 59 dogs in Hong Kong over a one year period. *Australian Veterinary Journal*, **82** (4), 211–214.

Morsy, T.A., Mazyad, S.A.M. and El-Sharkawy, .I.M.A., 1998a. The larvicidal activity of solvent extracts of three medicinal plants against third instar larvae of *Chrysomya albiceps*. *Journal of Egyptian Society of Parasitology*, **28** (3), 699–709.

Morsy, T.A., Shoukry, A., Mazyad, S.A.M. and Makled, K.A., 1998b. The effects of volatile oils of *Chenopodium ambrosioides* and *Thymus vulgaris* against the larvae of *Lucilia sericata* (Meigen). *Journal of Egyptian Society of Parasitology*, **28**(2), 503–510.

Mukandiwa, L., Eloff, J.N. and Naidoo, V., 2012. Evaluation of plant species used traditionally to treat myiasis for activity on the survival and development of *Lucilia cuprina* and *Chrysomya marginalis* (Diptera: Calliphoridae). *Veterinary Parasitology*, **190** (3–4), 566–572.

Narayan, M.A. and Pillay, M.R., 1936. Some notes on cutaneous myiasis in animals in Madras Presidency. *Indian Journal of Veterinary Science and Animal Husbandry*, **6**, 261–265.

Norris, K.R. and Murray, M.D., 1964. Notes on screwworm fly (Diptera: Calliphoridae) as a pest of cattle in Pupa New Guinea. CSIR Division Entomology Technical paper.

Nour, A.H., Sandanasamy, J.D., and Nour, A.H., 2012. Larvicidal activity of extract from different parts of neem (*Azadirachta indica)* against *Aedes aegypti* mosquito larvae. *Scientific Research and Essays*, **7** (31), 2810–2815.

Schnur, H.J., Zivotofsky, D. and Wilamowski, A., 2009. Myiasis in domestic animals in Israel. *Veterinary Parasitology,* **161**, 352–355.

Singh, A. and Singh, Z., 2015. Incidence of myiasis among humans- a review. *Parasitology Research,* **114** (9), 3183–3199. doi: 10.1007/s00436-015-4620-y.

Sinha, S., Murthy, P.S.N., Rao, C.V.N., Ramaprasad, G., Sitaramaiah, S., Kumar, D.G. and Savant, S.K.I., 1999. Simple Method for enrichment of azadirachtin from neem seeds. *Journal of Scientific and Industrial Research*, **58**, 990–994.

Spradbery, J.P. and Kirk, J., 1992. Incidences of Old World screw-worm fly in the United Arab Emirates. *Veterinary Research,* **127**, 33–37.

Wardhana, A.H., Kumarasinghe, S.P.W., Arawwawala, L.D.A.M. and Arambewela, L.S.R., 2007. Larvicidal efficacy of essential oil of betel leaf (*Piper betle*) on the larvae of the old world screwworm fly, *Chrysomya bezziana* in vitro. *Indian Journal of Dermatology*, **52** (1), 43–47.

Zumpt, F., 1965. Myiasis in Man and Animals in the old world.(Butterworths, London), pp 267.